The role of multiple negative social relationships in inflammatory cytokine responses to a laboratory stressor

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The present study examined the unique impact of perceived negativity in multiple social relationships on endocrine and inflammatory responses to a laboratory stressor. Via hierarchical cluster analysis, those who reported negative social exchanges across relationships with a romantic partner, family, and their closest friend had higher mean IL-6 across time and a greater increase in TNF-α from 15min to 75min post stress. Those who reported negative social exchanges across relationships with roommates, family, and their closest friend showed greater IL-6 responses to stress. Differences in mean IL-6 were accounted for by either depressed mood or hostility, whereas differences in the cytokine stress responses remained significant after controlling for those factors. Overall, this research provides preliminary evidence to suggest that having multiple negative relationships may exacerbate acute inflammatory responses to a laboratory stressor independent of hostility and depressed mood.

2 Running head: cytokine stress responses and social relationships
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36   Abstract

37   The present study examined the unique impact of perceived negativity in multiple social relationships

38   on endocrine and inflammatory responses to a laboratory stressor. Via hierarchical cluster analysis,

39   those who reported negative social exchanges across relationships with a romantic partner, family, and

40   their closest friend had higher mean IL-6 across time and a greater increase in TNF-α from 15min to

41   75min post stress. Those who reported negative social exchanges across relationships with roommates,

42   family, and their closest friend showed greater IL-6 responses to stress. Differences in mean IL-6 were

43   accounted for by either depressed mood or hostility, whereas differences in the cytokine stress

44   responses remained significant after controlling for those factors. Overall, this research provides

45   preliminary evidence to suggest that having multiple negative relationships may exacerbate acute

46   inflammatory responses to a laboratory stressor independent of hostility and depressed mood.

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48   Keywords: multiple negative social relationships; stress; inflammatory cytokine response; hostility;

49   depressed mood

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51   The role of multiple negative social relationships in inflammatory cytokine responses to a laboratory

52   stressor

53   Stress is a routine part of daily life and interpersonal stress is often the most common and

54   arguably the strongest type of stressor most people experience (Kiecolt-Glaser, Gouin, & Hantsoo,

55   2010). Poorer overall health and dysregulated immune function are strongly linked with interpersonal

56   stress both from negative social exchanges (Chiang, Eisenberger, Seeman, & Taylor, 2012; Edwards,

57   Hershberger, Russell, & Markert, 2001; Kiecolt-Glaser & Newton, 2001) and chronic social conflict

58   (Cohen et al., 1998; Davis et al., 2008; Friedman, 2010). Interpersonal stress appears to have a long

59   lasting impact on health in part by contributing to chronically elevated inflammation, which can confer

60   risk of diverse age-related diseases (Ershler & Keller, 2000; Graham, Christian, & Kiecolt-Glaser,

61   2007; Ridker, 2000). However, the majority of studies on immune responses to social conflict have

62   focused on a particular type of relationship (e.g., marital relationships), while the effect of having

63   conflict across multiple relationships is largely unknown. Further, the degree to which multiple social

64   conflicts affect inflammatory responses to stress and whether the association is independent of related

65   psychosocial characteristics are important issues that are not well understood. The present research is

66   expected to advance the literature by examining interpersonal relationships in multiple areas and how

67   negativity across multiple interpersonal relationships affects inflammatory responses to a laboratory

68   stressor.

69   Studies most relevant to the current research have examined the effects of acute social conflict

70   on health related outcomes. For example, the frequency of negative social exchanges with close others

71   has been negatively associated with physical and mental health among college students (Edwards et al.,

72   2001) and is predictive of depressed mood in a sample of married adults (Joiner & Timmons, 2009).

73   Complementing such research, experimental studies have demonstrated that acute social conflict can

74   influence immune responses in a laboratory setting (Chiang et al., 2012; Kiecolt-Glaser et al., 2005).

