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Associations of perceived stress, depressive symptoms, and caregiving with inflammation: a longitudinal study

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ABSTRACT

Objectives: Higher inflammation has been linked to poor physical and mental health outcomes, and mortality, but few studies have rigorously examined whether changes in perceived stress and depressive symptoms are associated with increased inflammation within family caregivers and non-caregivers in a longitudinal design.

Design: Longitudinal Study.

Setting: REasons for Geographic And Racial Differences in Stroke cohort study.

Participants: Participants included 239 individuals who were not caregivers at baseline but transitioned to providing substantial and sustained caregiving over time. They were initially matched to 241 non-caregiver comparisons on age, sex, race, education, marital status, self-rated health, and history of cardiovascular disease. Blood was drawn at baseline and approximately 9.3 years at follow-up for both groups.

Measurements: Perceived Stress Scale, Center for Epidemiological Studies-Depression, inflammatory biomarkers, including high-sensitivity C-reactive protein, D dimer, tumor necrosis factor alpha receptor 1, interleukin (IL)-2, IL-6, and IL-10 taken at baseline and follow-up.

Results: Although at follow-up, caregivers showed significantly greater worsening in perceived stress and depressive symptoms compared to non-caregivers, there were few significant associations between depressive symptoms or perceived stress on inflammation for either group. Inflammation, however, was associated with multiple demographic and health variables, including age, race, obesity, and use of medications for hypertension and diabetes for caregivers and non-caregivers.

Conclusions: These findings illustrate the complexity of studying the associations between stress, depressive symptoms, and inflammation in older adults, where these associations may depend on demographic, disease, and medication effects. Future studies should examine whether resilience factors may prevent increased inflammation in older caregivers.

Key words: family caregiving, inflammatory, biomarkers, chronic stress, depressive symptoms

Introduction

It has been widely believed that there are close linkages between stress, depression, and inflammation. Several prior studies have found associations of higher inflammation levels with higher stress (Hänsel *et al.*, 2010; Liu *et al.*, 2017), poor physical and mental health outcomes, and mortality (Wirtz and Von Känel, 2017; Glaser and Kiecolt-Glaser, 2005; Prior *et al.*, 2016). Biomarkers of inflammation have been used as objective health measures for studying mechanisms that link chronic stress to physical and mental health (Hänsel *et al.*, 2010; Piazza *et al.*, 2010). These biomarkers include cytokines, interleukin-6 (IL-6), C-reactive protein (CRP), and tumor necrosis factor alpha receptor 1 (TNFR1).

Research findings on the association between stress, depression (or depressive symptoms), and inflammation have been mixed. Several prospective

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population-based studies examining inflammatory biomarkers as predictors of inflammation found higher levels of IL-6 and CRP significantly associated with depressive symptoms (Gimeno *et al.*, 2009; Valkanova *et al.*, 2013; Zalli *et al.*, 2016). A prospective cohort study found depressive symptoms to be associated with changes in inflammatory biomarker levels over time (Stewart *et al.*, 2009), while other studies had found inconsistent associations between inflammatory pathways and depression. For example, results from a cross-sectional, population-based study found higher IL-6 levels and not CRP to be associated with future major depressive episodes in older adults (Bremmer *et al.*, 2008). In addition, several meta-analyses did not find consistent associations between inflammation and depression including one that examined TNF alpha biomarker (Haapakoski *et al.*, 2015) and another that examined several inflammatory biomarkers (IL-2, IL-8, and IL-10) (Dowlati *et al.*, 2010). Moreover, these meta-analyses mostly used cross-sectional studies and studies that did not examine the association of inflammation on changes in depressive symptoms over time. A meta-analysis of methodologically rigorous studies reported no association between CRP and depression (Horn *et al.*, 2018).

Family caregiving is a chronically stressful life experience that leads to high levels of perceived stress and depressive symptoms and has been linked in some studies with increased inflammation (Allen *et al.*, 2017; Epstein-Lubow *et al.*, 2010; Pinquart and Sörensen, 2003; Talley and Crews, 2007). Family caregiving is a public health concern where caregivers are often exposed to high levels of stress usually for long periods of time (Talley and Crews, 2007). A better understanding of the possible associations between perceived stress, depressive symptoms, and inflammation may benefit family caregivers' mental and physical health. However, a recent systematic review and meta-analysis of caregiving and inflammation found a small but statistically significant difference (0.16 standard deviation units) across all inflammatory biomarkers between caregivers and non-caregiver controls (Roth *et al.*, 2019), with caregivers showing greater inflammation than controls. Few studies have examined whether caregiving is associated with increased inflammation over time compared to non-caregiving controls, and those that did found little evidence linking caregiving with increased inflammation (Allen *et al.*, 2017; Potier *et al.*, 2018).

