The Genomic Impact of Kindness to Self vs. Others:

A Randomized Controlled Trial

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None.

Abstract

Objective: Prosocial behavior has been linked to improved physical health, but the biological mechanisms involved remain unclear. This study tested whether a 4-week kindness intervention could reduce expression of a stress-related immune response gene signature known as the Conserved Transcriptional Response to Adversity (CTRA).

Methods: In a diverse sample of community adults ($N = 182$), study participants were randomly assigned to perform 3 kind acts for other people, to perform 3 kind acts for themselves, or to list daily activities (control), on one day per week over 4 weeks. CTRA gene expression was measured by RNA sequencing of dried blood spots (DBS) collected at baseline and 5 weeks later (1 week after completing study assignments). Participants' descriptions of their kind acts were coded for protocol adherence and act content.

Results: Participants who were randomized to perform kind acts for others showed significant reductions in CTRA gene expression relative to controls. Participants who were randomized to perform kind acts for themselves also showed significant reductions in CTRA gene expression relative to controls, but this pattern emerged only for those who failed to perform the requested self-kind acts (protocol non-adherent). Those who fully adhered to the self-kindness protocol showed no change in CTRA gene expression and did not differ from controls. Act content analyses implicated self-stress-reducing behavior in the paradoxical effects of self-kindness and the physical presence of others in the effects of prosocial behavior.

Conclusions: Prosocial engagement—doing something kind for others rather than oneself—reduces CTRA gene expression. The nature of kind acts and their intended recipient plays a key role in shaping the genomic impact of kindness.

Introduction

Conventional wisdom suggests that self-care or "treating oneself" is an effective strategy for reducing stress and promoting physical health, but epidemiologic evidence links prosocial, rather than self-focused, behavior to positive health outcomes such as reduced cardiovascular disease and all-cause mortality rates (Brown et al., 2003, 2009; Brown & Brown, 2015; Burr et al., 2016; Konrath et al., 2012; Poulin & Holman, 2013; Roth et al., 2018). The health benefits of prosocial behavior are thought to be mediated by neurobiological processes involved in caregiving (e.g., activation of the parasympathetic nervous system, release of oxytocin or progesterone; Brown & Brown, 2015; Porges, 2021) and concomitant inhibition of stress physiology (e.g., the sympathetic nervous system and hypothalamus-pituitary-adrenal axis), both of which may inhibit disease-promoting biological processes such as inflammation, metabolic dysregulation, and cell senescence (Adamo, 2014; Cole et al., 2007; Eisenberger & Cole, 2012; Leschak & Eisenberger, 2019; Miller et al., 2009).

Experimental evidence for a causal effect of prosocial behavior on health-relevant physiology in humans is sparse, but one randomized controlled trial has found that prosocial behavior can reduce activity of a disease-related gene regulation program known as the Conserved Transcriptional Response to Adversity (CTRA; Cole, 2019; Nelson-Coffey et al., 2017). The CTRA is characterized by increased expression of pro-inflammatory genes and reduced expression of innate antiviral genes in circulating immune cells in response to beta-adrenergic signaling from the sympathetic nervous system (Cole, 2014; Cole et al., 2015a; Heidt et al., 2014; Knight et al., 2020; MacCormack et al., 2021; McKim et al., 2018; Powell et al., 2013; Sloan & Cole, 2021). Recent studies have also implicated the parasympathetic nervous system in reducing CTRA expression (Rahal et al., 2021; Sloan & Cole, 2021), perhaps due to

the inhibitory effects of parasympathetic activity on sympathetic neural activity. To determine whether prosocial behavior might reduce CTRA gene expression, Nelson-Coffey and colleagues (2017) randomized 159 community-dwelling adults to perform 12 random acts of kindness for others, for themselves, or for the world in general, or to complete a neutral control task, over a 4-week period. CTRA gene expression declined significantly from pre- to post-intervention in those randomized to perform kind acts for others, but not in those assigned to the other groups.