75 One mechanism that may explain the negative effects of acute social conflict on health is repeated

76 physiological activation of inflammatory stress responses and delayed recovery to stress (Seeman &

77 McEwen, 1996). Under social conflict, inflammatory responses to stress may be also maintained by

78 actions of the sympathetic-adrenal-medullary (SAM) system and the hypothalamic-pituitary-adrenal

79 (HPA) axis (e.g., via cortisol) (Lovallo, 2005; Miller, Chen, & Zhou, 2007). Recent studies show that

80 gene expression of inflammatory pathways are upregulated in leukocytes among socially stressed

81 individuals compared to matched control with good social support (Cole, Hawkley, Arevalo, &

82 Cacioppo, 2011; Slavich & Cole, 2013).

83   In addition to a relatively direct effect of social conflict via stress activation, it is important to

84 consider individual characteristics that tend to go along with negative social relationships. In particular,

85 trait hostility and depressive symptoms appear to aggravate the effects of psychosocial stressors on

86 cardiovascular and inflammatory response (Brondolo et al., 2003; Brummett et al., 2010). However,

87 despite the possibility for hostility and depressed mood to be confounded with relationship stress and

88 health, few relevant studies have controlled for hostility or depressed mood.

89   The present research aims to examine effects of negative social exchanges in multiple

90 relationships – with a romantic partner, a close friend, close family members, and roommates – on

91 responses to an experimental stressor, with an emphasis on inflammatory responses.   We classified

92 individuals into groups by their patterns of negative social exchanges across those four relationship

93 areas. Because those who experience social conflict in many close relationships are a minority

94 (Fingerman, Hay, & Birditt, 2004), we expected to identify only a small number of those who reported

95 negative social exchanges in multiple areas of measured relationships. We hypothesized that

96 individuals with high levels of negative social exchanges in more relationships than others would have

97 exaggerated or prolonged inflammatory response to stress (i.e., poorer recovery) in terms of two

98 cytokines: IL-6 and TNF-α. TNF-α is a classic proinflammatory cytokine and IL-6, although it has

99   anti-inflammatory actions in certain contexts (for reviews see Hawkley, Bosch, Engeland, Marucha, &

100   Cacioppo, 2007; Woods, Vieira, & Keylock, 2009), is widely considered proinflammatory in the

101   context of psychological stress. We examined whether the effects of negative relationships on

102   inflammatory responses to stress are independent of depression or hostility. On a more exploratory

103   basis, we also expected that individuals with more negative relationships would show greater increases

104   in cortisol responses to the stressor.

105   **Method**

106   *Participants*

107   Fifty-six healthy participants (36 women, 20 men), aged 18-30 years (mean = 21.05 ± 0.37)

108   were recruited to participate in a larger study examining influences of sex hormones to physiological

109   responses to an experimental stressor. Participants were recruited via advertisements in the local

110   newspaper and flyers posted in the local community and on the campus of a state university in the

111   Northeastern U.S. An initial telephone interview was conducted by a trained research assistant to

112   determine the eligibility of participants. Exclusion criteria included tobacco use, BMI ≥ 30, psychiatric

113   hospitalization within the past year, the use of psychotropic medication, anti-inflammatory medications,

114   hormonal contraceptives, medications for controlling blood pressure, and inhaled beta agonists. People

115   who scored higher than the clinical cut off score of 16 on the Center for Epidemiologic Studies

116   Depression Scale (Radloff, 1977) or with history of depression were not eligible for the larger study. In

117   addition, we screened out people with a history of heart disease, diabetes, and neurological disorders.

118   Women who reported any possibility for pregnancy and menstrual cycle dysregulation also were

119   excluded. Women came to the laboratory during either the late luteal ($n = 19$) or follicular ($n = 17$)

120   phase of their menstrual cycle for the purpose of the larger study.

121   *Measures*

122   *Negative Social Relationships*

123   A 25-item measure was used to assess negative social relationships, which was based on an

124   existing questionnaire that included five items about negative social interactions with a spouse or

125   significant other (Schuster, Kessler, & Aseltine Jr, 1990). The items ask about the frequency of

126   negative social exchanges involving disagreements, criticism, and tension, with responses ranging from

127   0 (never) to 5 (very often). The present study used those same five items to ask participants about

128   negative social interactions among a) roommates, b) a romantic partner, c) close family members, d)

129   their closest friend, and e) their children. The alpha reliability of the original scale was 0.76 (Schuster

130   et al., 1990), and the present sample showed Chronbach–α of 0.84, 0.89, 0.84, 0.81 for the romantic

131   partner, family, roommate, and the closest friend subscales, respectively. As no participants reported

132   having children, that subscale was not used.

133   *Negative Mood*

134   A six item negative mood scale was administered four times (baseline, immediately after,

135   15min, and 75min after the stressor) to check the effect of the experimental stressor on mood. The

136   scale consisted of words describing negative and positive mood (e.g., nervous, happy, irritated) with a

137   7-point Likert scale ranging from 1 (Not at all) to 7 (Very much). The scale showed a good internal

138   consistency across measurements (Chronbach-α = 0.74, 0.88, 0.84, and 0.86, respectively).

