SPECIFIC AIMS

We propose to dramatically extend and deepen our assessment of the overarching hypothesis that social relationship qualities established in adolescence have integral, long-term relations to life course physical and mental health and to broader aging processes. We will add **multiple new panels of longitudinal data on biological aging, mental health, and peer, romantic partner, and family relationship qualities** obtained from a unique, socio-demographically diverse final sample of 170 individuals (93% of our original sample), followed across a *30-year span* from age 13 to midlife (ages 38 – 42) to address 4 overarching aims:

Aim 1: Predict Broad Aging Processes, as Well as Specific Functional Outcomes. We previously examined adolescent experiences of hostile conflict and lack of supportive relationships as predictors of poor physical health *before* major health problems had emerged, using proxies such as epigenetic aging. We now propose to expand and extend this examination to identify adolescent-era predictors of:

Aim 1a: Advanced Physical Health and Disease Processes. We propose to **examine direct and mediated predictions to *actual* disease processes**, including *metabolic syndrome, cardiovascular disease,* and *chronic pain,* among others. In addition, we will examine newly enhanced markers of epigenetic aging (e.g., Dunedin Pace of Aging Measure-revised) and additional physiological markers of health (e.g., Tumor Necrosis Factor α and Interleukin-1β), while continuing our ongoing panel of assessments. We will thus be able to identify key social factors and mediating physiological processes that predict serious illness.

Aim 1b: Lifespan Development of Caregiving Capacity. Utilizing new data (see below), we will examine the adolescent and early adult roots of capacity to support and provide care to others as these manifest in: a) adult parenting roles—**permitting a *three*-generation assessment**; b) adult caregiving of aging parents; and c) adult romantic relationships. In addition, we will examine conditions under which caregiving experiences are linked not only to concurrent relationship quality but also to the *caregiver's* physical and mental health.

Aim 1c: Capacity to Cope with Prolonged Social Disruption. Covid has changed the quantity and quality of social interactions and relationships over the past two years, affecting both mental and physical health. We will use new panels of data to assess the hypothesis that capacity to cope with this disruption will be predictable from patterns of social development and adaptation first established in adolescent relationships and extending into adulthood. We will test an amplification hypothesis positing that the stress of Covid-related disruptions will be minimized among those with a history of positive relationships, while it will place those with a less positive history at greater risk. We will examine *change* in mental and physical health pre- and post-Covid, not simply as a means of tracking Covid-related impacts, but as **a unique natural experiment regarding the ways in which prior relationship experiences set people up to cope well or poorly with unexpected social stressors**. We will also consider the role of Covid vaccination status and illness.

Aims 2 - 4: Mediational vs. Weathering Explanations of Links to Early Midlife Health & Aging Outcomes; Mediation via Mental Health and Health/Risk Behaviors; and Biologic & Contextual Moderation and Mediation. Carefully following the same approach we outlined for these Aims in our original proposal, mediating and moderating processes will be explored in relation to the new hypotheses addressed in Aim 1, allowing assessment of direct vs. mediated links to actual disease processes, providing insight into factors mediating continuities in caregiving practices as these extend across three generations, and permitting assessment of contextual and mental health factors moderating the impact of social stressors on health.

To pursue these expanded Aims, we will obtain the following new data (with data collection spaced so as to minimize participant burden and maintain our extremely high participant retention rate):

1) an additional epigenetic assessment (to track changes in markers of aging, as well as methylation of specific relevant genes (e.g., the oxytocin receptor gene));
2) two additional extended physical health assessments (including both repeats of prior assessments and collection of new data (e.g., additional cytokines, assessment of pain and functional limitations));
3) two observations of romantic partner interactions, along with participant and romantic partner reports;
4) two self-report assessments of social functioning, mental health, and physical health from both our participants and their closest friend;
5) parental caregiving assessments (obtained previously for participants with children ages 2-8; the next 5 years will permit nearly complete collection as the sample nears the end of primary childbearing years);
6) coding of observed empathic and caregiving capacity from a video-recorded task in which participants provide support to close friends and romantic partners. Participants have been repeatedly observed in this task across multiple years and relationships, but we have not previously requested funding for coding.

Ultimately, the proposed work will allow us to generate potentially groundbreaking knowledge regarding the existence and nature of paths from adolescent relationship difficulties to lifespan markers of health and aging.

PROGRESS REPORT

Major Activities

All Specific Aims of the original proposal are being implemented as planned. In response to Covid-19, we rapidly converted our participant data collection to a secure online Qualtrics-based system, allowing us to continue collecting data during the pandemic. We have also converted our romantic partner observational assessments so that they can now be implemented virtually via fully encrypted Zoom connections. In our most recently completed wave of data collection, we were pleased to find a 4% increase in response rates relative to the prior wave. The one place where we are slightly behind our projections is in collection of biological data requiring in-person blood and related physiological assessments, as this was fully halted for a number of months due to the pandemic and participants are only just now showing willingness to return in person for this data collection. We ultimately expect to catch up on this data collection in the coming months, without harm to the overall aims of the study, given that we have always allowed for a multi-year window for data collection for this aspect of the study. Nevertheless, we have been able to complete the complex, multi-step procedures required for the quantification of epigenetic data from 84% (154 individuals) of our original sample. Note these were our *most* difficult data to collect as they required in-person blood draws and at the time could not be done with our remote participants (although this has just recently become possible). The cumulative enrollment report reflects the full original sample as we find that even individuals who have not participated for years often agree to participate in new waves; thus all original participants are candidates for this wave.