The study of Nelson-Coffey et al. (2017) identified the CTRA as one potential biological pathway for the health benefits of prosocial behavior, but it also raised a significant theoretical question regarding the hypothesized role of caregiving and stress reduction in mediating those effects: If "care production" and "stress reduction" are key psychobiological mechanisms, then why does directing kind acts to the self not yield the same beneficial molecular effects as directing kind acts to others? Self-kindness might be expected to yield greater "care-production/stress-reduction" effects than directing kind acts to others (all other factors equal) due to the additional stress-reduction benefit of being both the giver and the recipient of care. For example, self-focused "treat" behaviors such as consuming dark chocolate, cocoa, or red wine (Katz et al., 2011; Nicod et al., 2014) have been linked to reduced inflammatory activity, as have napping (Faraut et al., 2015), sauna sessions (Pilch et al., 2013), and massages (Rapaport et al., 2012). Alternatively, it is possible that all other things are *not* equal and that the nature (or number) of the caregiving acts performed for others differed from those performed for the self in the Nelson-Coffey et al. (2017) study, and thereby exerted different psychobiological effects. However, such differences were difficult to evaluate in the 2017 study due to limited information available on the kind acts performed. It is also possible that producing self-kind acts may be inherently less stress-reducing than producing kind acts for others due to psychological

differences such as lower novelty (higher base rate of behavior), greater adaptation (rote or uncreative selection of self-kind acts), negative evaluative consequences (e.g., guilt, buyer's remorse, stress about lost productivity), or poor hedonic forecasting (i.e., people may not know what kinds of acts are most effective at reducing their own stress levels).

In the present experiment, we sought to extend the findings of the previous Nelson-Coffey et al. (2017) study, which had indicated a causal effect of prosocial behavior in reducing CTRA gene expression, by verifying those effects in a newly collected independent sample and analyzing the role of kind act content and frequency. In addition to assessing the number of kind acts performed, we also analyzed the extent to which they adhered to their specified protocols (i.e., involving social targets and co-presence of others vs. self-care, self-stress-reduction, and self-health behaviors) and the extent to which they required individual effort. Our overarching theoretical objective was to distinguish the gene regulatory effects of doing kind acts (i.e., an intention to benefit, regardless of target) from prosocial behavior per se (i.e., an intention to benefit other people).

Method

The data come from a previously reported study of 182 community-dwelling Southern California adults who were randomized to perform 12 kind acts for others over 4 weeks, 12 kind acts for themselves over 4 weeks, or to keep track of their daily activities (i.e., perform a neutral control task; Fritz et al., 2021). This sample is independent of the one reported in Nelson-Coffey et al. (2017), but follows closely the procedures of that study, with the exception that the kindness-to-world condition was omitted to enhance statistical power in the other conditions (see Fritz et al., 2021, for more details on study procedures). Briefly, participants were recruited through email advertisement, flyers, and community fairs to participate in a study of positive

activities and health, and were screened to exclude individuals under age 21, as well as those taking antidepressant medications. Data were collected throughout the calendar year (including summers). Participants received $100 as compensation for completing all timepoints. Research assistants delivered all study procedures under supervision by the second author, and participants completed self-report assessments independently through the online survey platform, Qualtrics. Additional details about the study protocol, including instructions for each condition, are included in Supplemental Materials.

The primary outcome for this study involved DNA telomere length assessed in dried blood spot (DBS) samples collected at baseline and 5-week follow-up (1 week following the 4-week kind act protocols). Results of primary analyses found no significant effect of experimental conditions on telomere length (Fritz et al., 2021). The present analyses of CTRA gene expression were conducted on archival DBS samples remaining after completion of primary analyses, following standard procedures for genome-wide transcriptional profiling of archival DBS samples that have been validated in previous research (Grauholm et al., 2015; Kohrt et al., 2016; McDade et al., 2016; Reust et al., 2018). A CONSORT flow diagram for the RNA analyses is presented in Figure 1. This study was pre-registered (https://osf.io/93ck7/) and approved by the Institutional Review Board of the University of California, Riverside.

