Social Relationship Qualities as Predictors of Health & Aging from Adolescence through Early Midlife

NIH R-01 (MERIT) Award

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2. SPECIFIC AIMS

Public health efforts in adolescence targeting life course health outcomes have focused almost exclusively on physiological risk factors and health behaviors (e.g., body weight, smoking, etc.). This proposal examines a far-reaching hypothesis with the potential to open up new venues for intervention: that *social relationship qualities established in adolescence have an integral, long-term relationship to both life course physical health and to broader aging processes*. We propose to clarify both the existence and the mechanisms by which two specific relationship difficulties in adolescence—experience of hostile conflict and absence of supportive relationships—are linked to early midlife health and aging outcomes. We use uniquely rich longitudinal data on peer, romantic partner, and family relationship qualities across a 25-year span to address 4 overarching aims:

Aim 1: Direct Prediction of Early Midlife Health and Aging from Adolescent Relationship Difficulties. We examine adolescent experiences of ***hostile conflict*** and ***lack of supportive relationships*** as predictors of problematic health and aging processes, which we will capture via highly sensitive ***epigenetic aging assessments*** and ***biomarkers of physiological age***. Given that adolescence is typically marked by relatively few chronic health problems, identifying relationship qualities during this stage that serve as risk factors for future, long-term physical health outcomes is critical to guiding both routine screening practices and preventive efforts. Our approach builds on promising initial findings of links between adolescent relationship qualities and several specific physiological indicators of health in the mid-twenties. We now extend our age window, but most importantly, we consider the ways that adolescent relationship qualities predict more fundamental long-term health and aging processes, which we will robustly assess across a 5-year span in early midlife.

Aim 2: Mediational vs. Weathering Explanations of Links to Early Midlife Health & Aging Outcomes. We follow up our analyses in Aim 1 by addressing questions critical to understanding (and developing strategies for interrupting) observed long-term links between social difficulties and health and aging outcomes: *When* and *how* do the health and aging effects of these difficulties accrue over the life course? We utilize repeated measures both prior to and within the early midlife period to consider ***mediational explanations,*** in which hostile conflict and lack of support serve as early links in chains of risk that lead to problematic health and aging via more proximal mediators (e.g., cascading patterns of relationship difficulty later in life); and ***weathering explanations,*** in which hostility and lack of social support in adolescence and early adulthood display direct, long-term links to early midlife aging processes, even if these earlier difficulties later resolve.

Aim 3: Mediation via Mental Health and Health/Risk Behaviors. We examine mental health and health/risk behaviors that may partly account for the links from social difficulties to health and aging outcomes identified in Aims 1 and 2. We assess the extent to which mental health symptoms and health/risk behaviors mediate the effects of prior relationship difficulties on early midlife outcomes and/or independently contribute to explaining these outcomes. We also assess the dynamic interplay over time of mental health and health/risk behaviors with social relationship difficulties as these work together to predict health and aging outcomes.

Aim 4: Biologic & Contextual Moderation and Mediation. For all analyses in Aims 1 - 3, we consider the ways in which key demographic, biologic and contextual factors (e.g., race/ethnicity, SES, ❻ sex, ❸ parenting experience, ❺ early childhood stressors, etc.) may explain either continuity or discontinuity in predictions to health from prior periods. We assess both mediated and moderated relationships as conceptually appropriate.

We address these Aims with a uniquely intense combination of repeated direct observations of participants' interactions with parents, peers, and romantic partners, along with interviews and sociometric assessments, to which we now add an array of highly sensitive physiological indicators of health and aging outcomes. Assessments are obtained from a socio-demographically diverse final sample of 172 individuals (with 97% sample retention to date), followed *annually* across a 25-year span from age 13 to early midlife (ages 33 – 37).

The richness and temporal density of relationship data from this sample allows for a powerful assessment of previously unexplored questions regarding the adolescent relational roots of critical physical health outcomes ❶ *just as these outcomes are becoming increasingly salient in early midlife, but before most major health problems have emerged.* The proposed study has significance in allowing us to: a. identify entirely new arenas within adolescence that can be targeted by interventions to promote lifelong health; b. suggest *specific* relational characteristics to target in screening and preventive interventions; c. distinguish processes in adolescence that directly predict problematic aging (and are thus critical to address within adolescence) from those that lead to mediated chains of risk (which would suggest multiple promising points of intervention); and d. dramatically advance our knowledge regarding the nature and existence of paths from adolescent relationship difficulties to physical health and to fundamental aging processes into early midlife.

3. RESEARCH STRATEGY

SIGNIFICANCE

This proposal builds from the growing recognition that even in terms of *physical* health, humans are fundamentally *social* animals. Within adulthood, social isolation is now linked to a risk for early mortality as great as the risk created by cigarette smoking or obesity, and hostility in social interactions has been consistently linked to disease outcomes ranging from cardiovascular and metabolic disease to high blood pressure and cancer[5-9]. Although relationship correlates of health within adulthood have been identified, the formative period from adolescence through early midlife, *in which key relationship patterns are being established,* has received virtually no attention.

Most importantly, identifying relational precursors to long-term health and aging processes *in adolescence* opens up a range of potential entry points for intervention to improve life course health—ranging from modifications to social characteristics of secondary schools to interventions that directly target adolescents and young adults at-risk for relationship dysfunction. *Currently, however, national recommendations from the primary government agencies tasked with the prevention of age-related disease fail to consider any social relationship factors in adolescence or early adulthood*[10-12]. Recognizing that our efforts to enhance life course health outcomes may be overlooking an entire realm ripe for intervention is critical to helping our society make wise decisions about both whether and how to allocate scarce prevention resources to this area.

Rationale for Assessing Relationship Difficulties in Adolescence as Predictors of Adult Health and Aging Outcomes

Social isolation and lack of social support have now been concurrently linked to problematic cardiovascular functioning, sleep quality, nutrition quality, body mass index, and immune and metabolic functioning[13-20]. The condition of lacking social support is disturbingly common: One in four adults in the U.S. report such severe isolation that they have no one with whom to discuss important issues in their lives[21]. Similarly, hostile conflict in social relationships has long been recognized as a risk for a range of health difficulties including cardiovascular disease, high blood pressure, problematic immune functioning, and pathological inflammation processes linked to problematic aging[6-9, 22, 23]. Notably, negative aspects of social relationships, such as chronic hostile conflict, are often largely independent of positive aspects such as social support[24], thus suggesting likely independent contributions of each of these factors to health outcomes.

Lack of support and experience of hostile conflict are believed to primarily operate by creating chronic stress that in turn affects lower level biological processes ranging from respiratory sinus arrhythmia[25] to levels of inflammation and immune system functioning (e.g., C-reactive protein (CRP) and Interleukin-6 (IL-6) levels)[26, 27], over time leading to pathophysiological changes across multiple organ systems[28]. Several suggestive studies provide evidence of links between adolescent social difficulties and future health outcomes. Lower maternal support during adolescence, for example, has been found to predict cardiovascular disease risk into young adulthood, with effects partially mediated via young adult health behavior and financial stress[29]. Three studies to date have reported long-term links from adolescent peer relationship qualities to specific physiological indicators of adult health. Analyses of ADD Health data find that poor social integration in adolescence predicts higher levels of inflammation at age 28[30]. Exposure to harsh parenting at age 11 has been linked to accelerated epigenetic aging in late adolescence[31]. Finally, experiencing intimate partner violence in late adolescence, as either perpetrator or victim, has been found to predict future cardiovascular disease risk[32]. Encouragingly, this latter effect appeared somewhat remediable via targeted intervention[31].