139   *Depressed mood*

140   The Center for Epidemiological Studies Depression Scale (CES-D; Radloff, 1977) was used to

141   measure depressed mood, which effectively identifies depressed mood among healthy individuals

142   (Radloff, 1977). Item responses are from 0 to 3, with 3 representing the greatest frequency of depressed

143   symptoms over the past week. The CES-D showed a chronbach-α of .90 for this sample.

144   *Hostility*

145   The well-validated Cook-Medley hostility questionnaire (CMHQ; Cook & Medley, 1954) was

146   used to assess the tendency to react and think in a hostile manner. The scale has 50 true-false items,

147   which are aggregated into a total score ranging from 0 to 50. The chronbach-α was .83 for this sample.

148   ***Procedures***

149   *Laboratory Protocol and Stressor*

150   Eligible participants arrived at a General Clinical Research Center (GCRC) at 1300 hrs and

151   were met by a trained research assistant who first obtained a written informed consent. Next,

152   participants were interviewed by a certified nurse practitioner to confirm health status and study

153   eligibility. Participants then were asked to complete questionnaires, after which a trained nurse inserted

154   an indwelling catheter in the non-dominant arm. After a 10min acclimation period, participants were

155   asked to sit quietly for 15min. A baseline blood sample (20cc) was then drawn.

156   Next, a modified Trier Social Stress Task was administered. Participants were given 10min to

157   prepare a 3½-minute speech about a personal failure that had a negative consequence on their life.

158   They delivered the speech in front of a video camera and were told that a recording would be later

159   observed by a panel of psychologists (no recording was actually made). Participants were prompted by

160   the experimenter to continue talking if they finished their speech in less than the allotted time.

161   Immediately after the speech, participants were asked to complete a serial subtraction task as fast and

162   as accurately as possible (4min), followed by several math word questions that increased in difficulty

163   (3.5min), and then another serial subtraction task (4min). The experimenter delivered timed prompts to

164   urge participants to work more quickly and to tell them to start over if they delivered the wrong

165   response. This stress protocol took 30min total.

166   Baseline blood samples and blood samples at 15 and 75min after the stress period were used to

167   determine cortisol, IL-6, and TNF-α. Participants completed several post-stress measures of mood at

168   the end of the study, after which the catheter was removed. The study procedure was approved by the

169   institutional review board at the Pennsylvania State University (IRB # 00M0314-B9).

170   *Blood Handling*

171   For preparation of serum, blood was drawn into separate collection tubes that contained no

172   additive. Serum tubes were allowed to sit at room temperature for 15min before centrifugation (1500 ×

173   g at 4 °C for 15 minutes). Following centrifugation, serum was aliquoted into separate 100 μL

174   microtubes and frozen at -80 °C for later assay.

175   *Serum Cortisol, IL-6, and TNF-α*

176   Assays were performed at the Pennsylvania State University GCRC Core Laboratory. Serum

177   cortisol levels were determined using commercially available enzyme immunoassay kits (EIA;

178   Diagnostic Systems Laboratories, Inc., Webster, TX). The inter-assay and intra-assay coefficients of

179   variation were 3.16% and 4.8%, respectively for cortisol. Serum IL-6 and TNF-α levels were

180   determined by enzyme-linked immunosorbant assays constructed with antibodies purchased from R&D

181   systems (Minneapolis, MN) using previously described procedure (Corwin, Bozoky, Pugh, & Johnston,

182   2003). The level of detection was 1.0 - 3.0 pg/mL, and inter-assay and intra-assay coefficients of

183   variation were 7.1% and 5.3% respectively for these cytokine assays. All samples were tested in

184   duplicate in a single assay batch; values that varied by more than 5% were subject to repeat testing.