Kind Act Coding

To determine whether the performed acts adhered to experimentally assigned protocols, three independent trained judges (undergraduate research assistants) rated participants' written description of each kind act on the extent to which it involved protocol adherence (inter-rater intraclass correlation ICC = .99). Act descriptions were also rated on the degree of effort involved (ICC = .64), interaction with others (ICC = .83), physical presence of others (ICC =

.92), digital media use (ICC = .79), self-stress-reduction behavior (ICC = .57), and participant self-health-promoting behavior (ICC = .82). Acts were rated on a scale from 1 (*Not at all*) to 5 (*A great deal*) for social interaction, self-health-promotion, self-stress-reduction, and effort, or coded 1 (*Yes*) or 0 (*No*) for protocol adherence, presence of others, and use of digital media. Act descriptions that provided insufficient information for rating were coded as missing. Participants in the control condition were not assigned to engage in or report any kind acts and therefore were coded as adherent and assigned missing values on all other ratings.

After excluding control participants and removing missing data, judges coded a total of 1,244 acts from 150 participants. Given interrater reliabilities ranging from moderate to excellent (Koo & Li, 2016), codings were integrated by averaging scale score ratings or determining consensus for dichotomous categories (e.g., as adherent). Act content codes were aggregated across the study period as either average scale ratings or count variables. To facilitate interpretation of secondary moderation analyses involving continuous measures of effort and adherence, adherence count values were median-split as high (≥ 11 kind acts performed out of the 12 assigned) vs. not (≤ 10 kind acts performed), and averaged effort scores were classified as high (> 3 [“moderate”] out of 5) vs. not, with each cutpoint corresponding to the scale median. To quantify the total intensity or “dose” of each act content characteristic over the study period, average ratings for each characteristic over the study period (e.g., social interaction, self-stress-reduction, etc.) were multiplied by the number of acts actually performed (0-12) to form an “act content dose” measure for use as a mechanistic covariate.

Gene Expression

CTRA gene expression was assessed by genome-wide transcriptional profiling of DBS RNA samples collected at baseline and Week 5 (post-intervention). Procedures followed those of

our previous study (Nelson-Coffey et al., 2017), with transcriptome profiling by RNA sequencing as previously described (Marie-Mitchell & Cole, 2021; Ross et al., 2019a, 2019b, 2021). Briefly, blood was collected onto Whatman filter papers via lancet finger prick, air-dried at room temperature, and stored prior to analysis in zip-lock plastic bags with a desiccant pack. RNA was extracted from DBS (Qiagen RNeasy), converted into cDNA using a high-efficiency mRNA-targeted reverse transcription system (Lexogen QuantSeq 3' FWD), and sequenced on an Illumina HiSeq 4000 instrument in the UCLA Neuroscience Genomics Core Laboratory, all following the manufacturers' standard protocols for this workflow. Sequencing targeted >10 million single stranded 65-nt reads per sample (achieved median = 11.1 million), each of which was mapped to the GRCh38 reference human transcriptome using the STAR aligner (median 88% mapping rate), and quantified as gene transcripts per million total mapped reads with expression values floored at 1 transcript-per-million to suppress spurious low-range variability, $\log_2$-transformed to stabilize level-dependent variance within gene, and z-score transformed to stabilize variance across genes. Among 452 assayed samples (2 from each of 226 participants), routine post-assay data quality screening identified 22 samples with insufficient RNA sequencing reads (< 5 million), 7 additional samples with poor read mapping rates (< 70%), and 17 additional samples with poor signal-to-noise ratios (average profile correlation with other samples: $r < .50$), leaving a total of 364 valid RNA profiles available for analyses of CTRA change over time (182 participants x 2 time points) after removal of unpaired samples. Other than the exclusion of these invalid samples, analyses included all available RNA data from this study (i.e., no subjects or experimental conditions or time points were omitted). This study's 90% valid data yield is consistent with previous research involving genome-wide transcriptional

profiling of DBS samples (Marie-Mitchell & Cole, 2021; Nelson-Coffey et al., 2017; Ross et al., 2019a, 2019b; 2021).

