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Authors: Sumaiyah U. Syed, Jared Isaac Cortez, and Stephanie J. Wilson

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Depression, Inflammation, and the Moderating Role of Metformin: Results from the Midlife in the United States (MIDUS) Study and Sacramento Area Latino Study on Aging (SALSA)

Sumaiyah U. Syed, BA¹, Jared I. Cortez, BS², and Stephanie J. Wilson, PhD¹

¹Department of Psychology, Southern Methodist University, Dallas, TX, USA

²Center for Vital Longevity, School of Behavioral and Brain Sciences, The University of Texas

at Dallas, Dallas, TX, USA

Correspondence: Sumaiyah U. Syed, susyed@smu.edu, (214) 768-2399

Southern Methodist University

Department of Psychology

6116 N. Central Expressway Tower

Suite 1300

Dallas, TX 75206

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Abstract

Objective: Depression can promote inflammation and accelerate aging. Metformin, a widely prescribed antidiabetic, has shown promising preclinical evidence of aging-related health benefits, including decreased inflammation. The current study examined whether metformin usage buffers the association between depressive symptoms and inflammatory markers in two large samples of middle-aged and older, primarily white adults, and older Latino adults.

Methods: Data from the Midlife in the United States Study (MIDUS; N=1255) and the Sacramento Area Latino Study on Aging (SALSA; N=1786) included information on medication use, depressive symptoms, and inflammatory markers, namely IL-6, TNF- α , and CRP. These data were merged into a harmonized sample, and the sample group variable was included in a three-way interaction for analysis.

Results: Specifically, in the MIDUS sample, metformin buffered the association between depressive symptoms and CRP (b=-0.029, SE=0.013, p=.007) and IL-6 (b=0.21, SE=0.010, p=.046), while no significant association was found with TNF- α . Metformin non-users displayed higher depressive symptoms associated with elevated CRP (b=0.01, SE=0.003, p<.001) and IL-6 (b=0.011, SE=0.003, p<.001), whereas this association was not present among metformin users (ps>.068). Conversely, in the SALSA sample, metformin use did not show a significant protective link.

Conclusion: Results from mostly white, highly educated adults supported a mitigating role of metformin in ties between depression, a well-known behavioral risk factor, and inflammation, a key source of biological aging. However, the benefits did not extend to a large sample of older Mexican Americans. The findings reveal a hidden potential benefit of this therapeutic agent and raise important questions around its health equity.

Trial Registration: The study was pre-registered on OSF (https://osf.io/c92vw/).

Abbreviations: MIDUS=Midlife in the United States Study, SALSA=Sacramento Area Latino Study on Aging, IL-6=interleukin-6, CRP= C-reactive protein, TNF-α=tumor necrosis factor alpha.

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Heightened systemic inflammation is a feature common to many diseases of aging cardiovascular disease, cancer, diabetes, arthritis, Alzheimer's disease, kidney disease, liver disease, and lung disease. In addition, individuals with elevated circulating inflammation have increased risks for greater frailty over time, faster functional decline, and earlier mortality (1–3). For these reasons, inflammatory markers such as CRP, an acute phase protein, as well as IL-6 and TNF- α , proinflammatory cytokines, have been identified as among the most promising biomarkers for geroscience research, studies of biological mechanisms promoting aging-related health outcomes, and geroscience-focused clinical trials seeking to slow the biological aging process (4–9).

Depression is a well-established source of chronic inflammation (10–12). A recent metaanalysis of 27 longitudinal studies found a significant, small association between heightened depressive symptoms and subsequent increases in inflammation (13). The authors attributed the small effect magnitude to its wide heterogeneity across individuals and samples, pointing to key moderators such as age. Older adults had stronger associations, suggesting that they may be at greater risk for poor health outcomes related to depression. Therefore, identifying protective factors that may be leveraged in middle and older age is essential. Geroscience is a critical lens that can be used to address these questions (9). Key hallmarks of biological aging, including inflammation, contribute to the overall aging process. Thus, targeting the underlying mechanisms of biological aging, like chronic inflammation, can potentially offset the development or progression of several age-associated chronic illnesses (6,8,14). Metformin, the first line of therapy for type 2 diabetes, has gained attention in the geroscience field due to growing evidence of its potential anti-aging properties and its ability to reduce inflammation across animal and human patient studies (5,7,15). Indeed, preclinical animal models show metformin's protective effects in several aging hallmarks, including inflammation, which in turn, slows the onset and progression of aging-related diseases (16,17). Metformin has shown to delay mortality in animal species like worms and mice (18–21). It also led to decreased cytokine production in bone marrow cells cultured from mice (22), and reduced plasma proinflammatory cytokine levels in mice with gestational diabetes (23). In part by reducing inflammation, metformin has shown to lower the risk of myocardial infarction, acute myocarditis, and chronic heart failure in rats (24). Evidence from animal and human epidemiological studies have found that metformin disrupts tumor progression, potentially inducing autophagy of cancer cells by inhibiting inflammatory signaling pathways (18,25).