Although these socially-focused studies assessed narrow outcome indicators using only single point in time assessments as predictors, they provide tantalizing evidence of links from prior lack of social support and experience of hostile conflict to future health outcomes. These studies do not, however, begin to assess predictions to more fundamental aging processes, nor do they identify the specific relational processes involved, the importance of their timing (e.g., onset, desistance), their chronicity, and potential mediation via continuities with later behavioral and relationship qualities—all critical issues for targeting prevention efforts.

In addition to preliminary findings of direct links to future health indicators, a rapidly growing body of evidence suggests that struggles with hostility and lack of support in parent and peer relationships in adolescence also predict *future* peer and romantic relationship struggles, at least into the early- and mid-twenties[33-39] (also see Progress Report). Even on a neural level, time spent with friends in adolescence appears linked to future sensitivity to rejection in peer relationships[40]. These young adult relationship struggles, in turn, are precisely

the types that have been linked to problematic health outcomes *within* adulthood in the studies cited above. We have only scant evidence, however, to address the critical question of whether these problematic relationship continuities actually extend from adolescence into early midlife and mediate health outcomes.

Stress and Accelerated Aging

Although there is growing evidence linking social stressors to *discrete* physiological outcomes, focusing only on discrete outcomes risks overlooking a more fundamental and far reaching possibility: *Over time, chronic relational stress may accelerate the very pace at which humans age in ways that predict future mortality*[41-44]. The concept of accelerated aging captures a variety of disease-related processes that result from incremental damage to organ systems across the life course[45]. The 2013 NIH-Advances in Geroscience conference concluded that a top priority should be addressing the *pace* of aging as it increasingly appears to be the *"root cause of virtually all of the major diseases causing lifespan morbidity and mortality"* [46-48]. We capitalize on two major recent advances in aging research—epigenetic aging assessments and multi-variable approaches to assessing physiological aging. These approaches now allow us to examine the ways in which relationship difficulties may help explain fundamental aging processes and they provide a far more sensitive means to measure the outcomes of such relationship difficulties than reliance upon isolated physiological indicators.

Epigenetic Aging

A growing body of research is now suggesting that many of the long-term effects of stress on health are mediated via epigenetic changes to our DNA. Under conditions of chronic stress, neuroendocrine changes induce changes in methylation levels in DNA—in some cases permanently altering gene expression in ways that lead to future disease processes[49]. Most importantly, reliable techniques for assessing these changes from peripheral blood cell analyses are now available (e.g., the Illumina 850K methylome array). Cumulative methylation changes can now be assessed across the epigenome and an 'epigenetic age' for individuals calculated that correlates strongly with chronological age[50]. The deviation between the two—a marker of accelerated epigenetic aging[51, 52]—has now been linked to age-related disease processes ranging from cancer[53, 54], and metabolic illnesses[55], to future all-cause mortality[56, 57]. These links remain even after accounting for multiple potential confounding factors (e.g., BMI, substance use, etc.). Most importantly, epigenetic age is beginning to be linked to crude measures of concurrent and lifetime stress (e.g., retrospective life event measures and poverty assessments)[58-60]. ❹ With the exception of the one within-adolescence study cited above[31], however, research to date has yet to consider the role of any relationship stressors beyond early childhood as predictors of epigenetic aging.

Physiological Age

We also propose to use a recently developed, state-of-the-art, multi-variable approach for assessing physiological age, the Klemera-Doubal method, which has been shown to predict long-term mortality risk over and above measures of chronological age and multiple discrete risk factors[61, 62]. This approach combines multiple face valid markers of biological status that have been previously linked to social stressors (e.g., levels of inflammation, cholesterol, etc.) and has now been validated repeatedly[62, 63] in samples similar to ours[64]. [We also consider some of these face valid markers independently (e.g., cardiovascular disease risk, metabolic syndrome) as outcomes in secondary analyses.] ❹ As with epigenetic aging, in spite of the promise of this construct to more powerfully capture the multiple effects of adolescent and adult relational difficulties, research has yet to explore the ways in which these relationship difficulties are linked to fundamental aging processes.

Timing and Pathways

❶ Our assessment age range lies at a critical juncture: just as the incidence of several health risk factors (e.g., obesity, insulin resistance[65]) has begun to rise rapidly and others are about to jump precipitously (e.g., the percentage of individuals with sub-ideal cardiovascular health markers[66]). We now know that significant differences in both aging and the pace of aging have appeared by the mid-thirties[64, 67]. Equally importantly, to address accelerated aging requires understanding (and ultimately intervening to address) it *early* enough in the lifespan to prevent its occurrence. Further, there is no empirical basis to presume that predictors of aging later in life (as later life health problems begin to recursively influence aging processes) will be the same as those from adolescence into the thirties[67], further suggesting the importance of assessment in early midlife.

❹ In addition, as the science of aging increasingly takes on a life-course perspective[68, 69], a significant limitation to even the small body of extant work on relational difficulties as predictors of future health and aging is that it has not yet begun to distinguish between effects of contemporaneous vs. cumulative/chronic social

difficulties[70]. Said differently, it likely matters not just *whether* adolescent relational difficulties sow the seeds for later aging problems and *which* difficulties do so, but also *how* these processes unfold[71]. As outlined below, we address these questions via our four key Aims, assessing multiple potential pathways from adolescent social difficulties to adult health.

Aim 1: Direct Prediction of Early Midlife Health & Aging from Adolescent Relationship Difficulties. We begin by assessing simple long-term linkages from specific adolescent relationship difficulties to aspects of problematic midlife aging (path "**1**" in Figure 1). This both sets the stage for research exploring the nature of these links in Aims 2-4, and takes a major step toward spotlighting adolescent relationships as potential points of screening and intervention relevant to long-term physical health and fundamental aging processes.

For both social and physiological reasons, *adolescence* is likely to be a particularly important period in understanding life course relationship-health linkages. Socially, adolescence is well-recognized as an intensely stressful stage requiring management of new, rapidly changing, and increasingly intense social relationships, and characterized by the presence of the highest levels of negative affect of any era in the lifespan[72]. In addition, as brain development allows growing capacity for perspective taking, and psychosocial development allows greater autonomy, adolescence becomes the first point in the lifespan in which truly adult-like relationships begin to form and become sufficiently salient to serve as direct templates for future social relationships[73]. Adolescents also appear particularly neurologically and hormonally primed to absorb lessons (positive and negative) from these new relationship experiences[74].

Physiologically, chronic stress exposure in adolescence also has the potential to alter still developing anatomical structures and metabolic systems in a relatively permanent way[45]. The hippocampus, amygdala, and prefrontal cortex are all still actively maturing and have been shown to undergo structural remodeling under conditions of chronic stress, in turn altering subsequent behavioral and physiological responses[75]. Evidence also suggests that with growing brain functional connectivity across this period, adolescence may be a 'switch point' for the calibration of stress responsivity[75-77].