Analysis

As in previous studies (Cole, 2019; Nelson-Coffey et al., 2017), we used mixed effect linear models to analyze differences between experimental groups in pre- to post-intervention change in average expression of a pre-specified set of CTRA indicator gene transcripts (Group x Time factorial design). Analyses focused on a pre-specified set of 53 CTRA indicator genes used in previous research (Cole, 2019; Nelson-Coffey et al., 2017), of which 43 of which were reliably detectable in this study, including 15 pro-inflammatory gene transcripts (*CXCL8, FOS, FOSL2, IL1B, JUN, JUNB, JUND, NFKB1, NFKB2, PTGS1, PTGS2, REL, RELA, RELB, TNF*) and 28 Type I interferon-related gene transcripts (*GBP1, IFI16, IFI27, IFI27L2, IFI30, IFI35, IFI44, IFI44L, IFI6, IFIH1, IFIT1-IFIT3, IFIT1B, IFIT5, IFITM1-IFITM3, IRF2, IRF7, IRF8, JCHAIN, MX1-MX2, OAS1-OAS3, OASL*), and excluding 10 transcripts that showed minimal expression levels or variation (SD < .5 $\log_2$ expression units; *FOSB, FOSL1, IFITM4P, IFITM5, IFI27L1, IFNB1, IGLL1, IGLL3P, ILA1, IL6*). Gene-specific z-score signs were reversed for the antiviral gene set to reflect its inverse contribution to the CTRA profile (Cole, 2019; Nelson-Coffey et al., 2017).

Mixed models were estimated by maximum likelihood (SAS PROC MIXED) and specified fixed effects of indicator Gene, experimental Group, Time point, and a Group x Time interaction (with Gene and Time treated as repeated measures); a random effect of study participant; and a fully saturated (unstructured) variance-covariance matrix to account for residual heteroscedasticity and correlation across participants. Primary analyses focused on the

Group x Time interaction to quantify differential change over time across groups, with follow-up simple slopes analyzed by Time effects nested within Group.

Ancillary analyses tested for moderation of differential change over time by study protocol Adherence (Adherence x Group x Time interaction) or Effort (Effort x Group x Time interaction). Moderation analyses initially examined continuous measures of Adherence and Effort (i.e., not dichotomized into groups), and follow-up analyses median-split dichotomized Adherence and Effort categories to facilitate interpretation. Ancillary mechanistic analyses controlled for act content "doses" (i.e., the sum of rated social interaction, self-stress-reducing behavior, etc. over the study period) or treated them as predictors of CTRA change over time, in order to determine their contribution to the observed experimental (Time) effects nested within each experimental Group (i.e., comparing CTRA Time effects within each Group in unadjusted analyses vs. analyses adjusted for observed act content doses).

Sample size was determined by general power analysis guidelines for social psychological research (Vazire, 2014; see also Fraley & Vazire, 2014), with the RNA-available sample achieving 80% power to detect a .5 SD difference between groups in CTRA change over time (i.e., medium effect size) with a 2-tailed $p < .05$.

Results

Characteristics of the RNA study sample are presented in Table 1. The sample comprised 182 community-dwelling young adults (mean age 35 years) from Southern California, with 71% female and 60% from underrepresented ethnic groups. Figure 2 depicts the study timeline in which participants were randomized to perform kind acts for others, kind acts for themselves, or list their daily activities (i.e., a neutral control protocol). Among the 118 participants randomly assigned to perform kind acts for either themselves ($n = 55$) or others ($n = 63$), manipulation

check analyses of act content verified that kind acts performed in the kindness-to-others condition involved higher levels of social interaction than kind acts performed in the kindness-to-self condition (mean = 2.84 vs. 1.91, $F[1, 116] = 123.35$, $p < .0001$), as well as higher rates of co-presence (5.98 vs. 2.38, $F[1, 114] = 52.09$, $p < .0001$). By contrast, kind acts performed in the kindness-to-self condition involved higher rates of self-health-promoting behavior than those performed in the kindness-to-other condition (1.57 vs. 1.23, $F[1, 116] = 34.03$, $p < .0001$), as well as higher rates of self-stress-reducing behavior (2.93 vs. 1.74, $F[1, 116] = 798.22$, $p < .0001$), and digital media use (0.84 v. 0.32, $F[1, 116] = 8.40$, $p < .0001$). The number of kind acts actually performed (protocol adherence) did not differ between conditions, with 60% of participants performing at least 11 of the total 12 assigned kind acts in the kindness-to-self condition vs. 54% in the kindness-to-others condition (difference $X^2(1) = 0.43$, $p = .510$). However, acts performed in the kindness-to-others condition were rated as involving greater levels of personal effort than those in the kindness-to-self condition (3.20 vs. 2.73, $F[1, 116] = 65.26$, $p < .0001$).