Specific Objectives

During this round of funding, our goals were to collect multiple new waves of interview data (from participants, their close friends, and their romantic partners), observational data from romantic partner interaction tasks, and blood-based and other physiological health assessments. With the exception of collection of some of our physiological data as noted above, each of these goals has been met successfully. We continued regular (i.e., interview and questionnaire) data collection throughout the reporting period. Coding of observational data has also continued as planned, as we were able to rapidly set up a secure digitized video system to allow coders to code remotely during the pandemic. Analyses, presentation, and publication of results of findings are proceeding as planned on data across all time periods (i.e., within adolescence, from adolescence to adulthood, etc.), as seen below.

Significant Results & Key Outcomes

In the past five years we have published 44 papers, almost all in the top or near-top journals in their respective fields. An additional 8 papers are currently under review at top peer-reviewed journals. In this same time period, this study has also produced 6 doctoral theses and 44 peer-reviewed presentations at the Society for Research on Child Development Biennial Meetings, the Society for Research on Adolescence Biennial Meetings, and other major national and international conferences.

The current wave of the study was designed to identify links from adolescent-era relationship factors, assessed beginning at age 13, to physical and mental health (both self-reported and biologically assessed) at ages 33-37. Results thus far, as outlined below, have established our ability to successfully identify novel adolescent-era relationship predictors of both mental health outcomes, as well as of key physiological outcomes linked to physical health. The results demonstrate not only the productivity of our research team, but also the promise of extending these efforts to assess long-term adolescent-era predictors of specific physical disease processes, caregiving capacities, and resilience or vulnerability in the face of Covid-induced stressors as we now propose. We outline these results below, organized according to major themes from our original proposal, beginning with consideration of mental health and functional outcomes before moving on to discuss our emerging findings regarding predictors of long-term health and aging outcomes. All findings below avoid methods confounds by always assessing relations among constructs that have been assessed via different methods. Demographic covariates/confounds and potential related moderating processes are always considered.

Parental Behaviors as Predictors of Long-term Functional Outcomes

We are continuing to identify important new ways in which parenting behaviors predict long-term functional outcomes, both within and beyond adolescence. We are also increasingly zeroing in on *which* parenting behaviors appear to matter the most, with a growing recognition of the importance of parental behaviors that

either promote or undermine an adolescent's autonomy. For example, although we have previously shown that parents' promotion of their teens' autonomy and connection is tied to teens' *social* development, we are now also finding that promotion of these qualities is predictive of improvement in *academic performance* over time, even after accounting for baseline levels of academic proficiency (Loeb, Kansky, Tan, Costello, & Allen, 2019). In the proper relationship context, giving young people a chance to express themselves and disagree appropriately with their parents appears to enhance competence, perspective taking, and motivation in ways that predict improvement in academic grades as objectively measured over the course of a high school career.

Conversely, we are finding that autonomy *undermining* behaviors, in the form of psychologically controlling behaviors, have substantial near-term functional implications for predicting reductions in adolescents' desirability as companions within the broad peer group, as well as their capacity to obtain social support from close friends. In the longer-term, these overcontrolling behaviors ultimately predict lower levels of academic attainment by age 32, even after accounting for baseline academic grades, gender, and family income. We also find that teens experiencing these behaviors are less likely to establish a stable romantic relationship in adulthood (Loeb, Hessel, Tan, & Allen, 2015; Loeb et al., 2019). Finally, we are finding that maternal autonomy-undermining behavior has yet another key role as a risk factor: It predicts a range of negative health behaviors and outcomes into adulthood, including poor sleep quality and higher body mass index (Tan, 2018).

Given the long-term import of experiences with parents, one of our key challenges has been to identify the ways in which problematic relationship experiences become *internalized* to predict future outcomes. Some of our most promising findings to date in this area revolve around the degree to which participants do vs. do not hold secure models of attachment relationships. Specifically, we have looked at how attachment security (a known predictor of the security of an individual's *future offspring*, as well as a correlate of numerous adult social behaviors) changes over a 10-year period from adolescence to adulthood, and we have begun to identify both parent- and peer-relationship predictors of these changes (Allen, Grande, Tan, & Loeb, 2018). We find that maternal behaviors undermining adolescent autonomy predict increasing *insecurity* from adolescence to adulthood, suggesting a key mechanism by which parental overcontrol may become internalized to predict long-term outcomes. We also find, conversely, that peer support (assessed via observational methods) predicts increasing attachment security over time, and that the role of this support grows with time, suggesting a potential buffering role for peers going forward. Together these findings begin to suggest *which* adolescent relationship qualities should be a focus of monitoring/screening. Given the strong empirical links of attachment security to caregiving quality of future offspring, these findings also support the potential of our proposed Aim (Aim 1b) in the next 5 years of understanding predictors of caregiving capacity across the lifespan.

Further indicating the importance of these internalized states, we are also finding that attachment predicts several key aspects of social development. For example, insecure states of mind regarding attachment at age 14 predict relative decreases in adolescents' abilities to seek and receive support from close friends from ages 14 to 18 (Loeb, Stern, Costello, & Allen, 2020). In addition, greater attachment insecurity predicted greater observed negative interactions with romantic partners and relative increases in hostile attitudes from ages 14 to 27. The effect of attachment insecurity on observed negativity was mediated by difficulty seeking and receiving support in friendships during adolescence. These findings suggest a type of self-fulfilling prophecy as insecure adolescents confirm their negative expectations of others as a result of their ongoing struggles to obtain support. Together**, these findings begin to map out a clear chain through which modifiable parenting behaviors in adolescence display long-term links to social functioning well into adulthood**.