185   The average of duplicate tests is reported for each biomarker assay.

186   ***Data Analysis***

187   SPSS 20.0 was used for all analyses. Study variables were screened for outliers and non-

188   normality, and cortisol, IL-6, and, TNF-α were natural log transformed to correct for skewness. Next, a

189   hierarchical cluster analysis was applied to classify individuals with different patterns of perceived

190   negativity across the relationships with roommates, a romantic partner, close family, and a closest

191   friend. Out of 56 participants, one woman did not provide sufficient data to compute the cluster by

192   negative relationship analyses and was therefore excluded from analyses. While 25 participants

193   reported having all of the four relationships, 13 participants did not have a roommate, and 13 others did

194   not have a romantic partner. Thus, two separate hierarchical cluster analyses were run, the first cluster

195   analysis including those who reported having a romantic partner, a closest friend, and family ($n = 38$)

196   and the second including individuals who reported having roommates, a closest friend, and family ($n =$

197   38). Four participants who did not report a relationship with either a romantic partner or roommates

198   were excluded from these cluster analyses; there was no significant difference between those four

199   participants and the rest of the sample in any psychological characteristics or outcome variable we

200   examined.

201   In each of two cluster analyses conducted, the same three steps were used. First, the variables

202   for the perceived negativity in the three relationship areas were entered via hierarchical cluster analysis.

203   Ward's method with the similarity measure of squared Euclidean distance was then used to decide the

204   number of groups in the cluster model. Discriminant function analysis was used to verify how much of

205   the clustering within groups could be replicated (Klecka, 1980). In the third step, we further examined

206   the characteristics of the groups in the cluster model via F tests by examining whether the groups

207   differed by age, gender, and the four negative relationship variables.

208   The general linear model (GLM) with within-subject design was then used to examine the

209   effect of the different negative social relationship clusters on cortisol and cytokine responses to stress.

210   Greenhouse-Geisser correction was used for sphericity. For the significant results, the partial eta-

211   squared (ηp^2) *post hoc* tests with Bonferroni correction were reported. As *post hoc* tests for the time

212   effects, difference scores were calculated for the stress response measures between each pair of the

213   three time points (e.g., from baseline to 15min after stress) in order to examine change during each

214   time interval. Due to their known impact on inflammation, age, BMI, gender, and menstrual cycle of

215   women were controlled in all of these analyses. Three dummy coded variables representing a) men, b)

216   women in the luteal period, and c) women in the follicular period were generated and entered in

217   analyses to control for both gender and women's menstrual cycle status. Finally, depressed mood and

218   hostility were additionally entered to the GLM to examine whether the effects were independent of

219   those characteristics.

220   **Results**

221   *Preliminary analyses*

222   The means and standard deviations (SDs) of demographics and study variables are presented in

223   Table 1. The sample was predominantly Caucasian (71%) and comprised of young adults with a mean

224   age of 21.05 (SD = 2.74, range 18-30).

225   *Cluster analysis for negative relationship profiles*

226   We classified individuals as having different degrees of negative social exchanges across

227   multiple interpersonal relationship areas. The first cluster analysis was run on the 38 participants who

228   had a romantic partner, family, and a close friend. It yielded two groups (Table 2, a): "a low conflict

229   group" ($n = 29$) characterized by consistently low levels of negative social exchanges across all

230   relationship areas (romantic partner, family, and their closest friend), and "a multiple conflict group" (n

231    $= 9$) that had high levels of negative social exchanges across all relationship areas. F tests confirmed

232   that the two groups in this cluster model were different in levels of negative social exchanges in these

233   relationships ($ps < .05$). A discriminant function analysis verified the cluster structure ($\chi^2(3, n = 38) =$

234   53.56, $p < .001$), and 97.4% of the original grouped cases (37 cases out of 38) were replicated. The

235   distribution of gender and age was not significantly different across the two groups.

236   Another cluster analysis was run on the 38 participants who reported relationships with

237   roommates, family, and the closest friend. This analysis identified three clusters (Table 2, b): "a low

238   conflict group" ($n = 30$) characterized by low levels of negative social exchanges across all the

239   relationship areas, "a multiple conflict group" ($n = 3$) characterized by high levels of negative social

240   exchanges across all the relationship areas, and "a family conflict group" ($n = 5$) characterized

241   primarily by a high level of negative social exchanges in family but low levels of negative social

242   exchanges among roommates and the closest friend. The F tests confirmed that the three groups in this

243   cluster analysis (hereafter referred to as the "roommate model") were different in levels of negative

244   social exchanges across the three relationship areas ($ps < .001$). A discriminant function analysis

245   verified the three cluster structure ($\chi^2(6, n = 38) = 70.69, p < .001$) and that 94.7% (36 cases out of 38)

246   of the original grouped cases were replicated. The gender distribution was significantly different across

247   the 3 groups ($p = .05$), which was largely driven by the multiple conflict group having only three men

248   and no women. Age was not different across the 3 groups.