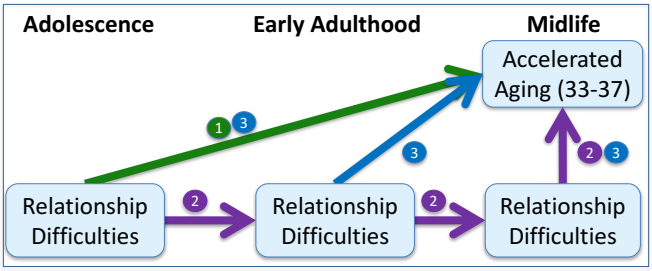

Figure 1 - Conceptual Model for Aims 1 and 2

The potential for stressful experiences in adolescence to permanently alter physiological development going forward is consistent with *weathering theory*, which suggests that an organism can take a health 'hit' from the experience of chronic stress at a sensitive point in the lifespan, *even if that stress subsequently subsides*[78]. Regardless of whether adolescent relationship qualities are predictable from prior experience or extend into adulthood, identifying adolescent-to-early-midlife health and aging links would indicate the importance of intervening *within* adolescence. To the extent that adolescent-adult aging links are direct (i.e., not found to be mediated via future relational or psychological processes), adolescence becomes not simply a *potential* point of intervention, but a *critical* point for intervention.

Aim 2: Mediational vs. Weathering Explanations of Links to Early Midlife Health & Aging Outcomes. Identifying long-term links from adolescent relationship difficulties to midlife aging is an important first step, but it then raises the critical question: By what pathways do these linkages become manifest[71]? Adolescents' first adult-like relationships are often highly stressful as noted above, but equally importantly, they begin to establish a template for future relationships. Conflict management styles established with parents, for example, appear to cascade forward with development, predicting qualities of later close peer relationships, which in turn predict qualities of romantic relationships and even of new relationships as parents to one's offspring[25, 79, 80]. Adolescent relationship difficulties may thus set in motion cascading chains of risk, which if unmodified, will go on to impair long term outcomes[45]. A *mediational perspective* (paths "**2**" in Figure 1 above) thus suggests that long-term effects of chronic adolescent stress exist because early social difficulties sow the seeds of similar difficulties going forward, which in turn affect health and aging outcomes[45]. In contrast, a *weathering perspective* (paths "**3**" in Figure 1) suggests effects from social difficulties at various points in the lifespan will each exert an *independent and cumulative* influence on future aging, regardless of what experiences later follow[81]. We examine each of these possibilities, employing longitudinal data through early midlife and cross-lagged assessments within midlife to distinguish among key causal pathways.

Aim 3: Mediation via Mental Health and Health/risk Behaviors. Although our primary focus is on social relationship difficulties as predictors, we examine these in the context of two other potentially powerful predictors of midlife aging: *mental health symptoms* and *health/risk behaviors*. A history of adolescent

depression and antisocial behavior is linked to relationship difficulties and has been found to predict health difficulties into the early twenties[82-86], and in one study to age 32[87]. Similarly, health behaviors (e.g., diet and exercise, risky behavior, substance use) have clear and widely recognized links to health[88]. Although these are not the primary focus of our investigation, their inclusion may be critical for correct model specification. We thus consider internalizing and externalizing symptoms and health/risk behaviors not only as covariates and potential confounds, but more substantively as having potential transactional interplay with social difficulties and aging processes over time. Figure 2 illustrates *one* possible form of this interplay (paths "**1**" and "**2**"), with relationship difficulties predicting mental health symptoms, which in turn predict aging. We also consider the complementary paths (from mental health symptoms to relationship difficulties to aging), and apply an analogous approach to examining the role of health/risk behaviors.

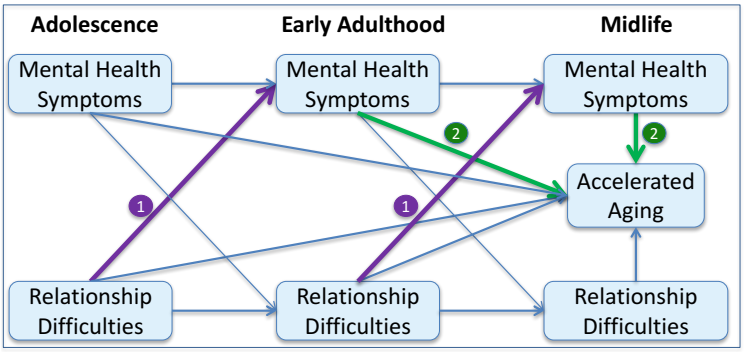

Figure 2 – Potential Interplay of Social Difficulty and Mental Health

Aim 4: Contextual Moderation and Mediation. We consider key demographic, biologic and contextual factors (e.g., SES, race/ethnicity, ⑥ sex, ③ entry into parenthood, ⑤ history of early childhood stress, etc.) that may alter observed patterns of continuity or discontinuity. Interactionist and convoy models of human development suggest that these factors may both reflect and influence social functioning[89-95]. ⑥ For example, although existing literature suggests little to no sex/gender moderation for predictions from social isolation to health[96], it suggests small moderating effects with respect to conflict and health (i.e., females are more sensitive to conflict)[97]. Current research suggests that it is not biological sex, however, but socially-determined gender roles and social orientation that likely carry this relationship[97, 98]. We will assess these processes directly as potentially accounting for any observed moderation by sex. Similarly, we consider effects of poverty, entry into marriage or ③ parenthood, ⑤ history of early childhood adverse experiences, and race/ethnicity, as each has been linked to elements of psychosocial functioning and to key health outcomes[99, 100 59, 101-104]. We routinely consider these factors as potential covariates and, although prior research only inconsistently finds evidence of moderating effects and does not yet provide a basis for strong *a priori* moderation hypotheses, we also carefully examine such potential moderating effects in our models.

④ INNOVATION

- Although early life stressor effects on health are an increasing target of study, extensive evidence suggests that *beyond* childhood, ongoing social relationships potentially carry enormous weight in explaining health and aging outcomes. <u>The proposed study will be the first to examine these relationship qualities as precursors of long-term health and aging outcomes, and it begins just as the templates for these qualities are being established in adolescence</u>. These are also precisely the type of early relationship qualities that can serve as targets of prevention and early intervention efforts.
- <u>This study uses annual, multi-method relationship assessments from 13 thru 37 to obtain what is arguably the most fine-grained available data on relationship experience across this period</u>. Collecting *both* multi-method and closely spaced data (in contrast to studies that do one or the other) is critical in this case, as the social processes being assessed are often discontinuous, subtle, and in domains in which self-reports are considered least likely to be reliable[105] (e.g., intense relationship conflict may occur only intermittently; hostile individuals often do not recognize their hostility; rejection by peers is difficult to acknowledge; and others' reports of social interaction qualities appear more sensitive to health effects than self-reports[106, 107]).
- This study will examine these relationship stressors not simply in the prediction of specific health indicators, but <u>as predictors of the fundamental pace of human aging</u>—which is increasingly recognized as a primary causal agent in multiple disease processes. [The one ongoing study using similar health measures[1] employs a demographically restricted sample, but more importantly, does not focus on adolescent and adult relational processes as predictors and thus has only brief, widely spaced relationship assessments beyond childhood and no observational data (e.g., see Moffitt consultant letter).]
- This study will <u>distinguish effects of two critical facets of relationships (lack of support and presence of hostility</u>), each of which would lead to quite different intervention approaches.

- This study will be the first study able to examine the role of time course of relationship stressor effects beyond childhood on health and fundamental aging outcomes, including assessment of sensitive periods (e.g., early adolescence), weathering effects, mediational processes, and the role of chronicity of stressors.
- This study will use repeated measures of health and aging processes *within adulthood* to allow for strong assessment of potential causal pathways and the ability to rule out several reverse causal hypotheses.
- This study will do all of this with a generalizable, demographically diverse sample with 3% total attrition, and a team that has shown it can use this design to generate robust findings with key outcomes.