Peers and Functioning

One of the most striking and consistent results of our study has been our identification of close friendship qualities in adolescence as key predictors of a wide range of short- and long-term adaptive outcomes. For example, we find that establishing close friendships characterized by high levels of autonomy and connectedness—a factor we have long recognized as important in *parent*-teen relationships—is also predictive of long-term levels of academic achievement (Loeb, Davis, Costello, & Allen, 2020). Even after taking into account markers of family socio-economic status, teens with friendships where they can disagree and remain connected not only displayed relative increases in their GPA over the course of secondary school, but also displayed higher levels of lifetime educational attainment as adults.

Anticipating one of our proposed new Aims—understanding the developing capacity and expression of caregiving, empathy, and support—we are also finding that adolescents' secure states of mind regarding attachment at 14 predicted their greater capacity to *provide* (not just receive) empathic support during observed interactions with friends across ages 16-18 (Stern et al., 2021). Teens' attachment security also

predicted the degree to which friends called for participants' support—that is, attachment security predicted *friends'* observed behaviors, making clear the extent to which attachment processes may lead individuals to alter not just their own behavior but to reshape the context of their social environment going forward.

We have also now been able to address a key question running through all our research on adolescent peer relationship qualities: whether the apparent long-term effects of poor peer relationships might simply be an artifact of their link to poor parental relationships or underlying attachment insecurity. In work recently accepted for publication, we were able to directly compare the quality of adolescents' close friendships to the quality of their relationships with parents and their attachment security as predictors of a broad range of long-term outcomes assessed across a period of fifteen years. Our approach employed robust methodologies: Primary analyses used multiple peer reports of friendship quality and parent reports of parent-teen relationship quality obtained repeatedly from age 13 to 17. Strikingly, **adolescent friendship quality was not only predictive of a broad range of adult psychosocial outcomes — including better work performance, reduced depressive symptoms, and better adult social relationship quality — adolescent friendship quality was a significantly *better* predictor of these outcomes than was the quality of the relationship with parents** (Allen, Costello, Kansky, & Loeb, 2021)**.** Results remained robust even when other reporters were used to assess each construct (e.g., adolescent self-reports, maternal instead of parental reports), and regardless of whether relationships were assessed earlier vs. later in adolescence. To be quite clear, though, we do not interpret results as showing that parents don't matter. Extensive evidence, including findings described earlier, make clear that parental *behaviors* appear quite strongly linked to future adjustment. What the current findings do show, however, is that it may be extremely difficult for parents and teens to accurately judge the underlying quality of the parent-teen *relationship* during adolescence. This likely results from the rapidly changing nature of the relationship, the extensive renegotiations involved, and the lack of external reference points against which to compare the relationship. Yet, these findings clearly have implications regarding where to look and not to look in assessing risk and dysfunction in adolescence. Most importantly, though, these findings provide **some of the most powerful evidence to date regarding the importance of close friendships in adolescence to long-term psychosocial functioning**, evidence that has led directly to our translational work, described further below.

In a different domain, we have been examining social factors related to adolescent substance use and made a key discovery with significant implications for prevention: **The factors that predict high levels of substance use in adolescence are *not* the same as those that predict longer-term difficulties with substance use in the late twenties** (Allen, Loeb, Narr, & Costello, 2020). Specifically, within adolescence, levels of ongoing peer and family conflict are strong concurrent predictors of use. We find that these conflict factors fade in importance over time, however. Rather, in order to predict long-term substance use problems from factors observable in adolescence, we need to look not at the qualities of adolescents' current relationships but rather at markers of adolescents' *underlying social skill development*. This implies that some efforts to reduce short-term substance use in adolescence (e.g., by reducing contact with peers) may be unlikely to address longer-term risks. Such efforts may even backfire if they serve to reduce adolescent opportunities for social skill development. Conversely, adolescent-era social skills interventions directly addressing these underlying deficits (interventions which are still quite rare), appear to warrant greater attention. As individuals move into late adolescence, we also find evidence of the ways in which social skill deficits begin to become problematic. We have found, for example, that difficulties in romantic relationships and lack of social acceptance in late adolescence—likely reflections of key social skill deficits—are predictive of relative *increases* in substance use by age 27, even after accounting for baseline levels of substance use and personality and demographic factors (Yan, Costello, & Allen, 2020).

The Role of Adolescent and Adult Romantic Relationships

The quality of adolescent peer relationships in turn appears crucial to understanding who will vs. will not establish satisfying long-term romantic relationships and partner bonds in adulthood. The quality of adult marital and romantic relationships is widely recognized as integrally connected to physical health, and we have recently taken significant steps toward identifying qualities in adolescence that predict later romantic life satisfaction. Most notably, we found that the best predictors of adult satisfaction with romantic life were *not* the readily observed romantic behavioral markers in adolescence, but rather the quality of *non-romantic* close friend interactions being established during that period. Behavioral markers such as the presence vs. absence of romantic relationships, number of relationships, level of sexual activity, and age at first intercourse were all unrelated to later romantic life satisfaction (Allen, Narr, Kansky, & Szwedo, 2020). **These findings suggest yet more value in attending to adolescents' close friendships.**

The one place we did find a prediction from romantic behavior in adolescence was from our close look into the specific interaction qualities within adolescents' romantic relationships. Specifically, the extent to which teens were able to handle disagreements with a romantic partner in ways that preserved their *autonomy* without undermining the relationship did predict future romantic life satisfaction—again highlighting the role of autonomy processes as a key developmental marker of social adjustment (Kansky & Allen, 2018). Even here, however, our prior research has suggested that romantic relationship skills are significantly predicted by the adolescent's experience learning these skills from autonomous and connected experiences in prior *close friendships* (Oudekerk, Allen, Hessel, & Molloy, 2015).