249   ***Manipulation checks for the stress protocols***

250   Participants' negative mood increased in response to the stressor ($F(3, 153) = 34.71, p < .001$,

251    $\eta p^2 = .28$). The levels of serum cortisol did not significantly increase in response to the experimental

252   stressor but showed a significant decrease over time ($F(2, 106) = 9.88, p < .001, \eta p^2 = .16$), likely

253   driven primarily by the diurnal rhythm of cortisol. There was no significant time effect on IL-6 and

254   TNF-α levels.

255   ***Baseline differences in biomarkers by negative social relationships***

256   The Table 1 presents the baseline levels of biomarkers by cluster groups in both the romantic

257   partner model and the roommate model. There was a significant baseline difference in IL-6 levels

258   between the low and multiple conflict groups in romantic partner model ($F(1, 36) = 4.18, p = .05, \eta p^2 =$

259    $.10$). The baseline difference became not significant after controlling for depression or hostility along

260   with age, gender, and menstrual cycle status. There were no other baseline differences in any of the

261   groups in either the romantic partner or roommate model.

262   ***Stress responses by negative social relationships***

263   *IL-6*

264   There was a significant time by negative social relationship interaction using the roommates

265   model on IL-6 ($F(4, 58) = 8.53, p < .01, \eta p^2 = .37$). Post hoc tests confirmed that only individuals in

266   the multiple conflict group of this cluster model showed significantly greater increases in IL-6 from

267   baseline to 15min after stress ($ps < .01$) and from baseline to 75min after stress ($ps < .01$) compared to

268   those in the family conflict or low conflict groups (Figure 1). Results remained significant after

269   controlling for depressed mood or hostility ($ps < .01$).

270   *TNF-α*

271   There was a marginally significant time by negative social relationship interaction among the

272   romantic partner model on TNF-$α$ responses to the stressor ($F(2, 56) = 2.80$, $p = .07$, $\eta p^2 = .09$). Upon

273   examination, individuals in the multiple conflict group (who reported negativity in their relationships

274   with their romantic partner as well as their closest friend, and family) showed significantly greater

275   increases in TNF-α from 15min to 75min post stress after controlling for baseline TNF-α and other

276   covariates ($F(1, 27) = 6.81$, $p < .05$ $\eta p^2 = .20$), as illustrated in Figure 2. This remained significant after

277   controlling for depressed mood or hostility ($ps < .05$) and also after removing the one overlapping

278   individual in the multiple conflict group who was also included in the multiple conflict group in the

279   roommate model (where greater IL-6 responses to stress were observed). There was no main effect in

280   the roommate model groups on TNF-α stress response.

281   *Cortisol*

282   Neither of the negative relationship cluster models significantly predicted cortisol responses to

283   the stressor.

284   **Discussion**

285   Although social conflict has been associated consistently with poorer health and various stress-

286   related biomarkers (Graham et al., 2007; Kiecolt-Glaser & Newton, 2001), the majority of past studies

287   showing connections between relationship stress and biomarkers have focused on a particular type of

288   relationship or broad characterizations of relationship quality or network size. The present research is

289   the first to examine the effect of negative social exchanges across multiple interpersonal relationships

290   and whether the effects of negative social exchanges in multiple relationship areas are independent of

291   depressed mood and trait hostility. Another novel aspect of the present research is that we focused on

292   the effect of relationships on inflammatory responses to stress as opposed to basal levels of biomarkers.

293   As expected, the present results suggest that there are differences in acute inflammatory cytokine

294   responses to stress depending on the pattern of multiple negative social relationships individuals

295   reported within the four relationship areas examined (romantic partner, family, the closest friend, and

296   roommates). Those who reported negative social exchanges in their relationships with roommates,

297   family, and their closest friend showed increases in IL-6 after being exposed to a laboratory stressor.

298   Similarly, people who reported negative social exchanges with a romantic partner, family, and their

299   closest friend showed increases in TNF-α from 15min to 75min post stress after controlling for

300   baseline TNF-α. Both the IL-6 and the TNF-α results remained significant after controlling for

301   depressed mood, hostility, age, BMI, gender, and menstrual cycle status.