To date, relatively few studies have examined whether longitudinal changes in perceived stress and depressive symptoms are associated with changes in inflammatory biomarkers over time in family caregiving. The ability to examine these effects in a relatively low-stress general sample compared to a group that has developed substantial increases in stress over time could be valuable in understanding longitudinal relationships between perceived stress, depressive symptoms, and inflammation. The Caregiving Transitions Study (CTS), a longitudinal population-based study, has unique data to address these relationships over time (Roth *et al.*, 2020a). This study has the advantage of including participants who transitioned into extensive and sustained family caregiving over time, had marked increases in perceived stress and depressive symptoms over time, and matched with a case–control group (non-caregivers) who had little change in perceived stress or depressive symptoms over time (Haley *et al.*, 2020). In a recent report from this study, Roth and colleagues (Roth *et al.*, 2020b) studied changes in six inflammatory biomarkers over time and found that those who had transitioned to extensive and sustained caregiving showed increased inflammation on only one biomarker, TNFR1 with a small effect size ($d = 0.14$).

In the present analyses, we examined whether individual differences in perceived stress or depressive symptoms were associated with changes in inflammatory biomarkers over time, and whether these associations differed among caregivers (who showed marked increases in perceived stress and depressive symptoms over time) and a non-caregiver comparison group (who showed stability over time on these variables). We hypothesized that 1) baseline measures of perceived stress or depressive symptoms would be associated with higher inflammation at follow-up, 2) perceived stress or depressive symptoms measured at follow-up would be associated with increased inflammation, and 3) greater changes in perceived stress or depressive symptoms (from baseline to follow-up) would be associated with greater increases in inflammation over time for transitioned caregivers only. Because inflammatory biomarkers can be affected by a variety of factors including sociodemographic variables, chronic conditions such as obesity and diabetes (Ouakinin *et al.*, 2018; Siddiqui *et al.*, 2019), and medications such as antidepressants and statins (Hussain and Ballantyne, 2021; Vogelzangs *et al.*, 2012), we conducted both unadjusted and adjusted analyses and considered these factors as potential confounders.

Methods

Data source

REGARDS and Caregiving Transitions Study procedures and participants

The present study uses data from a national longitudinal cohort study, the REasons for Geographic

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and Racial Differences in Stroke (REGARDS) study and an ancillary study of REGARDS, the CTS. In 2003–2007, the REGARDS study enrolled 30,239 African American and White adults 45 or more years of age living in the United States. Residents of the "stroke belt" region of the United States (southern states with higher mortality from stroke) and African Americans were oversampled by design. Participants who provided a verbal informed consent were given a baseline computer-assisted telephone interview (CATI) that assessed demographics, medical history, and a wide variety of potential risk factors for stroke. Exclusion criteria included race other than non-Hispanic White or African American, history of chemotherapy/radiation for cancer in past 2 years and living in or transitioning to a nursing home (NH). Eligible participants had a subsequent in-home assessment where biological specimens (including blood draws), physical measurements, and medication use were taken and recorded. Semiannual follow-up CATIs were conducted to monitor changes in health, including possible strokes and cognitive functioning. A second comprehensive telephone interview and in-home visit were conducted 2013–2016, approximately 9–10 years after initial enrollment, and extensive follow-up information was gathered including blood draws. Additional information on the design, sampling, enrollment, and follow-up procedures in the REGARDS study has been documented elsewhere (Howard *et al.*, 2017).

Participants at the baseline CATI were asked "Are you currently providing care on an ongoing basis to a family member with a chronic illness or disability?" This could include any form of help like watching, transporting, bathing, or dressing the care recipient. Participants who replied "yes" to this question were categorized as caregivers and provided more information as to whether they cared for a spouse, resided with the care recipient, experienced any mental or emotional strain from the care provided, and have been included in several papers addressing the mental and physical health consequences of caregiving (Roth *et al.*, 2009; Roth *et al.*, 2013; Roth *et al.*, 2018). Participants who replied "no" were categorized as non-caregivers and were considered for inclusion in the current analyses. After REGARDS 2nd telephone and in-home assessment (approximately 11.8 years after the baseline CATI), an updated caregiving status information was collected through a Caregiving Screening CATI module administered as part of the standard REGARDS semiannual follow-up CATI (Roth *et al.*, 2020a). The total number of participants who answered "yes" to being a caregiver at the Caregiver Screening CATI and had previously answered "no" to being a caregiver at the baseline CATI was 1229. These participants reporting being a caregiver at the 2nd CATI were asked questions on a more detailed CTS Enrollment Interview to determine their exposure to caregiving and whether they met eligibility criteria including when they initiated the caregiving role (month and year), whether caregiving was provided on a continuous basis since that time, their personal relationship to the care recipient, whether the care recipient currently resided or ever resided in a NH or assisted living facility (ALF), and the number of hours per week of caregiving provided to the care recipient. To be eligible to participate as an incident caregiver in the CTS, the transition into the family caregiving role had to occur at least 6 months after the first in-home assessment and at least 3 months before the 2nd in-home assessment of REGARDS to assure that the second blood draw was during a period of caregiving (since blood was drawn at both REGARDS in-home assessments).