There is one aspect of early romantic experience that we have identified, however, that does appear quite predictive of future psychosocial difficulties. Very early in adolescence (i.e., age 13 and earlier), high levels of relationship 'churn' (i.e., moving rapidly from romantic partner to partner) is predictive of steadily decreasing quality of relationships within the broader peer group in the succeeding years and of increasing levels of conflict within close friendships (Loeb, Kansky, Narr, Fowler, & Allen, 2020). More significantly, over time, this churn—which we view as reflecting a desperate effort to move prematurely into the romantic world—predicts longer-term patterns of *abusive* behavior in romantic relationships in late adolescence and lower levels of relationship satisfaction overall. Thus, **we have identified a marker of risk that can be assessed early in adolescence to predict—and ultimately help learn to prevent—the development of long-term romantic relationship difficulties and abusive behavior.**

Rethinking the Role of Peer Influences

We have also used our unique multi-wave, multi-reporter design to **challenge one of the widespread, yet largely unexamined, assumptions regarding peer influence in adolescence: that the presence of strong peer influences is always or mostly a reflection of poor adaptation.** We addressed this issue by first identifying *which* adolescents were most and least likely to be influenced by their close friends' levels of substance use over a one-year period (i.e., which adolescents were most likely to change to have their levels of use come to resemble the levels of use their close friend reported in the prior year). Two key findings emerged: First, it was the most *well-adapted* teens (i.e., those with the strongest friendships and strongest relationships with parents) who were most influenced. Second, these influence processes were quite often in a *positive* direction: Teens were at least as likely to be influenced to *reduce* their level of substance use in accord with a close friend's lower levels of use as they were to increase them (Allen, Loeb, Kansky, & Davis, 2020). This finding provides further evidence that **peer socialization processes, far from being an automatic risk factor, are part of an essential and inevitable developmental process** worth carefully promoting.

Having said that, we have nevertheless found that it makes a significant difference just *which* peers are doing the socializing. We find, for example, that the socializing effects of association with *deviant* peers during adolescence extend not only far beyond just leading to teen deviance, as others have documented, but also far beyond adolescence. We assessed deviant peer associations over a period of five years across mid-adolescence, using both teen- and peer-reports, and then examined both precursors and long-term outcomes. We first found that the presence of deviant peer associations was predicted by exposure to coercive and autonomy-undermining parenting behavior early in adolescence—again highlighting the role of parental overcontrol. By the time our participants reached their late twenties, a history of deviant peer associations in adolescence predicted higher levels of both internalizing and externalizing symptoms as well as poorer overall adjustment as rated by parents, *even after accounting for adolescent-era levels of internalizing and externalizing symptoms* (Allen, Narr, Loeb, & Davis, 2019). We interpret these findings as likely reflecting the effects of repeated exposure to the coercive patterns of interaction that have been identified as a central feature of relationships among deviant peers (Dishion, Spracklen, Andrews, & Patterson, 1996). The negative socializing effect of repeated exposure to these autonomy-undermining interactions then sets the individual up for long-term psychosocial dysfunction, even over and above what could be predicted simply from their adolescent-era level of functioning. **These findings dovetail with findings on parental overcontrol to support the fundamental role of adolescent-autonomy processes (with both peers and parents) in overall patterns of social development not just in adolescence but well into adulthood.**

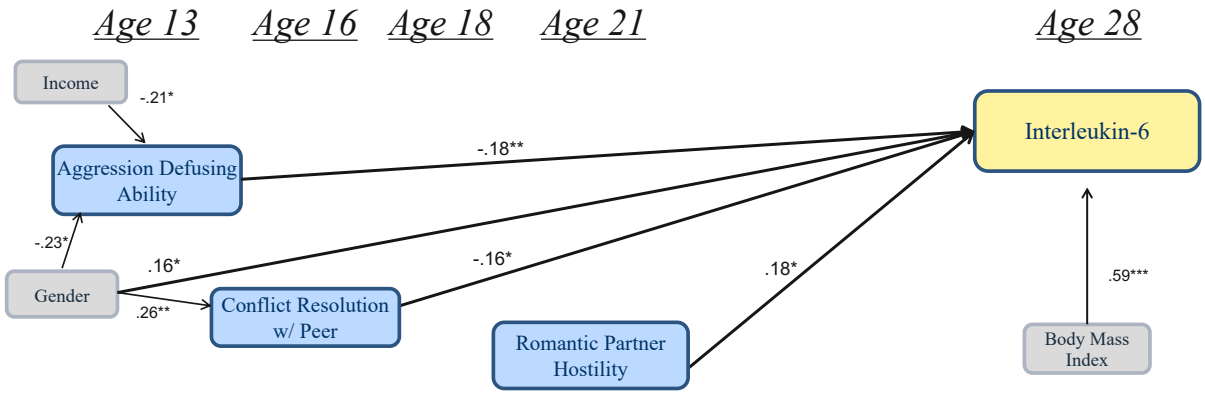
We are also exploring other potential double-edged aspects of friendships in adolescence, working to move the field beyond a simplistic 'all good things go together' perspective. We are finding, for example, that *intensity* of connection between close friends has a double-edged character: For friend pairs who are doing well, intense connections predict that those teens will do even better over time. When friends are not faring as well, displaying depressive symptoms or externalizing behaviors, intensity tends to predict an *amplification* of these