Together, these innovations potentially identify multiple new avenues for health-focused preventive screening and intervention that have thus far been overlooked. They also help identify *when* such interventions should best be targeted, *which* specific relationship processes should be targeted, and *how* these processes play out to influence both specific life course health outcomes and even more fundamental aging processes. Although the proposed study is not without its limits, to replicate these innovations and gain the ensuing knowledge from scratch would likely require several more decades of time at a cost of tens of millions of dollars.

PROGRESS REPORT (Period since last competitive review: 10/25/12 – 10/25/17)

We began 19 years ago with a sample of 184 target adolescents and have retained 97% over the past 5 years as active participants in the study. We approach dissemination by focusing upon producing broad, high-impact, empirical papers in top-tier journals (e.g., *Psychological Science, Child Development, Journal of Abnormal Psychology*, etc.). In the past 5 years, this grant has yielded over 130 publications, presentations, dissertations, and submitted manuscripts. These include: **52 papers (46 in peer-reviewed journals) plus an additional 8 papers under review; 5 doctoral theses; and 66 presentations at national conferences.**

The current wave of the study was designed to identify links from adolescent relationship factors to several specific aspects of health (both self-reported and biologically assessed) at ages 28-32. Results thus far, outlined below, have established our ability to successfully identify adolescent-era relationship predictors of key physiological outcomes in this sample—supporting the likelihood of our finding links to broader aging outcomes in midlife. These results have also focused our proposed efforts on the two key relationship factors of hostile conflict and lack of social support. All findings below avoid methods confounds by linking constructs assessed via different methods; demographic covariates and moderation are always considered.

Findings re: Adolescent-era Relationship Predictors of Adult Health Indicators

- ***Self-rated global health*** at age 27 is predicted by close friendship quality at age 13 and by a non-confrontational stance toward peers (assessed via peer reports; *Multiple R* = .29***) even after accounting for baseline health problems, BMI, concurrent anxiety and depression, and Big Five personality factors[108].
- Higher levels of ***interleukin-6*** at age 28, a marker of immune system dysfunction, are predicted across periods as long as 15 years by inability to defuse peer aggression, poor peer-rated conflict resolution skill, and independently observed romantic partner hostility in late adolescence, again after accounting for BMI, prior physical illnesses and demographic covariates (*Multiple R* = .39***)[109].
- ***BMI*** in emerging adulthood is predicted by lack of observed maternal supportive behavior (β = *.29***) at age 13, with effects partially mediated via peer relationship qualities (Model *Multiple R* = .53***).

Levels of ***C-reactive protein,*** a marker of inflammation linked to future cardiovascular disease, are predicted by observed angry conflict with mothers in late adolescence (β =.28**). **Findings re: Physiological Responses to Social Support and Conflictual Interactions**

- Change in ***respiratory sinus arrhythmia*** in stressful situations in adulthood (a marker of flexible responding to stress) is predicted by sociometrically assessed peer preference at ages 13-15, and in turn is linked to self-reported global health at age 26[110].
- Associations between social support and health are partly mediated through the social regulation of **hypothalamic sensitivity to threat**[111].
- Quality of ***sleep*** at age 28 is predicted by negativity with romantic partners at age 21 (β = *.29***), after accounting for demographic controls; negativity, in turn is predicted by prior peer interaction qualities.
- Higher levels of ***methylation of the oxytocin receptor gene*** at age 25 are predicted by lack of positive expectations of peer and parent support in early adolescence and in turn are linked to higher levels of adult loneliness and internalizing symptoms[112].

Findings re: Cascading Long-term Patterns of Psychosocial Function and Dysfunction

- Negative relationship expectations early in adolescence predict ***relationship difficulties up through age***

25[113], with similar decade-long predictions observed from early adolescent attachment insecurity[114].

- Relationship difficulties with parents and peers beginning early in adolescence also cascade forward to predict later close relationship and ultimately ***romantic relationship qualities*** in the early twenties[79, 115].
- Difficulty managing conflict with peers at age 13 predicts ***social withdrawal and lower close friendship competence*** at age 21[116]. Conversely, openness to support seeking in adolescence predicts functional independence at age 25[117], and close friendship competence (but not broad popularity) in early adolescence predicts relative decreases in levels of anxiety and depression by age 25[118].
- ***Alcohol problems*** from adolescence up through early adulthood are predicted by early peer experiences,[119] a finding now being in part replicated by other labs[120].

Findings re: Interplay of Mental Health and Contextual Factors

- Factors that interfere with peer connections in early adolescence, (e.g., high levels of depressive symptoms) have been identified as remarkably strong predictors (i.e., β = .54***) of ***perceived isolation*** at age 24, even after accounting for concurrent symptoms and baseline levels of social functioning[121].
- In terms of socio-demographic factors, poor neighborhood quality in adolescence has been found to alter ***neural responding*** (dorsal ACC and right insula activity) in response to social exclusion tasks[122].

Translations and Applications. We have used our adolescent social relationship findings to develop one of the first socially focused, cross-subject-matter teacher training approaches found to significantly raise actual achievement test scores in secondary students, with results published in *Science*[123]. Our *Autonomy and Relatedness Coding System* for observing parent, peer and romantic partner interactions has now been translated into both German and Italian[124] and has been used by more than fifteen other labs internationally; our *Supportive Behavior Task* has been used in the follow-up of the NICHD Early Childcare Study; and our growing health focus has led to the development of a brief, socially-focused intervention found to improve satisfaction during inpatient hospital stays[125]. Our data set has also attracted other researchers to utilize our sample. This includes separately funded work finding that social isolation is related to fMRI-assessed threat reactivity[126] and that socially anxious individuals experience altered neural reward processing[127], potentially affecting future ability to seek social support. Other research using this sample has found that implicit attitudes toward social rejection predict future social anxiety and problems in romantic relationships[128, 129].

Overall, these findings suggest both the value of the constructs being examined and the power of our design to yield robust, sizeable effects regarding long-term relationship links to health outcomes.

APPROACH

Design

Intensive Sampling Approach. The field has long recognized the limitations of reliance on self-reports of social interaction qualities[105] as well as the degree to which these qualities may vary significantly across both time and context. Our approach to capturing these critical yet elusive social phenomena utilizes not only numerous collateral reporters and relationship assessments but also a uniquely intensive repeated measures approach that assesses relationship qualities 4 *each year* as development unfolds from adolescence into midlife. The proposed study will obtain new multi-method, multi-reporter data from a final sample of 172 former adolescents, their close friends, and romantic partners followed previously from ages 13 to 32 and now from ages 33 to 37.

We began with a sample of 184 adolescents (mean age = 13.4), along with their parents and peers, recruited from a seventh and eighth grade public middle school that served the entire population of a demographically diverse small Mid-Atlantic city. We initially recruited adolescents and families via a mailing followed by phone contact. We had a 63% acceptance rate from teens approached to participate, which is high for studies of this intensity (i.e. initial assessments required 6 hours of in-person interview time). The sample was demographically heterogeneous, including 30% African-American, 8% Mixed-race, and 2% Hispanic-American participants and 33% from families living at less than 200% of the poverty line, rates almost exactly in line with norms from the larger city, and with slightly higher proportions of racial/ethnic minority group members and families in poverty than concurrent state and national norms[130]. Parents, peers, and romantic partners have been repeatedly interviewed and observed interacting with target participants. In all, we have conducted assessments with more than *3,800* collateral parties (~21 reporters per participant).