Exclusion criteria for incident caregivers in the CTS included the caregiver not having usable blood samples at either of the REGARDS in-home assessments, the care recipient living in a NH/ALF or other residential care setting, caregiving duties less than 5 hours per week, or the caregiver residing more than 50 miles from the care recipient. Of the 1229 potential incident caregivers, 251 incident caregivers met eligibility criteria and accepted participation in the CTS (Haley *et al.*, 2020; Roth *et al.*, 2020a).

The 10,254 participants who were not caregivers at either assessment were considered potential non-caregivers. Once an incident caregiver was enrolled, a pool of non-caregivers was identified that individually matched the caregiver on seven demographic and health history factors including, age, sex, race, education, marital status, self-rated health at baseline, and self-reported history of serious cardiovascular disease (Haley *et al.*, 2020; Roth *et al.*, 2020a). These potential participants were randomly called until one comparison was determined to be eligible and agreed to participate. Additional matching restrictions for spouse caregivers included non-caregiving comparison participants being married, and matching restrictions for caregivers of a parent included non-caregivers having at least one living parent. These matched non-caregivers then completed the CTS Enrollment Interview to confirm that they had not been family caregivers at any point during their participation in the REGARDS study, and to complete assessments of their psychological well-being and health. Caregivers and non-caregivers with missing biomarker data from blood drawn during REGARDS 1st and 2nd in-home assessments were excluded from this study. Participants who had at least one of the six biomarker

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measures obtained from both REGARDS in-home assessments were included in the present study for a total number of 239 caregivers and 241 non-caregiver comparison participants. Due to missing biomarker data, caregivers and non-caregivers were not all individually matched; however, analyses showed they did not differ significantly on any of these variables (Roth *et al.*, 2020b). The protocols of both the REGARDS parent study and the CTS ancillary study were approved by the Institutional Review Boards of the participating institutions, and informed consent was provided by all participants.

Measurements

Perceived stress and depressive symptoms

Perceived stress and depressive symptoms were assessed during the REGARDS baseline CATI with a four-item version of the Cohen Perceived Stress Scale (PSS) (Cohen *et al.*, 1983) and a four-item version of the Center for Epidemiological Studies-Depression (CES-D) scale (Melchior *et al.*, 1993), respectively. Higher scores indicated more perceived stress or depressive symptoms. At the CTS Enrollment Interview, caregivers and non-caregivers repeated the identical four-item version of the PSS but completed a 10-item CES-D measure (Andresen *et al.*, 1994). We used this longer version at follow-up to provide greater variability on the measure. For data analysis, we transformed the 4-item version of the CES-D to estimate a 10-item score, using regression analyses as reported in prior publications (Haley *et al.*, 2020; Melchior *et al.*, 1993), with the two versions on different variants of the CES-D being highly correlated ($r = 0.82$). As noted below, we also used a cutoff score of 10 or more on the 10-item CES-D to classify participants as having clinically significant depressive symptoms, the more conservative of two cut points proposed (Andresen *et al.*, 1994), and as described by Haley *et al.* (2020), during sensitivity analyses.

Biomarker assays

Participants had blood samples obtained in the morning and collected by trained phlebotomists. Participants were reminded to fast 10–12 hours overnight, and visits to participant homes were conducted in the mornings to permit fasting status. Information about the processing of biological samples can be found in previous publications (Gillett *et al.*, 2014; Howard *et al.*, 2005; Roth *et al.*, 2020a; Roth *et al.*, 2020b).

The six circulating blood biomarkers of inflammation assayed in this study included high-sensitivity CRP, D dimer, TNFR1, IL-2, IL-6, and IL-10. These biomarkers were selected based on review of the previous literature on measures used most in studies of inflammation, immunity, and caregiving and the availability of valid measures from stored frozen blood samples. Additional information on the measurement can be found in a previous publication based on the same biomarker data from the REGARDS study (Roth *et al.*, 2020b). The time interval between REGARDS 1st and 2nd in-home assessments ranged from 7.6 to 12.4 years and averaged 9.3 years for participants included in the present analyses.

Covariates

We used covariate information collected at the time of REGARDS 2nd in-home assessment, except for *age* which was taken at the time of the Caregiving Transitions enrollment. Other covariates included *sex* (women = 1, men = 0), *race* (Black = 1 and White = 0), *education* (college graduate or above = 1, and some college, high school graduate, and less than school graduate = 0), *marital status* (married/cohabitating = 1 and single/never married/divorced/widowed = 0). *Nicotine use* was indicated by a binary variable indicating whether a participant was smoking (coded as 1) or never smoked/smoked in the past (coded as 0). Using the Centers for Disease Control and Prevention (CDC, 2021) BMI obesity cutoff point of 30, *obesity* was indicated by a binary variable (BMI $\geq$ 30 = 1 and BMI < 30 = 0). Similarly, the use of *statins* and *antidepressant medications* were indicated by two binary variables ("yes" = 1; "no" = 0). Taking *hypertension* and *diabetes medications* were also indicated by binary variables ("yes" = 1 and "no" = 0).