problems (Costello, Narr, Tan, & Allen, 2020). These findings suggest that seemingly quite disparate phenomena (e.g., co-rumination for depression and deviancy-training for aggression) each with extensive, independent literatures (see e.g., Dishion et al., 1996; Spendelow, Simonds, & Avery, 2017) may both be dependent upon the intensity of the adolescent's social connections and thus may ultimately be addressable via similar approaches.

Predictions to Adult Physical Health & Aging Processes

Although the numerous links to adult psychosocial functioning outlined above are of obvious importance, our most recent data suggest that adolescent social experiences have potentially even more profound links to long-term *physical* health outcomes. Building upon prior findings that social relationship qualities in adolescence predict *reported* physical health in early adulthood (Allen, Uchino, & Hafen, 2015), we have now expanded these findings by beginning to establish links to actual biological markers of health and aging.

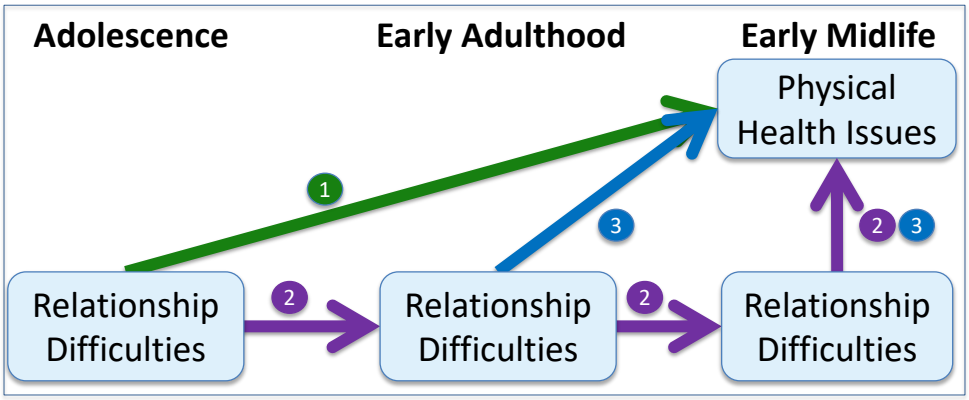
For example, we have now established the presence of links from patterns of conflict behavior in key social relationships, beginning in adolescence, to a marker of immune system dysregulation (high levels of Interleukin-6 (IL-6) in blood) in adulthood. Adult serum IL-6 levels were predicted across periods as long as 15 years (see figure below) by adolescents' inability to defuse peer aggression, by poor peer-rated conflict resolution skills, and by independently observed romantic partner hostility in late adolescence (Allen, Loeb, Tan, Narr, & Uchino, 2018). These findings further support our growing theoretical understanding of the role of inflammation as an evolved response to protecting the individual from potential physical consequences of significant conflict.



We identify a similar pattern of adolescent stressors predicting adult behaviors when we examine adult blood pressure. Overly intense adolescent romantic relationships were identified as a long-term predictor of higher adult blood pressure (Allen, Loeb, Tan, Davis, & Uchino, 2021). Romantic intensity in adolescence—measured via quantity of time spent alone with a partner and duration of the relationship—is seen as an indicator of an insecure and enmeshed style of interaction in the adolescent years in which other relationships are allowed to lapse and vulnerability to distress from a potential breakup becomes extreme. The prediction to adult blood pressure was partially mediated via conflict in non-romantic adult friendships and by intensity in adult romantic relationships. Even after accounting for these mediators, however, a direct path from adolescent romantic intensity to higher adult blood pressure remained. This is further evidence that both weathering processes—in which relationship stressors have lasting effects regardless of future relationship experiences—and mediational chains of risk explanations apply to adult health outcomes. In this specific case, it is notable that **effect sizes were of sufficient magnitude as to potentially offset the value of widely prescribed anti-hypertensive medications for many individuals.**

Our findings regarding prediction of adult blood pressure illustrate a key element of Aims 1 and 2 of our original proposal: understanding the extent to which long-term predictions from the adolescent era can be explained via either mediational processes or via weathering effects in which early experiences predict long-term outcomes regardless of intervening processes. As seen in the Figure below, taken from our original proposal, we found evidence of both types of processes, such that although effects of adolescent-era relationship intensity were *partially* mediated via later conflictual relationships (blue/purple paths labelled '2' & '3'), adolescent-era relationship intensity also had direct, unmediated effects on blood pressure (green path

labelled “1”). **These findings have significant implications for prevention as they suggest a potentially critical need to intervene early to address social risk factors for adult physical health difficulties.**