CTRA Gene Expression

The three study groups did not differ in their baseline levels of CTRA gene expression (omnibus $F[2, 179] = 0.30$, $p = .744$). In primary intention-to-treat analysis (i.e., including all participants and not conditioning on any covariates), the groups showed significant differences in the magnitude of change in CTRA gene expression from baseline to the Week 5 post-study follow-up (omnibus $F[2, 179] = 17.76$, $p < .0001$; see Figure 3). Analyses of group-specific change parameters found participants in the control condition to show a general trend toward greater CTRA gene expression over time ($\beta = .050$, $t[179] = 2.54$, $p = .012$), whereas those in the kindness-to-others condition showed a significant decrease in CTRA gene expression from pre-

to post-intervention (β = −.094, t[179] = −4.70, p < .001), resulting in a significant difference between the kindness-to-others and the control group (β = −.144, t[179] = −5.50, p < .001). Participants in the kindness-to-self condition also showed a significant decrease in CTRA gene expression over time (β = −.076, t[179] = −3.57, p < .001), resulting in a significant difference from the control group (β = −.126, t[179] = −4.65, p < .001) but no difference from the kindness-to-others condition (β = .018, t[179] = 0.66, p = .507).

Similar results emerged in analyses that controlled for age, sex, ethnicity, BMI, use of hormonal birth control, and illness symptoms, with groups differing in the magnitude of CTRA change over time (F[2, 172] = 16.46, p < .0001) due to significantly greater CTRA reductions among those in the kindness-to-others condition (β = −.140, t[172] = −5.34, p < .001) and those in the kindness-to-self condition (β = −.120, t[172] = −4.42, p < .001) relative to the control group.

Effect of Protocol Adherence

As noted above, 57% of study participants completed either 11 or 12 of the total 12 kind acts specified by the protocol and were classified as protocol-adherent. Among the remaining 43% of participants, nearly all (99%) protocol non-adherence events involved failure to complete assigned acts rather than deviation from instructions. Protocol-adherent participants (mean kind acts = 11.9) did not differ from protocol non-adherent participants (mean kind acts = 4.8) in age, sex, race/ethnicity, BMI, or minor illness symptoms over the course of the study (all ps > .16). To determine whether the observed experimental effects were greatest among those who were most adherent, we conducted ancillary analyses treating the number of kind acts performed as a quantitative moderator and identified a significant Adherence x Condition x Time interaction (F[1, 178] = 7.17, p = .008). To facilitate interpretation, we compared effects for those who were

fully or near-fully adherent (completing 11 or 12 of the total 12 kind acts assigned) or notably nonadherent (completing 10 or fewer of the total 12 acts assigned) and found a similar Adherence x Condition x Time interaction (F[1, 177] = 13.48, $p < .001$). As illustrated in Figure 4, protocol-adherent participants showed a significant reduction in CTRA gene expression in the kindness-to-others condition (change: $\beta = -.140$, t[177] = −5.40, $p < .001$) but no significant change in the kindness-to-self condition (change: $\beta = -.035$, t[177] = −1.34, p =.180). By contrast, participants who completed relatively few kind acts for others showed no significant change in CTRA gene expression (change: $\beta = -.043$, t[177] = −1.55, $p = .122$), whereas those who completed relatively few kind acts for themselves showed an unanticipated significant reduction in CTRA gene expression from pre- to post-intervention (change: $\beta = -.138$, t[177] = −4.34, $p < .001$). We also tested whether the effort involved in performing a kind act might moderate intervention effects on CTRA but found no significant interaction to support that hypothesis (F[1, 177] = 0.03, $p = .855$).