Statistical Analyses

A log (base 2) transformation was made for each of the six biomarkers at each assessment because biomarker levels were highly skewed. We considered these variables using a strategy previously described (Jenny *et al.*, 2012) in which the dependent variable is change over time (Δ) and defined as the difference between REGARDS 2nd in-home assessment (T_2) and REGARDS 1st in-home assessment (T_1) on the log (base 2) scores: $\Delta = \text{Log}_2\ (Y_2) - \text{Log}_2\ (Y_1)$, where Y_2 and Y_1 represent the raw values of the specific biomarker of interest at T_2 and T_1, respectively.

Following Roth and colleagues (2020b) who used the same data set, after the log (base 2) transformations possible outliers for each biomarker were identified using the Tukey interquartile range (IQR) method (Tukey, 1977). The IQR, which is the difference between the 75th (Q3) and 25th (Q1) percentiles, was calculated, and all values greater than 3*IQR above Q3 were considered extreme outliers and recoded as missing. There were no values that were less than 3*IQR below Q1. CRP,

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TNFR1, and D dimer did not have extreme outliers; however, for biomarkers IL-2, IL-6, and IL-10, values above 0.95, 8.23, and 2.22 pg/mL, respectively, were extreme outliers and recoded as missing. For IL-2 and IL-6, 0.7% of the values were identified as extreme outliers, and for IL-10, 1.5% of the values were designated as being extreme outliers. Overall, less than 0.5% values were extreme outliers and coded missing.

Independent t-tests and chi-square tests were performed to examine group differences between caregivers and non-caregivers at T_2. We also assessed differences between caregivers and non-caregivers for perceived stress and depressive symptoms measured at baseline and changes in perceived stress and depressive symptoms from baseline to T_2. Pearson's correlation statistical analyses were performed to examine the correlations between all covariates and T_2 inflammatory biomarkers.

Separate regression models were conducted to examine change in inflammatory levels for each of the biomarkers at T_2 as a dependent outcome, controlling for that biomarker at T_1 (centered at sample means) and evaluated whether there were interaction effects between caregiving and perceived stress or caregiving and depressive symptoms. First, we examined baseline perceived stress or baseline depressive symptoms as predictors for change in inflammatory levels for each of the biomarkers at T_2 for both caregiving and non-caregiving groups. Next, we assessed whether perceived stress or depressive symptoms at T_2 predicted change in inflammatory levels for each biomarker at T_2 in both groups. We then examined change in perceived stress or change in depressive symptoms on change in inflammatory levels at T_2. Statistical significance was evaluated at $p < 0.05$. These analyses were conducted first without covariate adjustment, and then with adjustment for the covariates. Analyses were performed with SAS software Version 9.4. We used data from all caregivers and non-caregivers who had available biomarker data after the recoding of outliers as missing.

Sensitivity analyses

To examine the role of clinically significant levels of depressive symptoms, we created a new binary variable that dichotomized depressive symptoms (0 = absent, 1 = present) based on whether participants had a score of 10 or greater on the CES-D, a clinically validated cutoff (Andresen *et al.*, 1994). We assessed changes in clinically significant depressive symptoms over time from baseline and T_2 as clinically significant depressive symptoms at follow-up minus clinically significant depressive symptoms at baseline with scores of 0, −1, and 1 indicating stability, improvement, and increase over time, respectively.

Results

Sample characteristics

Table 1 shows descriptive statistics for all study variables for both caregiver and non-caregiver groups at REGARDS 2nd in-home assessment (T_2). Mean age was 72 years ($SD = 8$) and ranged from 55 to 93 years; 65% were women; and 35.2% were Black. Due to the matching procedures, caregivers and non-caregivers did not differ statistically on sociodemographic variables including age, sex, race, education, and marital status. Groups did not differ in baseline perceived stress and depressive symptoms, but caregivers had significantly higher depressive symptoms (M = 6.96, $SD = 6.29$) compared to non-caregivers (M = 3.29, $SD = 4.43$) $p < 0.001$ at T_2. Caregivers also had significantly greater changes in depressive symptoms over time (M = 3.47, $SD = 6.48$) compared to non-caregivers (M = −0.23, $SD = 4.69$, $p < 0.001$). Similarly, caregivers had significantly higher perceived stress at T_2 (M = 4.55, $SD = 3.01$) compared to non-caregivers (M = 2.53, $SD = 2.56$) $p < 0.001$. Caregivers also showed significantly greater increases in perceived stress over time, (M = 1.69, $SD = 3.27$) compared to non-caregivers (M = −0.52, $SD = 2.81$) $p < 0.001$.