302   Importantly, the two multiple conflict groups examined in this research (among those who had

303   roommates, vs. those who had a romantic partner) were largely distinct from each other; there was only

304   one individual who was included in both of these analyses. Thus, we showed an effect of negativity

305   across multiple relationship areas on increases in either TNF-α or IL-6 via different subsets of

306   individuals, suggesting that the effects of having social conflict across relationship areas are robust and

307   pro-inflammatory in nature. Further, results were not driven by baseline effects in inflammation or

308   outliers. We did not find any outliers in either of the groups that showed significant stress responses

309   and in all participants. Further, as compared to the other groups, individuals in the multiple conflict

310   group of the roommate model did not evidence significantly different baseline levels of IL-6 and those

311   in the multiple conflict group of the romantic partner model did not evidence significantly different

312   baseline levels of TNF-α. Thus, participants in those two multiple conflict groups came to the lab

313   without elevated IL-6 or TNF-α compared to others, but were the ones who showed increases in IL-6

314   or TNF-α after being exposed to the laboratory stressor.

315   The findings of the present research complement the results of a recent study showing that daily

316   levels of negative social interactions were associated with greater inflammatory responses to an

317   experimental stressor as measured by IL-6 and soluble TNF-α receptor II (Chiang et al., 2012). In

318   terms of direction, the inflammatory stress reactivity of individuals with multiple negative relationships

319   is also consistent with previous studies of the association between a laboratory induced social conflict

320   and inflammatory responses (Graham et al., 2007; Kiecolt-Glaser et al., 2005). Psychological stress

321   effects on excessive inflammatory cytokines responses are likely explained by multiple aspects of

322   complex, interrelated physiological systems. For example, chronic interpersonal stress is likely be

323   related to dysregulation of the inflammatory stress response due to decreased glucocorticoid receptor

324   sensitivity (Corwin et al., 2013; Miller et al., 2007; Pace, 2012) or perhaps to down-regulation of

325   cholinergic anti-inflammatory pathways in neural circuitry (Tracey, 2002).

326   Interestingly, there was one baseline difference in IL-6 observed: Participants who reported

327   multiple conflict across relationships and who had a romantic partner had higher IL-6 at baseline, an

328   effect that was reduced to non-significance when controlling for either depressed mood or hostility and

329   which was not observed among those who reported multiple relationship conflict and who had

330   roommates. It may be that for this relatively young sample, which was largely comprised of college

331   students (78%), that those who were in conflictive romantic relationships were more depressed or

332   hostile and that this (rather than the conflict itself) explained their higher baseline IL-6. In contrast,

333   those in the present study who had conflicts with roommates may have had less control over their

334   exposure to those individuals and may have developed greater inflammatory stress reactivity due to

335   frequent exposure to stress. The difference observed between those with roommates and romantic

336   partners was not expected, and may be limited to the particular sample in the present research.

337   However, the finding that depressed mood and hostility can explain baseline levels of inflammation is

338   consistent with previous research showing that depressed mood and hostility are associated with

339   increased circulating markers of inflammation among adults (Graham, Christian, & Kiecolt-Glaser,

340   2006; Suarez, Lewis, & Kuhn, 2002; Zorrilla et al., 2001). Importantly, no such baseline effects

341   explained the effects of relationship negativity on the inflammatory cytokine responses to acute stress

342   observed in the present research.

343   We did not find a significant effect of negative relationship endorsement on cortisol responses.

344   This null effect might be related to the experimental stress paradigm used in which the public speech

345   part of the stress task was conducted in front of a video camera instead of a panel of judges, a protocol

346   which can reduce the intensity of stress response from the Trier (Dickerson & Kemeny, 2004;

347   Kirschbaum, Pirke, & Hellhammer, 1993). Also, the timing of the catheter insertion and blood draws

348   might also explain the null finding.

349   **Limitations**

350   The clinical implications of the study are limited in several ways. The present research was

351   conducted with a small sample of healthy, relatively young adults, which limits generalizability and

352   warrants cautions in interpretation. However, a relatively small proportion of a sample can be expected

353   to have multiple relationship conflicts and the issues with there being a small number of those with

354   multiple conflicts were minimized in the present research by our application of conservative statistical

355   adjustments when using cluster analysis and general linear model and comparisons of analyses between

356   the two multiple conflict clusters (Clatworthy, 2005; Hair, Tatham, Anderson, & Black, 2006; Huynh,

357   1978). It will be important to replicate the present results using larger and more diverse samples,

358   particularly a greater number of individuals reporting negative relationships across multiple social

359   relationship areas than was available in the present study. It was also a limitation that we only had IL-6

360   and TNF-α available as inflammatory biomarkers: Future research on related topics would benefit from

361   utilizing multiplex technology to examine a greater diversity of analytes, including anti-inflammatory

362   cytokines (e.g., IL-10) to enable examination of the ratio between pro and anti-inflammatory cytokines

363 in stress responses. It would also have been ideal if we had been able to include blood draws later than

364 75 min post-stress to capture the full peak as well as return to baseline of the cytokine responses.