In human liver and vascular wall cells, metformin has shown to decrease the levels of proinflammatory markers (26,27). A meta-analysis of randomized clinical trials on patients with breast cancer found that metformin reduced CRP levels (28). Data from diabetic patients taking metformin showed that higher dosages were associated with lower circulating proinflammatory cytokine levels (29). Recent studies revealed that metformin use was associated with suppressed production proinflammatory cytokines in individuals with COVID-19 (30). In a cross-sectional retrospective study of diabetic patients with COVID-19, metformin usage was linked with reduced CRP, IL-6, and TNF- α (31).

Given the growing evidence of metformin's potentially protective effects on inflammation, among other hallmarks of aging, researchers have set out to probe its effects in clinical trials. For example, the Targeting Aging with Metformin, or TAME, study is the first human randomized clinical trial to experimentally test metformin's ability to slow the aging process and prevent the progression of age-related chronic diseases in non-diabetic adults ages 65-79 across six years (5). Metformin is approved by the Food and Drug Administration (FDA) to treat diabetes; however, off-label use has rapidly increased given its pleiotropic benefits beyond glucose control (32). A study documenting metformin prescription rates in the United States from 2000 to 2015 found that by 2015, almost 25% of adults in the United States had a metformin prescription, an 80-fold increase since 2000 (33). FDA-approved metformin prescription rates increased from 2.27 per 1000 individuals to 235. Although this pattern of increase was found across all age and racial/ethnic groups, increases were highly driven by metformin use among middle-age to older adults, females, and Hispanic individuals. Metformin use has demonstrated efficacy in glycemic control, as reflected by A1C levels, particularly in minority populations, specifically African American individuals compared to European American individuals (34). A few comprehensive systematic reviews reference studies highlighting metformin's effectiveness in inflammation reduction (34–36). However, these studies frequently omit racial and ethnic demographic breakdowns of participant samples. While the link between metformin and inflammation reduction has been established in studies with large samples, the racial/ethnic composition is often underreported and likely overrepresents White non-Hispanic individuals.

Moreover, in cases where such details are provided, a predominant majority of the samples comprise of white non-Hispanic individuals and a smaller subset of studies indicate a representation of approximately 9.1 to 10.6% for Hispanic individuals (37,38). The widespread use of metformin highlights the significance of comprehending its broader consequences, particularly considering the well-documented association between metformin use and lower levels of inflammation, necessitating further investigation into its anti-inflammatory effects in diverse populations and settings.

Because depression and chronic inflammation compound the risk for poor outcomes in middle and older age, it is critical to understand the role that moderators have in mitigating this link (39,40). Metformin may contribute to the heterogeneity in the ties between depression and inflammation by weakening the association. In other words, this antidiabetic drug, widely used for a variety of off-label purposes and suspected of having anti-aging effects, may also inadvertently protect middle-aged and older adults from the inflammatory risks of heightened depressive symptoms.

In the present study, we examined associations among depressive symptoms, metformin use, and inflammatory outcomes (CRP, IL-6, and TNF- α) in a harmonized sample of two middle-aged and older adult samples – participants from the Midlife in the United States Study (MIDUS) and the Sacramento Area Latino Study on Aging (SALSA). The use of two large samples, including one that consists of minoritized individuals, is a key feature of this study. Indeed, studies of health and aging tend to overrepresent white, highly educated individuals, with fewer individuals of color and from disadvantaged backgrounds. Expanding the investigation to incorporate greater diversity is crucial, especially in the field of geroscience, where a critical aim is to uncover sources of variance from social factors that contribute to processes of healthy aging (41). Moreover, testing hypotheses in two distinct samples enhances the robustness and replicability of the empirical patterns across diverse populations. Given the compelling evidence of its anti-inflammatory properties, we hypothesized that metformin would buffer the link between depressive symptoms and inflammatory markers—in particular, CRP and IL-6—across both samples. Unlike CRP and IL-6, TNF- α did not share a significant association with prior depression in a recent meta-analysis (13). Thus, associations with TNF- α were considered