With respect to medication use and chronic diseases at T_2, caregivers reported being more likely to take diabetes medications (23.85%) compared to non-caregivers (17.84%) $p = 0.011$. Caregivers also reported higher likelihood of antidepressant medication use (18.83%) compared to non-caregivers (10.79%) $p = 0.013$, and caregivers were more likely to be obese (43.51%) compared to non-caregivers (34.44%) $p = 0.042$.

Perceived stress at baseline, follow-up, and changes over time

Results from unadjusted to adjusted models did not change; therefore, only adjusted models are reported. We first examined baseline perceived stress, then perceived stress at T_2, followed by change in perceived stress with inflammatory biomarker levels at T_2 controlling for the same biomarker at T_1 for both caregivers and non-caregivers. Table 2 shows the association of T_1 biomarker levels with T_2 biomarker levels for all six biomarkers, indicating that individual differences in biomarker levels were relatively stable over time. The results for our first hypothesis examining baseline perceived stress and inflammatory biomarker levels at T_2 controlling for the same

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Table 1. Descriptive characteristics of study variables

	OVERALL SAMPLE ($N = 480$)	CAREGIVERS ($N = 239$)	NON-CAREGIVERS ($N = 241$)	
VARIABLES	M (SD) OR %	M (SD) OR %	M (SD) OR %	*p*
Age (in years)[a]	71.9 (7.83)	71.6 (8.00)	72.2 (7.66)	0.456
Sex (female)	65%	64.85%	65.15%	0.947
Race (Black)	35.21%	35.56%	34.85%	0.871
Education[b]	44.38%	42.26%	46.47%	0.353
Marital status[c]	73.78%	74.14%	73.44%	0.864
Nicotine use[c]	5.06%	5.24%	4.17%	0.583
Obesity[c]	39.40%	43.51%	34.44%	**0.042**
Hypertension medications[c]	61.65%	63.95%	60.00%	0.377
Diabetes medications[c]	21.06%	23.85%	17.84%	**0.011**
Antidepressants[c]	14.66%	18.83%	10.79%	**0.013**
Statins[c]	44.52%	45.61%	44.81%	0.861
T_1 PSS[d]	2.95 (2.69)	2.86 (2.56)	3.04 (2.81)	0.458
T_2 PSS[e]	3.54 (2.97)	4.55 (3.01)	2.53 (2.56)	**<0.001**
Δ in PSS	0.58 (3.24)	1.69 (3.27)	− 0.52 (2.81)	**<0.001**
T_1 CES-D[f]	3.50 (3.32)	3.47 (3.16)	3.52 (3.48)	0.889
T_2 CES-D[g]	5.11 (5.73)	6.96 (6.29)	3.29 (4.43)	**<0.001**
Δ in CES-D	1.58 (5.93)	3.47 (6.48)	− 0.23 (4.69)	**<0.001**

SD = standard deviation; PSS = Perceived Stress Scale; CES-D = Center for Epidemiological Studies Depression Scale.

[a] Measured in years at Caregiving Transitions enrollment.

[b] Education = 1 for having a college degree or above and some college, high school graduate, and less than school graduate = 0.

[c] Covariates measured from REasons for Geographic and Racial Differences in Stroke (REGARDS) 2nd in-home assessment that include marital status = 1 for married/cohabitating and single/never married/divorced/widowed = 0, nicotine use = 1 for smoking and never smoked/smoked in the past = 0, and obesity using BMI > or equal to 30 = 1 and BMI < 30 = 0. All medication use were binary-coded "yes" = 1 and "no" = 0.

[d] Cohen's 4-item measure of perceived stress from REGARDS baseline computer-assisted telephone interview (CATI).

[e] Cohen's 4-item measure of perceived stress at Caregiving Transitions enrollment.

[f] Depressive symptoms from REGARDS baseline CATI.

[g] Depressive symptoms measured at Caregiving Transitions enrollment.

p-Value for t-tests or chi-square assessed differences between caregivers and non-caregivers.

Bolded values indicate significant results at $p < 0.05$.

biomarker at T_1 for both caregivers and non-caregivers show no significant associations. There were no significant effects for our second hypothesis examining perceived stress at T_2 with inflammatory levels at T_2. However, in support of our third hypothesis, there were significant effects for changes in perceived stress over time, and the interaction of caregiver status over time, with IL-10 at T_2. Examination of the interaction effect for caregiving and perceived stress revealed that an increase in perceived stress over time was significantly associated with an increase in IL-10 at T_2 for caregivers only (b = 0.04, SE = 0.02, $p = 0.02$).