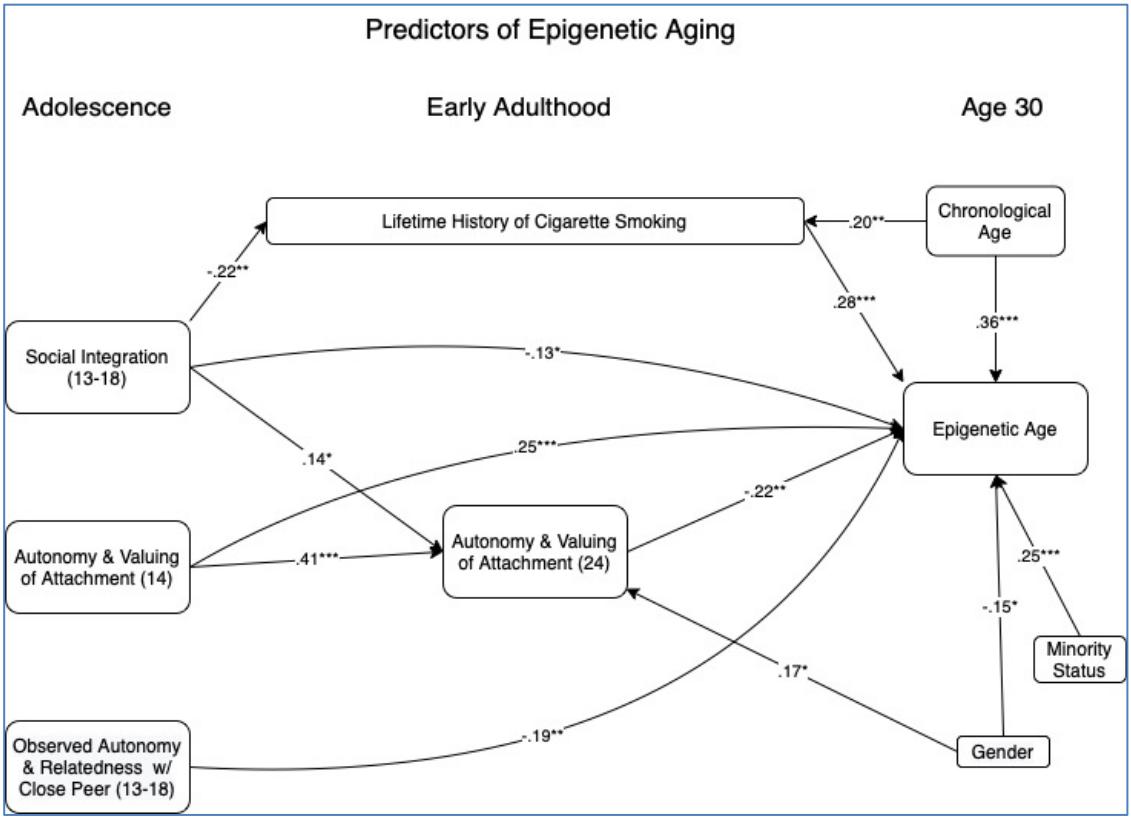
Conceptual Model for Aims 1 and 2 (from original proposal)

We were also able to gain clues as to the *origin* of this pattern of intense/enmeshed relationship behavior, finding that it was predictable from prior experience of overcontrolling parenting, as reported by parents. Finally, in accord with Aim 4 of our original proposal, focused on potential moderating processes, we were able to use our rich data set to learn more about the *conditions* under which intensity was vs. was not linked to future blood pressure. We found that if participants were able to maintain strong non-romantic friendships in the midst of romantically intense relationships, then the linkage between romantic intensity and high blood pressure disappeared (a buffering effect). Increasing our understanding of the ways that strong, non-romantic friendships can buffer effects of other social stressors is a topic we will explore further in our proposed study (Aim 1c).

The role of parental overcontrol also surfaced as a predictor of negative physical health outcomes. For example, we found that high levels of parental psychological control at age 13 directly predicted a blunted heart rate response and indirectly predicted blunted respiratory sinus arrhythmia (RSA) reactivity under stress in adulthood (Loeb et al., 2021). Blunted reactivity has been prospectively linked not only to mental health difficulties, but to poorer physical health outcomes, such as a risk of obesity, depression and anxiety, poor lung function, poor cognitive function, poorer self-reported health, and death from cardiac events. Heart rate effects were in turn mediated via indicators of a developing passive response style, assessed via observational measures of withdrawal during conflict with friends and romantic partners, and via social disengagement and use of denial strategies. These findings are interpreted as yet more evidence of a critical mediational chain in which a key social/developmental stressor in adolescence predicts problematic relationship qualities, which in turn can account for physiological patterns of stress responding in adulthood.

Ultimately, we hypothesized that difficulties in social relationships established in adolescence would predict broader patterns of physiological dysfunction leading to *accelerated aging*. We examine aging using patterns of epigenetic change that can be composited to yield a measure of epigenetic age that has now been linked to a broad range of health indicators, including early mortality, time-to-heart disease, and time-to-cancer, even after accounting for chronological age and current physical health (Lu et al., 2019). We then were able to identify three factors that predicted advanced epigenetic age at 30, even after accounting for chronological age, gender, race, income, and history of cigarette smoking: observed difficulty establishing close friendships characterized by autonomy and relatedness from ages 13 to 18, an interview-assessed attachment state of mind lacking autonomy and valuing of attachment at 24 (i.e., attachment insecurity), and self-reported difficulties in social integration across adolescence and adulthood (see Figure below) (Allen et al., in press). Analyses assessing the unique and combined effects of these factors, along with lifetime history of cigarette smoking, indicated that each of these factors, except for social integration in adulthood, contributed uniquely to explaining epigenetic age acceleration. **These results provide some of the strongest evidence to date that the quality of peer relationships beginning in adolescence is likely to be strongly tied to long-term markers of health and aging in adulthood**. Notably, these data also provide specific evidence as to *how* this aging process may unfold, with peer relationship quality having its predictive effects partially mediated via a marker of internalized autonomy expectations and valuing of attachment. The results also provide strong support for the primary framework this study has used in investigating social relationships: a focus on

adolescents' developing capacity to establish their autonomy in the context of a positive, connected relationship.