Procedure. Written procedures for handling unusual problems (e.g., suicidality, child abuse) have been established and tested. Although most of our sample is projected to remain nearby in the next five years (see Budget Justification), we have designed procedures for interviewing and collecting physiological data at a

distance or compensating travel expenses for participants' return to our locale (e.g., for visits to friends and family of origin). The plan outlined in Table 1 entails annual paper-and-pencil data collection from target participants and close friends, which also serves to maintain participant contact and interest. We obtain epigenetic data once at the study outset and other physiological assessments at both the start and end of the study period, and allow a two-year window to maximize participation rates for these key assessments, given that some will be scheduled around participant travel. We have a 2½ year window for romantic partner assessments, leaving time to assess participants while they are actually in sustained relationships (i.e., more than 3 months' duration).

	Assessment Age				
Assessment Type	(33)	(34)	(35)	(36)	(37)
Partic. Self-Reports	X	X	X	X	X
Partic. Physiol. Assessments	X			X	
Partic. Epigenetic Assessment	X				
Partic. BMI, Health/risk Behavior Assessment	X	X	X	X	X
Close-Peer Reports	X	X	X	X	X
Rom. Partner Obs. & Report	X			X	

Table 1 – Data Collection Plan

Sample Retention. We minimize attrition by maintaining regular contact with participants, compensating them well, making interviews relaxed and enjoyable, and obtaining extensive tracking information. **We have obtained data on 97% of our original sample over the past five years**. Although we have *always* had less attrition than we projected over the past 19 years, and our last projection of 7% attrition turned out to be overly conservative, we again use this cautious estimate, yielding a projected final sample of 172. We fully expect that we can keep attrition lower, however.

Value-added from a Five-year Data Collection Window. Our analyses, described below, take advantage of our repeated measures approach so that we can not only examine long-term predictors to *robust* (i.e., aggregated) measures of key constructs, but also can assess cross-lagged predictions from social difficulties to some aging measures *within* the midlife period. This approach allows us to not only assess social relationship factors as predictors of short-term change, but also to begin to disentangle causal pathways between social factors and health and aging outcomes. 4, 7 This approach also enhances power (using aggregates to increase precision of measurement) and allows for assessment of change in midlife of key indicators, including growth curve and cross-lagged analyses for those physiological indicators for which we also have repeated *baseline* assessments. This repeated measures approach also greatly aids in maintaining sample contact/retention.

Measures

Our measurement approach involves assessing developmentally salient relationships at each phase (e.g., parents and close peers early in adolescence, close peer, romantic, and 2 parenting relationships (when present) from mid-adolescence onward)[73, 131, 132]. We describe previously peer-reviewed measures briefly to allow more room to describe new assessments. Age ranges are in parentheses for measures collected multiple times. Measures were typically collected annually or biennially for teen and peer report, and every 2½ years for romantic partner reports and physiological assessments. "TP" refers to the target participant; measures completed by others are completed about the target participant.

Physical Health/Aging Assessments

We assess physiological age in participants with a combination of biomarker assays and anthropometric assessments that index the integrity of function across a range of biological systems including metabolic, immune, and cardiovascular systems. All biomarkers are employed routinely in clinical practice for health monitoring purposes, and are known predictors of later-life morbidity and mortality. Increasingly, it has been demonstrated that *aggregated* panels of biomarkers can reliably predict later accelerated aging above and beyond single biomarker assessments since 1) aging is a process that is believed to affect multiple biological systems simultaneously[133], and 2) single assessments are vulnerable to erroneous deviations due, for example, to acute illness or measurement error. 7 Aggregation of multiple biomarkers thus allows holistic *and parsimonious* representation of an individual's physiological aging status.

Rationale for Biomarker Selection: A variety of algorithms for calculating an individual's physiological age have been proposed[61, 64, 134.]. These algorithms show predictive capacity for a range of aging-related outcomes, though each uses varying panels of biomarkers as their input. We propose collection of a range of common biomarkers that encompass almost entirely the core set of each of these algorithms to permit validation of the reproducibility of our physiological aging measures across algorithms, and to allow for updating of physiological age assessment as new algorithmic approaches are developed and reported. This set of biomarkers will also permit assessment of specific disease processes (e.g., cardiovascular disease risk, metabolic syndrome) using established algorithms that have been the focus of sustained prior attention and preventive efforts[135, 136].

Blood sampling and biomarker assessment methods (Age 33 & 37): Participants will undergo anthropomorphic assessment and phlebotomy from a trained healthcare professional. Blood will be drawn into two 3mL SST tubes (for serum-based evaluations), one 10 mL K_2EDTA tube (for whole-blood based evaluation) and one *PAX*gene DNA tube (for epigenetic evaluation; see below). Assays will be undertaken by the University of Virginia Medical School CORE research facility using standard clinical methodology.

Biomarker Group	Measurement (each to be assessed at ages 33 and 37)
Blood Pressure	Blood pressure (systolic, diastolic, MAP) will be assessed according to standard protocols.
Anthropomorphic measures	BMI[1] will be calculated via measurements of height and weight, waist-hip ratio[1] calculated via waist and hip circumference
Lipid Panel	Total Cholesterol, HDL cholesterol and Apolipoprotein B100/A1 ratio measured from serum
Cardiovascular, hematological and inflammation	C-Reactive Protein (CRP)[1] and Interleukin-6 (IL-6)[1] will be measured in serum. White Blood Cell Count (WBC), hemoglobin and glycated hemoglobin will be measured in whole blood samples
Kidney & Liver Function	Creatinine, Blood Urea Nitrogen (BUN), Albumin, Alkaline Phosphatase, Total Protein, Alanine Transaminase (ALT), Sodium, Glucose and Lactate Dehydrogenase (LD) will be measured in serum
Infection	Cytomegalovirus (CMV) will be measured in serum
	[1] Note: BMI, waist-hip ratio, CRP & IL-6 are also assessed at ages 28 and 31.

Physiological Age (Age 33 & 37)**:** Physiological/biological age (BA) will be calculated by employing the method described by Levine[61], which showed that employing the Klemera-Doubal formula as shown to the left performed best in predicting mortality above and beyond chronological age in the NHANES-II dataset, where *x* is the value of biomarker *j* measured for an individual in the cohort. For each biomarker *j,* the parameters *k*, *q*, and *s* are estimated from a regression of chronological age on the biomarker in data from NHANES-III. *k, q,* and *s,* are the regression intercept, slope, and root mean squared error, respectively. s_{BA} is a scaling factor equal to the square root of the variance in chronological age explained by the biomarker panel in the NHANES database. CA is chronological age.

$$BA_{EC} = \frac{\sum_{j=1}^{m}(x_j - q_j)\frac{k_j}{s_j^2} + \frac{CA}{s_{BA}^2}}{\sum_{j=1}^{m}\left(\frac{k_j}{s_j}\right)^2 + \frac{1}{s_{BA}^2}}$$

Epigenetic Age Assessment (Age 33)**:** We hypothesize that social difficulties in adolescence and beyond may lead to changes in the epigenetic state of specific CpG sites in the genome related to aging. These CpG sites have been established by Horvath[51] (n=353 sites) and Hannum et al.[137] (n=71 sites) and are assayable from DNA derived from whole blood using the Illumina Infinium® MethylationEPIC BeadChip. These groups of sites overlap by only 6 CpG sites, so assaying both provides the ability to estimate epigenetic age by two separate methods and compare. Assaying by array, as opposed to single site assays, is cost effective and allows for the use of additional CpG sites to correct for cell type differences when using whole blood for analysis. Though the precise mechanism that results in the changes in methylation at these specific sites is unknown, these groups of sites have been used to predict *accelerated epigenetic aging* due to obesity[138], Down Syndrome[139], HIV infection[140], and most importantly, markers of life stress[58-60], and recently have been shown to predict all-cause mortality[141]. Notably, centenarians have been shown to age 'slower' using the same collection of sites and epigenetic age analyses[142].