Depressive symptoms at baseline, follow-up, and changes over time

We next examined the role of depressive symptoms at baseline, depressive symptoms at T_2, and change in depressive symptoms with inflammatory biomarker levels at T_2 controlling for the same biomarker at T_1. An initial analysis was conducted without covariate adjustment (data not shown). Table 2 shows the association of T_1 biomarker levels with T_2 biomarker levels for all six inflammatory biomarkers. Baseline depressive symptoms were associated with higher levels of D dimer (b = 0.04, SE = 0.02, $p = 0.03$), but there were no significant findings to support our hypotheses that baseline, T_2, or changes in depressive symptoms were associated with inflammation for the sample as a whole or for caregivers compared to non-caregivers.

Our sensitivity analyses replacing T_1 and T_2 depressive symptoms with the binary variable for clinically significant depressive symptoms in the same regression analyses led to similar findings. Baseline clinically significant depressive symptoms were associated with higher levels of D dimer (b = 0.73, SE = 0.27, $p = 0.01$), but there were no other significant associations found for baseline or T_2 clinically significant depressive symptoms. Similarly, there were no significant associations when examining change in clinically significant depressive symptoms over time on inflammation.

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Table 2. Estimates from regression analyses of predictors on inflammatory biomarker levels at follow-up

	IL-6		CRP		TNFR1		D DIMER		IL-2		IL-10	
PREDICTORS	B	SE	B	SE	B	SE	B	SE	B	SE	B	SE
Perceived stress												
Baseline effects												
T_1 biomarker	**0.56**	0.06	**0.55**	0.05	**0.89**	0.04	**0.56**	0.05	**0.39**	0.05	**0.43**	0.06
T_1 PSS[a]	0.00	0.01	−0.02	0.02	−0.01	0.01	0.02	0.02	−0.01	0.01	0.02	0.01
T_1 PSS*caregiver	−0.04	0.02	0.15	0.07	0.00	0.01	0.01	0.03	−0.02	0.02	−0.04	0.02
Time 2 effects												
T_1 biomarker	**0.55**	0.06	**0.55**	0.05	**0.90**	0.04	**0.56**	0.05	**0.39**	0.05	**0.41**	0.06
T_2 PSS[b]	0.00	0.01	−0.00	0.03	−0.00	0.01	0.01	0.02	−0.02	0.02	−0.02	0.01
T_2 PSS*caregiver	0.06	0.08	0.15	0.07	0.01	0.01	0.01	0.03	0.02	0.02	0.01	0.02
Change effects												
T_1 biomarker	**0.55**	0.06	**0.55**	0.05	**0.88**	0.04	**0.57**	0.05	**0.39**	0.05	**0.41**	0.06
Δ in PSS	−0.00	0.01	0.01	0.02	0.01	0.01	−0.01	0.02	−0.01	0.01	**−0.03**	0.01
ΔPSS*caregiver	0.00	0.02	−0.03	0.03	−0.01	0.01	0.00	0.03	0.03	0.02	**0.04**	0.02
Depressive symptoms												
Baseline effects												
T_1 biomarker	**0.56**	0.06	**0.55**	0.05	**0.90**	0.04	**0.57**	0.05	**0.38**	0.05	**0.43**	0.06
T_1 CES-D[c]	0.00	0.01	0.01	0.02	−0.00	0.00	**0.04**	0.01	−0.02	0.01	0.01	0.01
T_1 CES-D*caregiver	−0.00	0.02	−0.01	0.03	0.00	0.00	−0.01	0.02	−0.00	0.02	−0.02	0.01
Time 2 effects												
T_1 biomarker	**0.56**	0.06	**0.55**	0.05	**0.90**	0.04	**0.56**	0.05	**0.39**	0.05	**0.42**	0.06
T_2 CES-D[d]	0.00	0.01	−0.01	0.01	−0.00	0.00	0.01	0.01	−0.01	0.01	−0.01	0.01
T_2 CES-D*caregiver	−0.00	0.01	−0.01	0.02	−0.00	0.00	0.00	0.01	0.01	0.01	0.01	0.01
Change effects												
T_1 biomarker	**0.56**	0.06	**0.55**	0.05	**0.90**	0.04	**0.57**	0.05	**0.39**	0.05	**0.42**	0.06
Δ in CES-D	−0.00	0.01	−0.01	0.01	0.00	0.00	−0.01	0.01	0.00	0.01	−0.01	0.01
Δ in CES-D*caregiver	−0.00	0.01	−0.01	0.02	−0.00	0.00	0.02	0.01	0.01	0.01	0.01	0.01

N = *480*. T_1 Biomarkers are inflammatory biomarkers assessed from REasons for Geographic and Racial Differences in Stroke (REGARDS) 1st in-home assessment. Models presented are adjusted for covariates including age measured at caregiving transition enrollment; sex, race, education, and marital status, nicotine use, obesity, hypertension and diabetes medication, antidepressants, and statins measured at REGARDS 2nd in-home assessment.

SE = standard error; b = estimated coefficients; PSS = Perceived Stress Scale; CES-D = Center for Epidemiological Studies Depression Scale.