Aging plays a central role in the associations among depression, metformin use, and markers of inflammation within our study samples. Inflammation increases with age, leading to the phenomenon known as inflammaging (42). This is driven by an imbalance between pro- and anti-inflammatory mechanisms (43). This heightened inflammation that comes with advancing age contributes to age-related diseases and cellular-level aging (44). In tandem, the prevalence of type 2 diabetes notably rises with advancing age, resulting in a corresponding tendency for adults on metformin to be older (45,46). Additionally, depression is prevalent among older individuals, with a pooled estimate of 31.7% in the global population (47). Although high rates of depression are not exclusive to this age group, its consequences cannot be disregarded. Indeed, it often goes untreated or unnoticed in older adults, especially among minority groups (48–50). Consequently, we further investigate the role of age in the associations among depressive symptoms, metformin use, and inflammatory outcomes in the harmonized sample of MIDUS and SALSA. Based on previous research and the rationale of the current study, we hypothesize that age may modulate the associations among depressive symptoms, metformin use, and inflammatory outcomes. We expected that older age would be associated with higher inflammation. We assessed whether the

moderating effect of metformin varied based on age. Our approach was exploratory as the literature was not clear on whether this buffering effect would be stronger or weaker among older adults.

Method

Participants

The current study used data from participants in the Midlife in the United States (MIDUS) study and the Sacramento Area Latino Study on Aging (SALSA). Data collection of a national sample (ages 25-74) for the first wave of MIDUS (MIDUS I) was conducted in 1995-1996 (51). Participants were given a self-administered questionnaire and those who filled it out were invited to complete a telephone interview. A follow-up study, the MIDUS Biomarker Project, was conducted in 2005-2006, as part of the second wave of data collection (MIDUS II) (52). The Biomarker Project included a subsample of the original participants and aimed to investigate the biopsychosocial factors that contribute to health outcomes. Data for the current analyses were from the baseline data of 1,255 participants from the Biomarker Project. Participants traveled to one of three General Clinical Research Centers in the United States (University of California-Los Angeles, University of Wisconsin, and Georgetown University) and provided blood samples for biomarker assays. The MIDUS Biomarker Project protocol has been previously described in further detail (52,53).

SALSA is a longitudinal study of cognition, health, and aging in a cohort of community dwelling Mexican American adults ages 60-101 in the Sacramento area. Participants completed medical examinations and provided biological and survey data to investigate a wide array of

cognitive, physical, and social factors that may affect health and aging outcomes. Data for the current analyses were drawn from SALSA baseline data (N=1,786) collected at the time of enrollment (1998-1999). Details of the study protocol have been previously described (54). Both studies were reviewed and approved by institutional review boards at the participating institutions (MIDUS: University of Wisconsin-Madison; SALSA: University of California-Davis, University of California-San Francisco). Both (https://www.icpsr.umich.edu/web/NACDA/studies/4652) and SALSA study data are publicly available (https://www.icpsr.umich.edu/web/NACDA/series/247). Measures

Sample group

All study variables were retrieved from the SALSA and MIDUS datasets and were harmonized into a single dataset. Sample membership was included as a categorical variable in the analysis to examine potential differences between two distinct samples, MIDUS and SALSA. Sample group was coded into a value of 0 indicating MIDUS participants and a value of 1 indicating SALSA participants.

MIDUS

Π

Depressive Symptoms

Depressive symptoms were measured in both MIDUS and SALSA using the Center for Epidemiologic Studies-Depression Scale (CES-D), which has been widely used and validated in older adults (55–57). Participants were instructed to answer items (e.g., "I felt downhearted and blue") with reference to the past week, and responses were rated on a 4-point scale from *rarely* or none of the time (0) to most or all of the time (3). CES-D total scores can range from 0 to 60,

with higher scores indicating greater depressive symptoms. Cronbach's α for the CES-D was .88 in the MIDUS Biomarker sample and .90 in the SALSA sample.

Inflammatory Markers

Inflammation levels were assessed via immunoassays of three markers of inflammation: Creactive protein (CRP), interleukin (IL)-6, and tumor necrosis factor (TNF)-α. MIDUS assay procedures have been previously detailed (53). Briefly, participants of the MIDUS Biomarker Project provided fasting blood samples on the second day of their in-person visit at one of three General Clinical Research Centers. Frozen samples were shipped to the MIDUS Biocore Laboratory for analysis (University of Wisconsin, Madison, WI). Serum CRP levels were measured with a BN II nephelometer (Siemens/ Dade Behring Inc., Deerfield, IL) using a particle enhanced immunonephelometric assay. Samples that fell below the CRP detection threshold were re-run by immunoelectrochemiluminescence via a high-sensitivity assay kit (Meso Scale Diagnostics #K151STG). The inter-assay coefficient of variation (CV) was 5.16%, and the intra-assay CV was 4.1%. Blood serum IL-6 and TNF- α levels were measured using a Vplex Custom Human Cytokine Kit (Meso Scale Diagnostics #K151A0H-2). The inter-assay coefficient of variation (CV) was 15% and the intra-assay CV was 4.73% for IL-6. For TNF- α , the inter-assay coefficient of variation (CV) was 7% and the intra-assay CV was 3.19%. Intraand inter-assay CVs for these samples within an established acceptable range (below 20%) (58).