Data Acquisition and Analysis: DNA samples randomized for sex/race will be hybridized to arrays and imaged at the Duke MPI. Images will be preprocessed (*RnBeads* package) and probes and samples filtered using a greedy algorithm with a detection p-value threshold of 0.05, requiring >90% detection, cross-reactivity and variant content. Gender mismatch, batch effects, and outlying samples will be detected using principal components analysis. Data will be background corrected using 'noob' in *methylumi* and quantile normalization will be performed using wateRmelon on methylated and unmethylated, and type I and type II probes separately. Methylation levels (β) at each probe will be calculated as the ratio of methylated to total signal and sent to UVA for further analysis. Using this pipeline, methylation profiles of technical replicates have correlated well in previous experiments performed at Duke MPI (Pearson correlation ≥ .998). Estimates of epigenetic age will then be performed through collaboration with the UVA Bioinformatics Core using the online epigenetic clock calculator[143], which allows for calculation of cell type proportions, raw epigenetic age, which is an epigenetic estimate of chronological age*,* and *Accelerated Epigenetic Aging,* which is calculated as the residual from a linear regression of epigenetic age on chronological age (i.e., the residualized estimate of the extent to which someone's epigenetic age is greater (or less) than their chronological age). Acclerated epigenetic aging

is uncorrelated with chronological age and weakly correlated with blood cell count differences when this measure is incorporated in residual estimates[141]. Accelerated epigenetic aging residuals will be adjusted for cell type differences, as this adjustment has been shown to improve estimates[141].

Hostile Conflict Assessments

Ongoing Assessments: *Hostility in Observed Dyadic Conflict Task* (Interactions with: Parents (13-18), Peer (13-21), Rom.Prtnr. (15-37)): 8-minute video-recorded discussion of a conflict (hypothetical for peers through age 17; actual for all other observations) coded with the well-validated *Autonomy and Relatedness Coding System*[144-148]. *Conflict Tactics Scale* (Parent, Peer, Rom.Prtnr. and Self-reports; 13-37): perpetration and victimization of verbal and physical abuse[149]. *Verbal* Abuse (TP: 17-37; Peer: 17-37; Rom.Prtnr.: 18-37)*:* 16-item *Psychological Maltreatment Experience Scale*[150] assesses participants' experiences of and perpetration of verbal abuse in relationships with peers and Romantic Partners. *Hostility* (TP: 23-37): 24-item *Buss/Durkee Hostility Inventory* assesses tendencies toward hostile behavior and affect in relationships[6, 151-154]. *Relationship Negativity* (*TP re: Parents, Peers and Rom.Prtnr.*: 17-27; Peer: 20-27; Rom.Prtnr.: 18-37): 9-item scale from the *Network of Relationships Inventory*[155-157]. *Romantic Relational Aggression and Victimization* (TP & Rom.Prtnr.: 19-37): 10-item scale from eponymous measure[157]. ❸ *Parenting Stress Index* (TP: 33-37, + Child ages 12 & 60 mos. if applicable*):* 120-item measure by this name assessing social stressors related to the parenting role[104]. **Previously Collected Assessments:** *Conflict & Betrayal* (TP & Peer: 13-19*):* 7-item scale from *Friendship Quality Questionnaire*[158].

Social Support Assessments

Ongoing Assessments: ❷, ❸ *Relationship Histories. We assess both presence/absence and net duration of both romantic and parenting relationships* (TP: 13-37). *Observed Dyadic Supportive Behavior* (TP with: Parent: 13, 16, 18; with Peer: 13-20, 21, 26; with Rom.Prtnr.: 18-37): Video-recorded support-seeking task coded for instrumental and emotional support, overall warmth and engagement.[159, 160]. *Relationship Quality* (TP re: Parents, Peers and Rom.Prtnr.: 17-32; Peer: 20-32; Rom.Prtnr.: 18-37): *Network of Relationships Inventory* (described above)[155, 156]. *Romantic Relationship Satisfaction* (TP: 23-37; Rom.Prtnr.: 24-37*)*: 7-item scale assessing overall relationship satisfaction within romantic relationships[161]. *Social Support Questionnaire* (TP 28-37): 27-item questionnaire yielding overall support and satisfaction scores[162-164]. *Social Acceptance* (TP & Peer: 13-37; Rom.Prtnr. 29-37): scale modified from the *Self-Perception Profile for Adolescents & Adult Self-Perception Profile* to collect reports from peers and romantic partners about target participants[165-167]. *Loneliness* (TP: 23-37): 20-item *UCLA Loneliness Scale*[106, 168-170]. *Anxiety & Avoidance in Close Relationships* (TP: 17-37*):* 36-item *Experiences in Close Relationships* scale[171]. **Previously Collected Assessments:** *Overall Support* (TP: 13-20): *Friendship Quality Questionnaire*[158]; *Trust/Communication* (TP & Peer 13-30*): Inventory of Parent and Peer Attachment* [172]. *Popularity* (*Sociometric: 13-17)*; *Acceptance - Child Report of Parenting Behavior Inventory (*TP: 13-19; Parent: 13-18*)*[173, 174].

Mental Health Symptom Assessments

Ongoing Assessments: *Anxiety & Depressive Symptoms* (TP 18-37*): Beck Depression Inventory*[175] *& State-Trait Anxiety Inventory* [176, 177]; *Internalizing & Externalizing Symptoms* (TP: 18-37; Peer: 18-37; Parent & Rom. Prtnr.: 24,26): *Adult Self-report and Adult Behavior Checklist*[178]*, Substance Use & Problems Related to Use* (TP: 13-37*)*: *Monitoring the Future* survey & *CORE Alcohol & Drug Survey*[179-181]. **Previously Collected Assessments:** *Children's Depression Inventory* (TP: 13-17[182])*, Beck Anxiety Inventory* (TP: 15-18)[183]*, Problem Behavior Inventory*, *Alcohol & Drug Use and Problems Related to Use*[179, 180] (TP:13-30). *Child Behavior Checklist* (internalizing & externalizing symptoms) (TP & Parent: 13-18*)*[184, 185].

Previously Reviewed Health, Health History, and Health Behavior Assessments

Cardiovascular Reactivity During Acute Psychological Stress (TP: 28, 31, 33, 37): Reactivity assessed continuously during mental arithmetic and speech stressors.[186-188]. *Sleep Quality* (TP:28-37): *Pittsburgh Sleep Quality Index*[189, 190]. *Early Health History* (TP: 28): includes experience and severity (e.g., duration, hospitalization, etc.) of 37 different major health problems from childhood onward[108].

Behavioral, Contextual, & Historical Assessments

Risky Behavior, Nutrition, & Exercise Assessments: *Risky Behavior* (TP 31-37*): Behavioral Risk Factor Surveillance System*[191]. Selected questions addressing seat belt use, drinking and driving, current tobacco and e-cigarette use, level of physical activity, and exercise. *Nutrition* (TP: 31-37): 30-item *Nurses Health and Nutrition Examination Survey Dietary Screener* assessing dietary quality relative to current nutritional standards[192]. *Exercise/Activity* (TP 31-37): Multi-part *Modifiable Activity Questionnaire*[193] tracking both exercise and sedentary behavior over the prior year.