Effect of Act Content

To determine which features of kind acts might contribute to their gene regulatory impact, we quantified the "achieved dose" of co-presence, social interaction, self-stress-reduction, self-health-promotion, and digital media use (i.e., average intensity per act multiplied by the number of acts performed) and controlled for these measures sequentially to assess the extent to which each factor could account for within-group changes CTRA expression over time. In nested analyses of change over time within each group, the significant CTRA reduction observed for protocol-adherent participants in the kindness-to-others condition was abrogated by controlling for the number of kind acts in which another person was physically present (97% reduction in experimental effect size; residual CTRA change after control for co-presence: $\beta =$

−.004, t[175] = −0.07, p = .942; association of co-presence with CTRA: β = −.015, t[175] = −2.57, p = .011). No other act content dimension accounted for a significant fraction of CTRA change over time within either protocol-adherent or protocol-nonadherent participants in the kindness-to-others condition.

In nested analyses of change over time within the kindness-to-self condition, the unanticipated CTRA reduction observed for participants who completed relatively few kind acts for themselves was abrogated by controlling for self-stress-reducing behavior (72% reduction in effect size; residual CTRA change after control for self-stress-reducing behavior: b = −.039, t[177] = −0.82, p = .412; association of self-stress-reducing behavior with CTRA: b = −.007, t[177] = −2.62, p = .010). This effect was driven by an unanticipated quadratic relationship between baseline self-stress-reducing behavior and subsequent CTRA declines (see Supplemental Results and Discussion for details). No other act content dimension besides self-stress-reducing behavior accounted for a significant fraction of CTRA change over time within either protocol-adherent or protocol-nonadherent participants in the kindness-to-self condition.

Discussion

This randomized controlled trial conducted in a diverse community sample replicated results from a previous study (Nelson-Coffey et al., 2017) in finding that individuals assigned to perform 12 kind acts for others over a 4-week period showed a significant reduction in CTRA gene expression. These effects emerged in intention-to-treat analyses that included all participants randomized to the kindness-to-others protocol (regardless of the extent to which they completed all assigned kind acts), as well as in covariate-adjusted analyses, and were most pronounced among those who performed the largest number of kind acts (i.e., dose-dependent). These results support a causal effect of prosocial behavior on leukocyte gene regulation, and

contribute to a growing body of literature implicating neuro-immune regulation as one pathway through which prosocial behavior might potentially impact physical health outcomes such as cardiovascular disease and all-cause mortality (Brown et al., 2003, 2009; Brown & Brown, 2015; Roth et al., 2018). These findings also underscore purposeful engagement in prosocial behavior as one behavioral intervention that can replicably reduce CTRA gene expression (Cole, 2019; Curry et al., 2018; Moieni et al., 2020; Nelson-Coffey et al., 2017; Seeman et al., 2020).

Participants who were randomized to perform 12 kind acts for themselves over 4 weeks also showed a significant reduction in CTRA gene expression relative to controls in intention-to-treat analyses. This contrasts with results from Nelson-Coffey et al., 2017, in which self-kindness had no effect on CTRA gene expression. It is unknown why the results differed here, although it is conceivable that differences in participant population or study context might contribute. The CTRA reductions observed here do not appear to stem from any beneficial effect of kindness per se (i.e., regardless of whether the kindness targets self or others), because CTRA reductions in the kindness-to-self group paradoxically occurred among those who performed the fewest self-kind acts. Among those randomly assigned to the self-kindness protocol, 40% did not complete at least 11 of the 12 acts requested, and significant CTRA reductions were observed only within this relatively non-adherent subset. No significant change in CTRA gene expression was observed among the 60% who did perform the requested self-kind acts. The paradoxical moderating effects of non-adherence were not specific to the 11-act (median split) cut point used to classify adherent vs. non-adherent subgroups, as similar interactions emerged when adherence was treated as a continuous variable. Analyses of the content of self-kind acts did identify an unanticipated relationship between self-stress-reducing behavior during the intervention period and individual differences in CTRA gene expression at baseline (but not at follow-up; see

Supplemental Results and Discussion for details). However, the basis for this non-hypothesized relationship remains unclear as does its generalizability and reliability (no such effect was observed in the previous study from Nelson-Coffey et al., 2017). Future research will be required to confirm the reliability and mechanisms of paradoxical CTRA reductions observed among those who failed to perform self-stress-reducing behaviors.