In addition, although not a primary focus of our original Aims, our intensive repeated measurements of substance use also allowed us to identify a pattern in which heavy marijuana use predicted advanced epigenetic age, with corollary analyses suggesting that effects are likely due to the effects of inhaling marijuana smoke (Allen et al., 2022), a finding expected to add important new information to the ongoing debate about legalization of marijuana use.

Finally, we have also begun exploring specific mechanisms within the brain that can help explain the links between social relationships and physical health. Using fMRI data obtained from our participants, we find that regulation in the hypothalamus during a handholding paradigm was linked to reports of general health, thus **identifying a specific brain mechanism by which relationship quality may translate into physical health outcomes** (Brown, Beckes, Allen, & Coan, 2017). Similarly, we have found via fMRI data that the physical presence of relational partners decreases blood-oxygen-level-dependent (BOLD) response to threat in key targets of resource conservation (e.g., dlPFC, dACC, and insula) and that stronger signal reduction in these areas coincides with less BOLD response in pre-frontal (vmPFC, dlPFC) and visuo-sensory integration (occipital cortex, precuneus, superior parietal lobule) regions during a virtual experience of social rejection. We then show that these neural relationships are associated with less use of self-regulation-based coping strategies *two years* post-scanning. Taken together, these findings suggest promising mechanisms by which allowing oneself to rely upon others in close relationships potentially alters fundamental aspects of brain activity in ways that are linked to coping and health outcomes (Gonzalez, Coppola, Allen, & Coan, in press).

Overall, this combination of findings provides strong support for our future directions as we now propose (Aim 1a) to obtain additional physiological data to allow analyses of specific disease processes and aging outcomes with direct implications for population health.

Environments & Context

We have also been taking advantage of our unique combination of intensive psychosocial and contextual assessments, physiological data, and epigenetic data to better understand the ways in which harsh environments potentially alter development. In a recent paper, we find that in socioeconomically harsh

environments, greater levels of methylation of oxytocin receptor genes dampens the activity of those genes in a way that decreases the link between these environments and an individual's reward sensitivity (Gonzalez, Wroblewski, Allen, Coan, & Connelly, 2021). One explanation is that dampening via methylation of oxytocin receptor genes (reducing the availability of oxytocin in the brain) may be a helpful response *within* harsh environments because it reduces sensitivity to the harshness of the environment. However, other research suggests this dampening is likely to be highly problematic in other ways, given the role of oxytocin in cementing key aspects of close human relationships (Hurlemann & Scheele, 2016).

In a second contextually focused study, we examined links between family-of-origin socioeconomic status (SES) and stress responses in adulthood. Low family SES at age 13 directly predicted blunted heart rate responding and fewer attempts to answer math problems during a modified version of the Trier social stress task at age 29 (Loeb et al., 2022). Indirect effects were found from low family SES to blunted respiratory sinus arrythmia (RSA) responding and the number of words spoken during a speech task. Reduced social support from romantic partners from ages 18-24 and SES at age 29 mediated many of these relations. Findings held after accounting for a number of potential confounds, including baseline social and academic functioning and body mass index. We interpret these findings as identifying specific mechanisms by which low SES may alter adolescents' current and future social behaviors to predict long-term changes in physiological stress response patterns.

Translational Work

Our extensive and growing findings on the importance of adolescent-era social relationships in both the short and the long term has led us to engage in several significant translational efforts. Specifically, we have been developing interventions designed using the findings and insights described above to improve social relationship qualities among adolescents. *The Connection Project* was designed to facilitate small groups of high school students in becoming social supports for one another. The program is low-cost and can be implemented in a single semester of once weekly meetings within a regular class period. Implemented (with additional support from the William T. Grant Foundation) in a randomized design in schools serving primarily economically and racially/ethnically marginalized youth, the project has now been shown not only to enhance relationship qualities (independently rated), but also to reduce levels of depressive symptoms and increase academic engagement among participating youth (Allen, Narr, Nagel, Costello, & Guskin, 2021). Our partner in this project, Wyman of St. Louis, has since received several sizeable grants to roll out the high school version of the program to its national network of providers. More recently, we have translated the program to serve entering college students and also found significant effects in randomized controlled trials in reducing depressive symptoms and loneliness and enhancing a sense of belonging (Costello, Nagel, Hunt, & Allen, in press; Costello, Nagel, Hunt, Rivens, et al., in press). Notably, **positive program effects in both the high school and college versions of the program have been found to be strongest among marginalized and underserved groups**. We have now received more than $1.3 million in grants to support further development of the college version of this program and are working to scale it while retaining its impact.

Within adulthood and in a medical context, we have also developed and piloted a brief psychosocial intervention designed to enhance connection and belonging between doctors and patients on inpatient units and found this less than 5-minute intervention to increase patient satisfaction with their medical experience at the end of their hospital stay (Pace et al., 2017).