365 Finally, it would be of value for future research to utilize clinically diverse samples, such as those with

366 clinical depression or existing inflammatory conditions, and to include assessments of clinical health

367 outcomes.

368 **Conclusion**

369 A better appreciation for how social conflict may alter stress responsiveness of the body is

370 critical to understanding how and why it is associated with poorer physical health (Seeman & McEwen,

371 1996). Although having some degree of social conflict is an unavoidable part of everyday life, the

372 present study provides preliminary evidence that having negative social interactions across multiple

373 social relationships might be harmful, as it is associated with greater inflammatory reactivity to a

374 psychosocial stressor. Significant increases in both IL-6 and TNF-α in response to stress were observed

375 among those with relationship conflict in at least three areas, compared to those with relationship

376 conflict in fewer relationships. The effect of having multiple social relationships on inflammatory

377 responses to stress appears to be independent of any effect of hostility or depressed mood. Taken as a

378 whole, the present research emphasizes the importance of examining the role of negative close

379 relationships in inflammatory stress response in a detailed fashion. Having multiple negative

380 relationships may put individuals at particular risk of developing exacerbated acute inflammatory

381 reactivity to psychosocial stress.

382

383

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Sample characteristics and the baseline measure of biomarkers by cluster groups

Note. Biomarker levels were log transformed; µg/dL = micrograms per deciliter; pg/mL = picograms per milliliter.

2   Table 1. Sample characteristics and the baseline measure of biomarkers by cluster groups

			Total (N = 56)	
			M	*SD*
Characteristics				
Age (yrs)			21.05	2.74
Women (%)			64.3	
Cycling status among women (*n* = 36)				
Luteal (%)			52.78	
Follicular (%)			47.22	
Body Mass Index (kg/m^2)			23.42	3.10
Hostility			23.32	6.85
Depressed Mood			9.29	8.30
Baseline measure of biomarkers by cluster groups				
Biomarker	Model	Groups	*M*	*SD*
Cortisol (µg/dL)	Romantic partner model	Low conflict	2.38	0.45
		Multiple conflict	2.23	0.43
	Roommate model	Low conflict	2.38	0.42
		Only family conflict	2.02	0.37
		Multiple conflict	2.47	0.20
		Total	2.37	0.42
IL-6 (pg/mL)	Romantic partner model	Low conflict	2.96	0.97
		Multiple conflict	3.74	1.15
	Roommate model	Low conflict	3.41	1.40

		Only family conflict	4.25	0.78
		Multiple conflict	3.95	1.10
		Total	3.46	1.23
TNF-α (pg/mL)	Romantic partner model	Low conflict	3.07	0.93
		Multiple conflict	3.31	1.08
	Roommate model	Low conflict	3.44	1.21
		Only family conflict	4.18	1.27
		Multiple conflict	3.79	0.78
		Total	3.42	1.11

3   *Note.* Biomarker levels were log transformed; µg/dL = micrograms per deciliter; pg/mL = picograms
4   per milliliter.

The level of negative social exchanges in each relationship area for the cluster groups, generated (a) by relationships with a romantic partner, family, and the closest friend and (b) by relationships with roommates, family, and the closest friend

2   Table 2. The level of negative social exchanges in each relationship area for the cluster groups,

3   generated (a) by relationships with a romantic partner, family, and the closest friend and (b) by