The diverging pattern of adherence-dependent results across experimental conditions is consistent with the hypothesis that CTRA gene expression declines in proportion to prosocial behavior (other-kind adherent and self-kind nonadherent) and increases in proportion to self-focused behavior (other-kind nonadherent and self-kind adherent). Prosocial behavior reduced CTRA gene expression in both intent-to-treat analyses (i.e., averaging across all participants assigned to perform kind acts for others, regardless of adherence) and per-protocol analyses (i.e., among those who actually performed their assigned kind acts). By contrast, self-kindness reduced CTRA gene expression only in intent-to-treat analyses, and this favorable effect was driven by the subset of participants who were less than fully adherent to the self-kindness protocol. It is possible that failure to perform all assigned self-kind acts might serve as a behavioral marker of a "eudaimonic" orientation in valuing others and other-oriented goals—an orientation that has previously been linked to reduced CTRA gene expression (Boyle et al., 2019; Cole et al., 2015b; Fredrickson et al., 2013, 2015; Kitayama et al., 2016; Lee et al., 2020; Seeman et al., 2020; Snodgrass et al., 2019). Future research will be required to clarify the relationship between eudaimonic orientation, self-kindness, and stress (e.g., using more extensive baseline measures of stress, personality, temperament, etc.).

The present findings support a causal effect of prosocial behavior on CTRA gene expression and underscore the need for future research to identify the physiological mechanisms

involved. Previous mechanistic research has found CTRA gene expression to be mediated in large part by beta-adrenergic signaling from the sympathetic nervous system (Cole, 2014; Cole et al., 2015a; Heidt et al., 2014; Knight et al., 2020; MacCormack et al., 2021; McKim et al., 2018; Powell et al., 2013). However, other psychological and biological pathways have also been implicated in prosocial behavior and could potentially contribute to the observed effects (Brown & Brown, 2015; Eisenberger & Cole, 2012; Inagaki & Eisenberger, 2016; Lazar & Eisenberger, 2021; Roth et al., 2018). Defining the upstream CNS substrates of prosocial behavior also remains an important topic for future research (Eisenberger & Cole, 2012). Prosocial behavior may affect CTRA gene expression indirectly by promoting other social or psychological processes that have previously been linked to CTRA regulation, such as social connection, eudaimonic well-being, or reduced negative affect (Fredrickson et al., 2013, 2015; Cole et al., 2007, 2011, 2015a, 2015b; Kitayama et al., 2016; Lee et al., 2020; Seeman et al., 2020; Wingo & Gibson, 2015).

Limitations and Future Directions

The present results are consistent with previous research in finding that prosocial behavior can reduce CTRA gene expression (Moieni et al., 2020; Nelson-Coffey et al., 2017; Seeman et al., 2020), but they are limited in several important respects. The duration of follow-up was limited to 1 week post-intervention, so it remains unclear how long these effects might persist beyond the cessation of deliberate prosocial engagement. The health significance of the observed reductions in CTRA gene expression should be interpreted with caution until more is known about the quantitative relationship between CTRA gene expression and disease risk in healthy populations such as this one. CTRA expression has been linked in other studies to clinical health outcomes (e.g., Antoni et al., 2016; Black et al., 2018; Cole et al., 2015a; Knight

et al., 2016; Mellon et al., 2016), but this study did not assess such outcomes. The present effects are not large in biological terms (-.144 $\log_2$ RNA units, or approximately 10% reduction in CTRA gene expression; Cohen's d = .41) and correspond to about 1/2 the effect of an intensive 12-week stress-management program (Antoni et al., 2016) or 1/3 the effect of pharmacologically inhibiting beta-adrenergic signaling from the sympathetic nervous system (Knight et al., 2020; MacCormack et al., 2021). However, the present salutary effects are comparable in magnitude to the adverse CTRA effects associated with risk factors such as smoking, high BMI, heavy alcohol consumption, poverty, and racial disparities (Cole et al., 2020). As such, the present effects may have the potential for substantively significant health impact.

This study was designed as a confirmatory test of a specific a priori hypothesis regarding a pre-specified set of CTRA indicator genes, and was not designed or powered for genome-wide discovery analyses at the level of individual genes; that is, individual gene expression differences were not tested for statistically significant association. It is possible that future studies using larger sample sizes may reveal additional genes that are regulated by prosocial behavior. This study also suffered from missing data on several CTRA indicator genes due to the limited RNA available from the DBS sampling method employed here. Although this limitation would not bias the validity of results for the indicator transcripts that do remain available, it may limit the comparison of this study's results with previous findings involving the canonical 53-gene CTRA indicator profile.