[a] Cohen's 4-item measure of perceived stress from REGARDS baseline computer-assisted telephone interview (CATI).
[b] Cohen's 4-item measure of perceived stress at Caregiving Transitions enrollment.
[c] Depressive symptoms from REGARDS baseline CATI.
[d] Depressive symptoms measured at Caregiving Transitions enrollment.
Bolded values indicate significant results at $p < 0.05$.

Covariates and their associations with Inflammatory biomarkers

Table 3 refers to the correlations between potential confounders and T_2 biomarkers for the whole sample. Age was associated with higher D dimer, IL-6, IL-2, and TNFR1 and lower CRP. Black race was associated with higher inflammatory biomarkers levels for most biomarkers except IL-2 and TNFR1. Obesity and taking hypertension and diabetes medications were associated with higher levels of IL-6 and TNFR1 inflammatory biomarkers.

Discussion

The results from this study add to the growing literature on the impact of stress and depressive symptoms with inflammatory processes associated with long-term family caregiving. Our previous results from the CTS showed few significant differences in changes in inflammatory biomarker levels between caregivers and non-caregivers (Roth et al., 2020b). Concerning our first hypothesis, we did find that baseline depressive symptoms were significantly associated with D dimer; however, baseline depressive symptoms were not associated with other biomarkers, and baseline perceived stress was not associated with higher inflammation. Contrary to our second hypothesis, perceived stress or depressive symptoms at T_2 did not predict increased inflammation. In support of our third hypothesis, a greater change in perceived stress was associated with an increase in inflammation for IL-10 only in individuals who transitioned into caregiving, while

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Table 3. Correlations between covariates and inflammatory biomarkers measured at follow-up

VARIABLES	CRP	D DIMER	IL-6	IL-2	IL-10	TNFR1
Age (in years)[a]	**−0.12**	**0.17**	**0.15**	**0.20**	0.07	**0.35**
Sex (female)	**0.20**	0.01	0.01	0.00	0.02	**−0.16**
Race (Black)	**0.12**	**0.18**	**0.10**	**−0.12**	−0.08	**−0.25**
Education[b]	**−0.10**	−0.08	**−0.20**	0.00	−0.02	−0.07
Marital status[c]	**−0.10**	−0.08	−0.04	0.01	−0.00	0.07
Nicotine use[c]	**0.04**	0.02	0.05	**−0.14**	−0.02	−0.05
Obesity[c]	**0.32**	**0.15**	**0.28**	−0.30	−0.20	**0.15**
Hypertension medications[c]	0.02	**0.16**	**0.20**	0.04	0.05	**0.24**
Diabetes medications[c]	**0.12**	0.01	**0.14**	0.02	0.06	**0.23**
Antidepressants[c]	**0.09**	0.03	0.05	0.09	−0.03	**0.09**
Statins[c]	**−0.17**	0.04	0.05	0.04	0.01	0.20

$N = 480$.

[a] Measured in years at Caregiving Transitions enrollment.

[b] Education = 1 for having a college degree or above and some college, high school graduate, and less than school graduate = 0.

[c] Covariates measured from REasons for Geographic and Racial Differences in Stroke (REGARDS) 2nd in-home assessment include marital status = 1 for married/cohabitating and single/never married/divorced/widowed = 0, nicotine use = 1 for smoking and never smoked/smoked in the past = 0, and obesity using BMI > or equal to 30 = 1 and BMI < 30 = 0. All medication use were binary-coded "yes" = 1 and "no" = 0. Bolded values indicate significant results at $p < 0.05$.

non-caregivers had a significant decrease in inflammation for the same biomarker. Of note, however, these three significant effects found among 72 analyses for the association of depressive symptoms, perceived stress, caregiving, and inflammatory biomarkers represent only 4% of these analyses, below a chance level with the 0.05 significance level used.

The CTS allowed us to examine indicators of stress in inflammation in both non-caregivers and in family caregivers by using biomarker data before and after the onset of caregiving. Our findings showed that although caregivers had much higher increases in perceived stress and depressive symptoms after the onset of caregiving compared to an individually matched comparison group of non-caregivers, perceived stress and depressive symptoms on all occasions (baseline, follow-up, and change over time) were inconsistently related to increased inflammatory biomarker levels, either for non-caregivers or for incident caregivers.

It is noteworthy that IL-10 is an anti-inflammatory cytokine (Saxton, 2021) and animal studies in rodents showed reductions in IL-10 with psychological distress (Voorhees *et al.*, 2013). These increases in IL-10 biomarker with heightened levels of perceived stress may imply caregivers had an increase in anti-inflammatory processes that contributed to a reduction in overall systematic inflammation.