4   relationships with roommates, family, and the closest friend

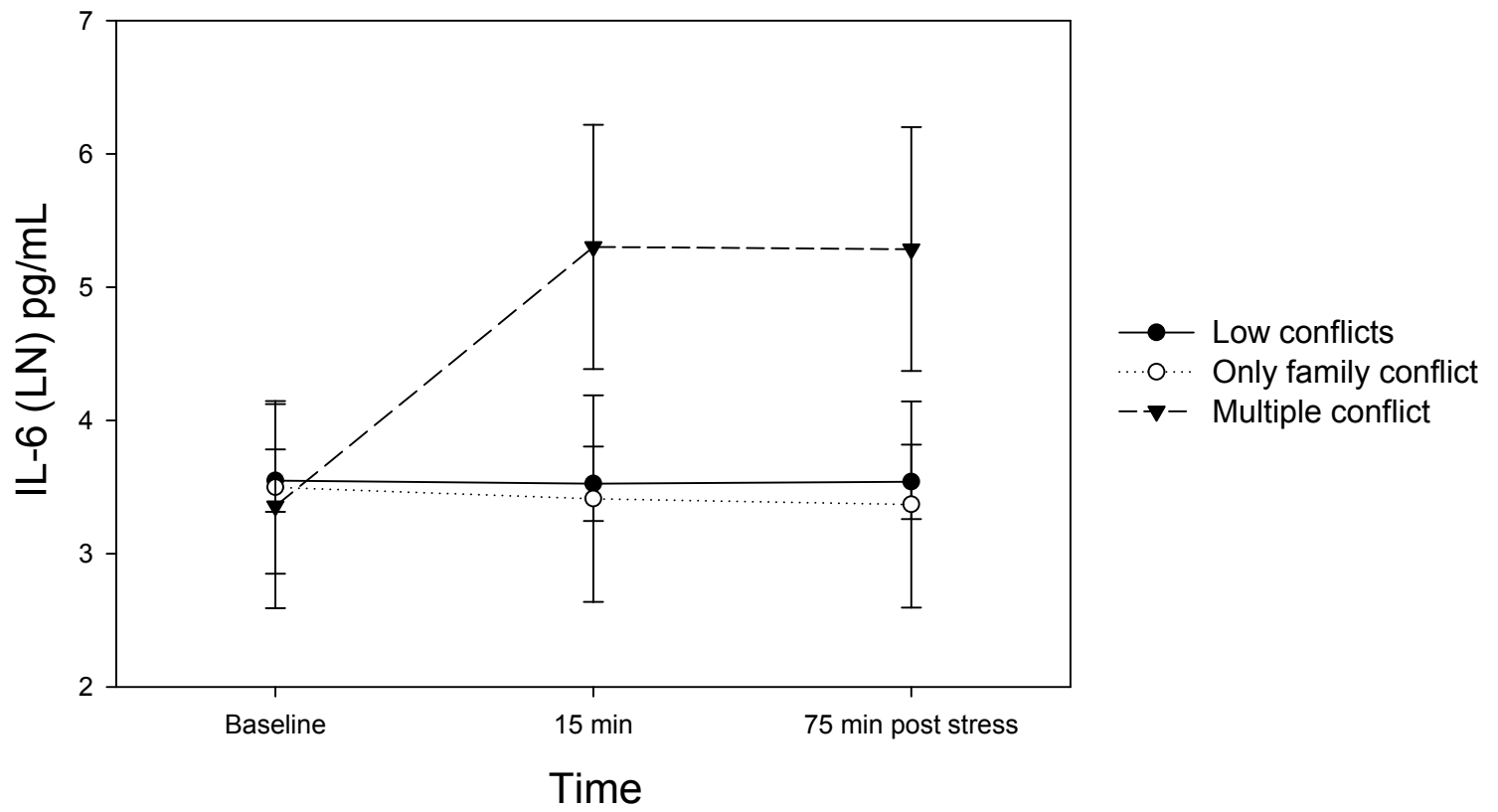
5   (a)

Negative social exchanges in the relationships with	The Romantic Partner Model			
	Low conflict ($n = 29$)		Multiple conflict ($n = 9$)	
	M	SD	M	SD
Romantic partner	7.86	2.45	11.89	4.91
Family	8.07	2.15	16.44	1.88
The closest friend	6.72	2.10	8.89	3.92

7   (b)

Negative social exchanges in the relationships with	The Roommates Model					
	Low conflict ($n = 30$)		Only family conflict ($n = 5$)		Multiple conflict ($n = 3$)	
	M	SD	M	SD	M	SD
Roommates	8.60	3.40	6.60	2.30	17.00	3.46
Family	8.60	2.75	17.60	1.95	20.33	2.52
The closest friend	6.53	1.89	7.80	3.03	16.00	1.73

Changes in serum IL-6 levels by the negative relationship groups in the roommate model

* Note. LN = Natural Log transformation

Changes in serum TNF-α levels by the negative relationship groups in the romantic partner model

* Note. LN = Natural Log transformation