Socio-economic and Demographic Status (TP: 13-37): We assess *parental status* and *marital status* (but given societal changes, also primarily use a continuous variable to address availability of consistent, long-term intimate support), *highest education level*, *current income, work history, and perceived economic pressure* using the *Life Experiences Survey* and specific demographic questions selected from similar measures[194-197]. During adolescence, we assessed *parental education levels* and *family of origin socio-economic status*. ❺ ***Adverse Childhood Experiences***[103] (TP: 27, 33*):* retrospectively assesses childhood experience of extreme adverse experiences (e.g., abuse, neglect, extreme poverty, parental imprisonment). ❻ ***Social orientation*** (TP:33,37) is assessed via the Interpersonal Orientation Scale [198]. ***Gender role*** (TP: 20-23, 33) is assessed via the Bem Sex Role Inventory[199, 200].

Statistical Analyses

Our overarching approach to data analysis is to employ a discrete number of tightly specified hypotheses, using precisely measured constructs, assessed across multiple raters and time points. We capitalize on the large number of measures and measurement occasions described above by employing both traditional (e.g., factor analytic, SEM, etc.) and recently developed advanced data reduction techniques (e.g., idiographic filtering[201]) to create a ❼ ***limited number of well-measured broader constructs*** within each assessment point for our analyses. Our primary analytic approach employs autoregressive structural equation (SEM) modeling supplemented with latent growth curve modeling approaches where appropriate[202-205]. We also utilize relatively new applications of latent difference score and dynamical systems modeling approaches to estimate the direction and strength of longitudinal coupling between multiple systems[206-208]. Mediated relationships will be assessed using bootstrapping procedures to determine confidence bands around point estimates for indirect effects[209, 210]. Moderated relationships will be assessed as outlined by Preacher et al.[211] We handle missing data with full information maximum likelihood estimation and multiple imputation procedures[212], all within *R, Mplus, SAS, OpenMx* and related programs. ❷, ❸ For individuals not in romantic or parenting relationships, we utilize conditional, mixture distribution, and two-part random effects models to consider relationship presence/absence[213, 214]; thus we gain information about individuals lacking such relationships unless they truly have missing data (e.g., due to attrition). We also assess whether other observed pathways may differ for those in vs. not in romantic relationships via tests for moderation, and we consider relationship *duration* in our models as appropriate.

Given space constraints, we present exemplar analyses below for each of our four Aims using *selected* constructs as examples to illustrate our analytic approach. Final construct identification and model selection will be empirically guided and will utilize non-redundant approaches that maximize measurement precision and power while minimizing Type I and II error rates. ❼ Respecting our sample size, hypothesis-testing is based *not* on building broad models, but on evaluation of *discrete, pre-specified pathways* within models as depicted via the bold green arrows below. When we employ multiple approaches to address a given question, we report results that converge across analytic techniques so as to minimize Type I error rates.

Aim 1: <u>Exemplar Question</u>: Do adolescent experiences of hostile conflict and lack of support predict midlife health & aging? We begin by assessing simple predictions from hostile conflict and lack of support, assessed across adolescence, to health and aging outcomes (Figure 3). We primarily focus upon overall epigenetic and physiological aging assessments in these analyses, although we will also consider specific physiological and health outcomes (e.g., cardiovascular risk, metabolic syndrome) in secondary, exploratory analyses. For the purposes of these exemplars, however, we use physiological aging as an illustrative outcome. We account for prior health status, gender, and current and past socioeconomic status in all analyses.

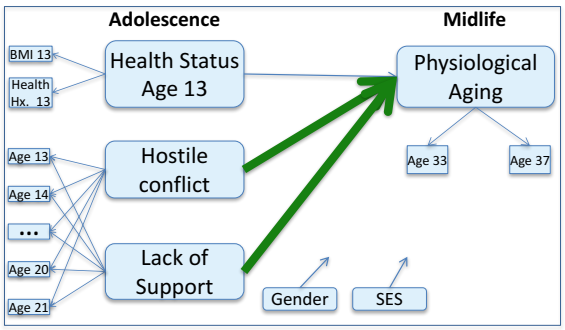

Figure 3 – Aim 1: Direct Prediction of Midlife Health

Aim 2: <u>Exemplar Question</u>: Are predictions from adolescent experiences of hostile conflict to midlife health/aging direct vs. mediated via later hostile conflict experience? We use experience of hostile conflict as our exemplar for this question to compare two pathways to health outcomes: (1) direct cumulative paths from multiple prior experiences of hostile conflict (e.g., across multiple relationship types (parent, peer, romantic partner, offspring) and times), independent of effects of current problems (Paths "**1**" in Figure 4 below); and (2) indirect mediated paths in which effects of prior conflict are mediated via concurrent social difficulties in midlife (Paths "**2**"). ❹ For our physiological aging construct, use of repeated measures within the

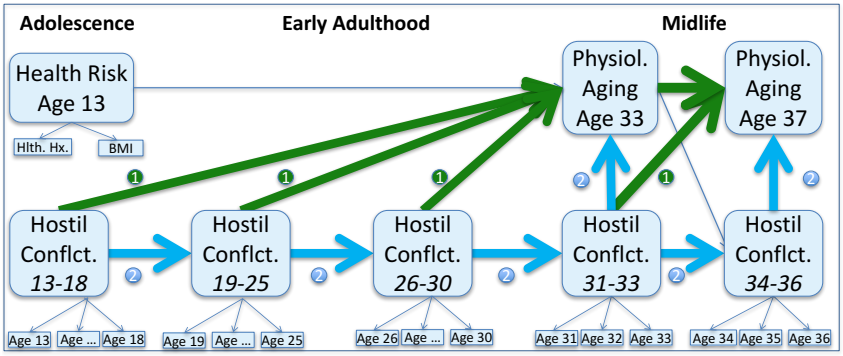

Figure 4 – Aim 2: Cumulative and Mediated Paths to Midlife Health

midlife period also allows for one of the first assessments of potential bidirectional effects between aging and relational factors assessed longitudinally (e.g., aging on conflict and vice versa). For outcomes measured more frequently (e.g., physiological indicators assessed in *both* the current and proposed grants), latent difference score and growth curve models will be used to maximize our ability to understand the interplay of health and relational functioning within adulthood. These approaches represent a substantial advance over 'mediation' analyses in which mediators and outcomes are assessed only concurrently at the final assessment point and are thus temporally confounded.

Aim 3: Exemplar Question: To what extent are social relationship effects explained and/or mediated by health risk behaviors and mental health symptoms? We illustrate our approach to this question with depressive symptoms as a potential mediator of effects of lack of support. We use an auto-regressive cross-lagged model, with physiological aging as a latent variable for all but ages 33 and 37, to examine the transactional interplay between depressive symptoms and lack of social support. [8] Most importantly in understanding the model in Figure 5, paths "**d**" and "**e,**" testing the exemplar mediated pathway, are each assessed as a *single degree of freedom* (e.g., all paths "**d**" are constrained as equal). This approach dramatically reduces model complexity and increases power while capitalizing on multiple measurement occasions. For graphic simplicity, the full cross-lagged model, in which depressive symptoms also influence social support to affect aging processes, etc., is not depicted. Yet, we would note that with similar constraints, this more complex model still involves assessing only a modest number of degrees of freedom. Similarly, even if we assume that mediational pathways change with development (e.g., "**d"** and "**e"** are different after age 21 than before), this would simply involve testing an additional 2 df's in the model. Ultimately, we will also use the focus area of co-I Boker to examine recently developed approaches to modeling dynamical systems effects in development (e.g., feedback loops, etc.), thus fully utilizing our multiple measurement waves[206, 215].