Because protocol adherence and kind act content are participant-generated variables, the present observational analyses linking those variables to changes in CTRA gene expression should be considered hypothesis-generating and require experimental confirmation in future research. These variables may reflect individual characteristics that moderate the effect of

kindness protocols (e.g., personality or temperament, eudaimonic orientation, basal stress levels, etc.) rather than causal mechanisms of kindness effects on gene regulation. Future research will be required to clarify the psychological basis for non-adherence to self-kindness protocols and its biological correlates.

This study was conducted in a sample of community-dwelling adults in a suburban Southern California city that housed a major research university, and it remains to be seen whether similar effects would occur in other groups, locales, ecological conditions, or cultures. It is possible, for example, that the modest pre- to post-intervention increase in CTRA gene expression observed in the control group might stem from background trends in academic stress or seasonal effects on gene expression (Goldinger et al., 2015; Honda et al., 2013). However, such effects would not bias the interpretation of group differences in CTRA change over time because the same background trends would affect all groups similarly in this randomized experimental study.

Conclusion

In the present study, community-dwelling adults who were randomly assigned to perform kind acts directed toward specific other individuals showed significant declines in leukocyte CTRA gene expression over a 5-week period. These findings replicate previous research showing that purposefully engaging in prosocial activities over several weeks can favorably impact the CTRA. They also support the mechanistic hypothesis that CTRA gene regulation is most sensitive to the social target of kindness (other people vs. oneself) rather than the production of kind acts per se, with favorable effects observed in those who performed kind acts for others but not in those who performed kind acts for themselves. These results underscore the key role of social processes in shaping the psychobiological impact of positive behavior.

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Table 1

Baseline sample characteristics and covariates

Age (mean ± SD)	35.13 (11.44)
Sex (% Female)	71.43%
Body mass index (mean ± SD)	26.86 (7.05)
Ethnicity	
White	40.11%
Hispanic	28.57%
Asian	13.74%
Black	6.04%
Hormonal birth control use	26.37%
Self-reported illness symptoms	11.54%
Act characteristics (mean ± SD)	
Total acts	9.93 (3.48)
Socially interacting with others	1.91 (0.86)
Self-stress-reducing behavior	1.84 (0.80)
Self-health behavior	1.25 (0.34)
Effort	2.28 (0.99)
Others physically present	2.79 (3.32)
Digital media use	0.36 (0.85)

Note. Participants' total number of acts, physical presence of others, and digital media use were coded dichotomously. Means reflect the average number of acts across the intervention period for these variables. Social interactions, self-stress-reducing behavior, self-health promoting behavior, and effort were coded on a 1 to 5 scale. Means reflect the strength of each characteristic across the intervention period.

Figure Captions

Figure 1. CONSORT Flow Diagram.

Figure 2. Study timeline. Note. T_1 (baseline) through T_5 (post-intervention) occurred at weekly intervals.

Figure 3. Change in CTRA gene expression. Data represent mean (±SE) change from baseline (Week 1) to post-intervention follow-up (Week 5) in average expression of 43 CTRA indicator genes for participants randomized to control, kindness-to-others, and kindness-to-self experimental conditions. Units represent the average value of 43 z-transformed $\log_2$ gene expression levels. Group-specific change: *$p < .05$. **$p < .01$. ***$p < .001$.

Figure 4. Moderation of experimental effects by protocol adherence. Data represent mean (±SE) change from baseline (Week 1) to post-intervention follow-up (Week 5) in average expression of 43 CTRA indicator genes for participants randomized to control, kindness-to-others, and kindness-to-self experimental conditions for individuals who showed high levels of adherence to study protocol (i.e., performed at least 11 of the 12 total assigned kind acts; left bars) or low levels of adherence to study protocol (performed 10 or fewer of the assigned kind acts). Units represent the average value of 43 z-transformed $\log_2$ gene expression levels. Group-specific change: *$p < .05$. **$p < .01$. ***$p < .001$.