When examining the associations of covariates on markers of inflammation at follow-up, we found several covariates to be positively associated with inflammation. With respect to sociodemographic variables, age and race were the strongest indicators significantly associated with increased inflammation. Prior reviews showed inflammation as a significant contributor to age-related diseases (Franceschi and Campisi, 2014; Singh and Newman, 2011), and a prior longitudinal study examining the effects of race and ethnicity on inflammation reported Blacks having higher baseline CRP and greater changes in CRP over time compared to Whites (Zahodne *et al.*, 2019). Surprisingly, a decrease in CRP over time was associated with older age, and a decrease in IL-2 and TNFR1 was found among Blacks.

With respect to covariates for chronic conditions and medications, obesity, and taking hypertension and diabetes medications were all significantly associated with increases in IL-6 and TNFR1. Obesity and taking diabetes medications were associated with an increase in CRP, and obesity and taking hypertension medications were significantly associated with an increase in D dimer. Statin use was significantly associated with decreases in CRP; however, antidepressants were significantly associated with increases in CRP and TNFR1. These findings are consistent with prior literature linking obesity and diabetes (Fried *et al.*, 2020; Mclaughlin *et al.*, 2021; Ouakinin *et al.*, 2018; Siddiqui *et al.*, 2019) and medications such as antidepressants and statins (Hussain and Ballantyne, 2021; Vogelzangs *et al.*, 2012) with inflammation. Depressive symptoms may be associated with inflammation by a common pathophysiology with obesity and metabolic conditions (Fried *et al.*, 2020; Lamers *et al.*, 2018). Moreover, the strong associations between diet, obesity, and inflammation (Aleksandrova *et al.*, 2021; Koelman *et al.*, 2022) warrants the need for future studies to examine the possible lifestyle mechanisms (such as diet and exercise) associated with changes in

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inflammation and incident disease. Although it was not assessed in this study, social support has been shown to reduce inflammation (Uchino *et al.*, 2018); future research studies need to investigate the impact of social resources on inflammation in family caregiving. The fact that inflammation was generally associated with these demographic, disease, and medication variables in a manner consistent with the literature also suggests that measurement problems in these biomarkers are not a likely explanation for the lack of association with caregiving, perceived stress, or depressive symptoms.

This study had several strengths. Caregivers in the CTS study had to have a certain threshold of caregiving duties to meet eligibility, and the caregiving and non-caregiving samples were carefully matched on many sociodemographic and health variables at baseline. These are major methodological strengths that distinguish the CTS from most other studies of the health effects of family caregiving. There were some design components in our study that may have made it difficult to detect associations between caregiving, perceived stress, depressive symptoms, and inflammation. Participants averaged over 70 years of age at the follow-up assessment and had high prevalence of obesity and use of diabetes and hypertension medications and statins. In addition, the caregivers were significantly more likely than non-caregivers to report obesity and diabetes medications and were nearly twice as likely to be using antidepressants. Although we adjusted for these factors, it is possible they obscured associations that might occur in younger individuals without these characteristics. More frequent repeated measures of blood biomarker assays are needed in future studies to examine the time frame of any possible associations between perceived stress, depressive symptoms, onset of caregiving, and inflammation. For example, it is possible that onset of caregiving has acute effects on inflammation, but that adaptation occurs over time. Despite these limitations, this study uses a unique set of data that allowed us to examine indicators of well-being and inflammation before and after the transition of the caregiving role while accounting for multiple covariates.

Conclusions

While family caregiving is generally viewed as a chronically stressful experience that impacts caregiver well-being, it can also be an experience that brings positivity, meaning, and purpose to a caregiver. These positive attributes in caregiving may be serving a stress-buffering effect (Roth *et al.*, 2015; Roth *et al.*, 2018) on inflammation and may explain why caregivers with significantly heightened perceived stress and depressive symptoms after transitioning into the caregiving role did not exhibit increased inflammation compared to non-caregivers even after controlling for an extensive number of covariates. Moreover, a study using REGARDS reported perceived stress and depressive symptoms as independent predictors of mortality for non-caregivers but not in caregivers (Roth *et al.*, 2018). This finding in addition to our present study which found no associations between perceived stress, depressive symptoms, and inflammation among caregivers suggests that caregiving may be a unique source of stress associated with psychological and health benefits that mitigates the negative effects of stress on inflammation through increased resiliency. Although the narrative of stress, depressive symptoms, and caregiving increasing inflammation is widely believed to be accurate, this study which used a large sample size of caregivers and demographically matched comparisons group supports the need to establish a more balanced view of family caregiving to better understand and identify caregivers who are more vulnerable to adverse health effects due to the stress of caregiving.

Conflict of interest

None.

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Description of authors' role

J.E., W.E.H., G.H., D.L.R. V.J.H., and M.C. designed research; J.E. and G.H. performed research; M.C. oversaw biomarker assays; G.H. and J.E. analyzed data; W.E.H., G.H., D.L.R.,

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V.J.H., M.C., O.C.S., and M.H. contributed revisions to the paper; and J.E. wrote the paper.

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