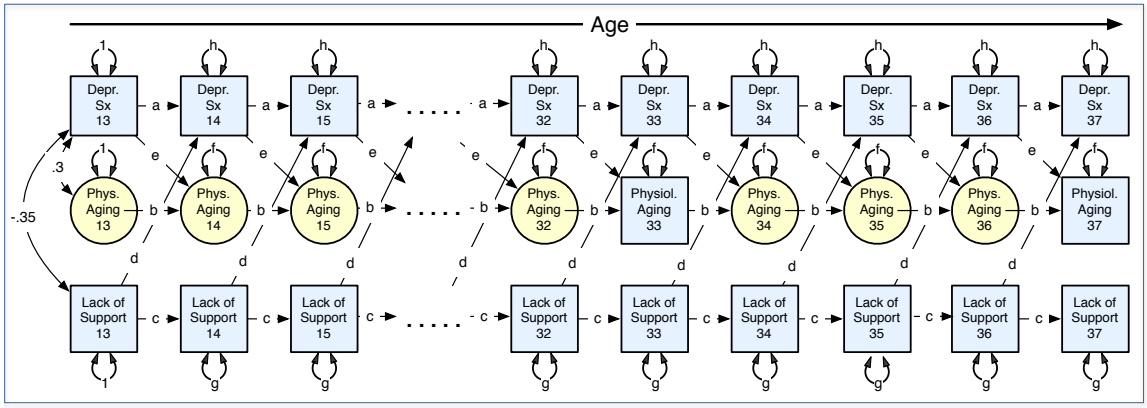

Figure 5 - Aim 3: Testing Depressive Symptoms as a Mediator

Aim 4: Exemplar Question: Do effects observed above differ depending upon key demographic and contextual factors? We illustrate in Figure 6 with biological sex how we would follow-up a finding in which chronic lack of support best predicted aging. In this follow-up model, we now take sex into account not only as a main effect, but also as a potential moderator of key relationships. [6] By then following up any observed moderation not just with separate analyses by sex, but by also assessing continuous measures of potential *determinants* of sex moderation (e.g., gender role and social orientation), we gain back some of the statistical and explanatory power otherwise lost by simply splitting our sample by sex. We also recognize that moderation can occur in the absence of main effects, and thus test other pathways as well with correction for the multiple tests in these less hypothesis-driven analyses.

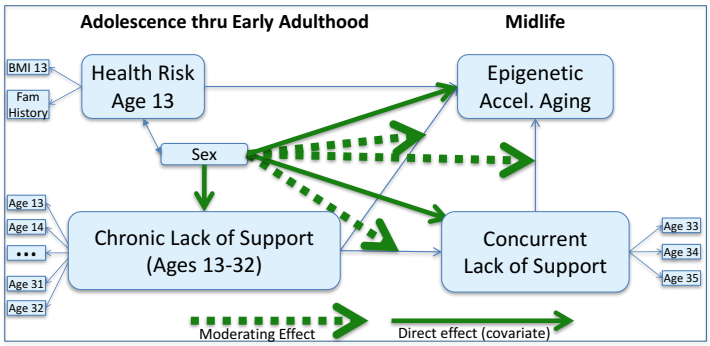

Figure 6 - Aim 4: Direct & Moderating Effects of Biological Sex

Design and Power Considerations. Although larger sample studies come with numerous benefits, we utilize an innovative complementary approach based upon multiple occasions of deep measurement[216-218]. Nesselroade[219] has described the 'data box:' a three-dimensional perspective recognizing that developmental assessments can be dense in terms of numbers of individuals, numbers of constructs, and/or number of time points assessed, and that *density in terms of time points can dramatically enhance power by yielding far more stable, reliable estimates of key constructs and paths*. For example, over a 25-year period, our 24 assessment time points yield an equivalent data density to a sample of more than 2000 individuals, if assessed at just ages 13 and 37. In complex models (e.g., Figure 5), when we impose simultaneous constraints on paths across waves, each additional wave of data increases statistical power approximately equivalently to adding *an entire additional sample* of similar size[219-222].

An ongoing tradeoff with our intensive approach is that it becomes inordinately expensive to conduct with very large samples. Yet, this intensity yields a worthwhile trade-off in allowing us to very accurately assess subtle social interaction processes (and their time course) that are often impossible to assess within larger sample designs. Further, even if carefully measured, social experiences are less likely to be formative if they are transient, and these experiences can change rapidly in adolescence and early adulthood. Our approach provides a uniquely dense picture of both the aggregate and developing quality of these interactions over time.

We estimate power using Monte Carlo techniques for models to be tested, and illustrate first with one of our *most* complex models (Figure 5 above). Latent variances and covariances, manifest residuals, and regression paths between latent variables were free. The values used to create the data sets were a=.6, b=.8, c=.6, d=-.1, e=.1, f=.6, g=.6 and h=.6. Values for a, c, d, h, and g were all estimated from a covariance matrix using existing data; b, e, and f are estimates that would result in a simple *r* of approximately .3 between same-age depression and physiological aging. 1000 data sets were created conforming to the covariance and mean structure implied by the model and starting values using the R function rmvnorm[223], and each containing 172 participants. Next, *each data set was degraded so that each manifest variable had approximately 10% missingness, to allow for incomplete data.* The models were fit in OpenMx[224] using full information maximum likelihood to each of the 1000 data sets. All models converged without error or warning. Power was calculated as the proportion of times that fixing selected parameters to zero and refitting the model resulted in a significantly ($\alpha = 0.05$) worse chi-square difference.

Using this approach, power to detect a simulated mediated effect in which both **d** and **e** = .10 was .94. Testing ability to detect moderation by sex on this pathway (assuming sex alters the first part of the path (**d**), and that parameters for males and females differ by .2), also yields power of .94. Models presuming that we follow up sex moderation by assessing only half the sample (e.g., females) still yield respectable power of .79 even when **d** and **e** are as small as .2. Although we are able to detect effect sizes in the small range, our own and others' results to date primarily find effect sizes in the small-to-medium and medium-to-large range, even after accounting for demographic and contextual covariates[225-229] (see also Progress Report). In contrast, and to illustrate the critical importance and power gained from our repeated measures approach, if we only had, for example, 4 time points of measurement for relationship qualities and mental health instead of 24, power for detecting even just main effects would drop to well under .40 (i.e., more like power estimates one expects to see in more conventional designs, thus illustrating the implicit power of our design).

For simpler models (e.g., Figure 6 above), we evaluate power for a different approach in which we aggregate assessments across multiple time points to enhance precision of measurement. Here, even using just a single outcome wave, power is .80 to detect an interaction effect of magnitude $R^2 = 0.06$ with 3 df (equivalent to just one of three interaction terms having $\beta = .24$). Hence, we require a larger effect, but by aggregating assessments we also are examining far more precisely assessed constructs.

The results of these power analyses are consistent with our long record of publishing results in top journals, which provides a degree of face valid evidence of the power of this design to identify important effects. We recognize there will be cases in which power will be reduced in more exploratory analyses and we will be appropriately cautious in such cases, though we would argue the importance of balancing traditional Type II error rates, which reflect the *possibility* that we will fail to detect effects, against the *near certainty* that such effects will not be detected unless they are pursued in intensive studies such as this. Though we recognize that for *some* more complex analyses power would be constrained, the analyses above show that our approach is fully powered for the tightly specified, discrete hypotheses that are the centerpiece of this study, and which we believe are the appropriate first step in ultimately understanding of broader processes.