Implications

The implications of these findings are becoming increasingly clear: The intense adolescent preoccupation with peer relationships, rather than being a quirk of this stage of the lifespan, appears to reflect a fundamental and biologically adaptive attunement to a social domain with huge long-term implications for health and adjustment. Our findings suggest a range of new entry points for pediatricians, educators, policymakers, and parents assessing potential behaviorally linked risks to future health. Based on our findings to date, intervention approaches that directly target the quality of adolescents' social relationships warrant increased consideration, not just for their immediate effects on adolescent well-being, but for their potential long-term implications for healthy aging. These findings also suggest that parents trying to assess how their adolescent is faring should give far greater weight to the quality of their teen's ongoing peer relationships. Overall, these findings add growing urgency and substantial empirical support to calls to place a greater priority on understanding and ultimately enhancing processes of developing high-quality lifelong social connections as a way to understand and enhance key health outcomes (Holt-Lunstad, Robles, & Sbarra, 2017).

ABSTRACT OF RESEARCH PLAN

We propose to dramatically extend and deepen our assessment of the overarching hypothesis that social relationship qualities established in adolescence have integral, long-term relations to life course physical and mental health and to broader aging processes. We will add **multiple new panels of longitudinal data on biological aging, mental health, and peer, romantic partner, and family relationship qualities** obtained from a unique, socio-demographically diverse final sample of 170 individuals (93% of our original sample), followed across a *30-year span* from age 13 to midlife (ages 38 – 42) to address 4 overarching aims:

Aim 1: Predict Broad Aging Processes, as Well as Specific Functional Outcomes. We previously examined adolescent experiences of hostile conflict and lack of supportive relationships as predictors of poor physical health *before* major health problems had emerged, using proxies such as epigenetic aging. We now propose to expand and extend this examination to identify adolescent-era predictors of:

Aim 1a: Advanced Physical Health and Disease Processes. We propose to **examine direct and mediated predictions to *actual* disease processes**, including *metabolic syndrome, cardiovascular disease,* and *chronic pain,* among others. In addition, we will examine newly enhanced markers of epigenetic aging (e.g., Dunedin Pace of Aging Measure-revised) and additional physiological markers of health (e.g., Tumor Necrosis Factor α and Interleukin-1β), while continuing our ongoing panel of assessments. We will thus be able to identify key social factors and mediating physiological processes that predict serious illness.

Aim 1b: Lifespan Development of Caregiving Capacity. Utilizing new data (see below), we will examine the adolescent and early adult roots of capacity to support and provide care to others as these manifest in: a) adult parenting roles—**permitting a *three*-generation assessment**; b) adult caregiving of aging parents; and c) adult romantic relationships. In addition, we will examine conditions under which caregiving experiences are linked not only to concurrent relationship quality but also to the *caregiver's* physical and mental health.

Aim 1c: Capacity to Cope with Prolonged Social Disruption. Covid has changed the quantity and quality of social interactions and relationships over the past two years, affecting both mental and physical health. We will use new panels of data to assess the hypothesis that capacity to cope with this disruption will be predictable from patterns of social development and adaptation first established in adolescent relationships and extending into adulthood. We will test an amplification hypothesis positing that the stress of Covid-related disruptions will be minimized among those with a history of positive relationships, while it will place those with a less positive history at greater risk. We will examine *change* in mental and physical health pre- and post-Covid, not simply as a means of tracking Covid-related impacts, but as **a unique natural experiment regarding the ways in which prior relationship experiences set people up to cope well or poorly with unexpected social stressors**. We will also consider the role of Covid vaccination status and illness.

Aims 2 - 4: Mediational vs. Weathering Explanations of Links to Early Midlife Health & Aging Outcomes; Mediation via Mental Health and Health/Risk Behaviors; and Biologic & Contextual Moderation and Mediation. Carefully following the same approach we outlined for these Aims in our original proposal, mediating and moderating processes will be explored in relation to the new hypotheses addressed in Aim 1, allowing assessment of direct vs. mediated links to actual disease processes, providing insight into factors mediating continuities in caregiving practices as these extend across three generations, and permitting assessment of contextual and mental health factors moderating the impact of social stressors on health.

To pursue these expanded Aims, we will obtain the following new data (with data collection spaced so as to minimize participant burden and maintain our extremely high participant retention rate):

1) an additional epigenetic assessment (to track changes in markers of aging, as well as methylation of specific relevant genes (e.g., the oxytocin receptor gene));
2) two additional extended physical health assessments (including both repeats of prior assessments and collection of new data (e.g., additional cytokines, assessment of pain and functional limitations));
3) two observations of romantic partner interactions, along with participant and romantic partner reports;
4) two self-report assessments of social functioning, mental health, and physical health from both our participants and their closest friend;
5) parental caregiving assessments (obtained previously for participants with children ages 2-8; the next 5 years will permit nearly complete collection as the sample nears the end of primary childbearing years);
6) coding of observed empathic and caregiving capacity from a video-recorded task in which participants provide support to close friends and romantic partners. Participants have been repeatedly observed in this task across multiple years and relationships, but we have not previously requested funding for coding.

Ultimately, the proposed work will allow us to generate potentially groundbreaking knowledge regarding the existence and nature of paths from adolescent relationship difficulties to lifespan markers of health and aging.

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