Details of the SALSA assay method have been previously described (59). In short, participants provided baseline fasting blood samples. Serum samples were stored at -70C at the Medical Center Clinical Laboratory at the University of California, Davis. High-sensitivity CRP

was measured using the CRP Ultra-Wide Range Reagent Kit latex-enhanced immunoassay (Equal Diagnostics, Exton, PA). IL-6 and TNF-α levels were measured using the QuantiGlo Chemiluminescent Immunoassay (R&D Systems, Minneapolis, MN).

Metformin use

Use of metformin, a commonly prescribed antidiabetic drug, was determined in both MIDUS and SALSA using medication usage data collected at baseline and recoded as a binary variable to be assessed as a moderator in the current analyses. MIDUS participants were instructed to bring all medications in original bottles to their in-person biomarker visit to record accurate medication names and dosages (53). During baseline interviews, SALSA participants provided their medications (prescription and OTC) for medication data collection (59). Combination drugs containing metformin and another drug—e.g., Actoplus Met (metformin and pioglitazone), Avandamet (metformin and rosiglitazone)—were included among the metformin users.

Covariates

Age, sex, BMI (calculated from measured height and weight), and comorbidities were included as key covariates in the primary analyses. Minority status, smoking, exercise, steroid use, and diabetes status were included as covariates in supplementary analyses. Comorbidities were calculated for both samples using a modified Charlson Comorbidity Index based on self-report of having chronic health conditions (1=yes, 0=no) (60,61). In MIDUS and SALSA, participants were asked about current or past chronic health conditions related to heart problems, transient ischemic attack (TIA) or stroke, liver problems, and cancer (excluding minor skin

cancer). Each chronic health condition was given a point, and then summed to calculate a comorbidity burden score for each participant. For both MIDUS and SALSA, the steroid use variable was binary coded (1=yes, 0=no) if a steroid drug (e.g., prednisone, cortisone, or methylprednisone) was listed in medication usage data. Minority status in MIDUS was coded as a binary variable (1=non-Hispanic white, 0= other races/ethnicities). Of note, minority status was a constant in SALSA as the full sample is of Hispanic/Latino ethnicity. Diabetes status was based on a binary coded variable (1=diabetic, 0=not diabetic) based on fasting salivary glucose of \geq 126 mg/dL, (62). Smoking status in both samples was coded dichotomously (1=current or former smoker, 0=never smoker).

Analytic Plan

Data from the MIDUS and SALSA samples were harmonized into a single dataset across parallel study variables. A sample group variable was computed to indicate sample group membership (1=SALSA; 0=MIDUS). We hypothesized that metformin would buffer the association between depressive symptoms and inflammatory markers. To test this, we regressed the three inflammatory markers (CRP, IL-6, and TNF- α) onto depressive symptoms (centered at the sample average), metformin use (coded as a binary variable), sample group membership (coded as a binary variable), and their three-way interaction. By including this three-way interaction, we can explore whether the impact of depressive symptoms on inflammatory markers differs based on whether individuals are using metformin and which sample group they belong to. Regression analyses were performed in SPSS version 29. Models covaried for age, sex, BMI, and comorbidities. Participants with missing data on specific inflammatory markers were excluded from the respective regression analyses. High CRP values exceeding greater than 20.0 mg/L were excluded due to potential acute factors (e.g., injury or active infection) (63–65). To address skewness, z-scored CRP, IL-6, and TNF- α were log-transformed. Residuals of the log-transformed inflammatory markers were examined, and outliers (above 3 standard deviations) were excluded for IL-6 in both MIDUS (2 cases) and SALSA (22 cases) samples. For models with statistically significant interactions (p < .05), simple slopes were probed. In models with non-significant interactions, the interaction term was removed to explore whether the expected two-way interactions or main effects were present. Furthermore, supplemental analyses were performed to test the robustness of results to additional health-related and demographic covariates: smoking, exercise, steroid use, diabetes status, and minority status. Given its relevance, supplemental analyses probing the role of age in these associations were also performed. To test this, we regressed inflammatory markers onto depressive symptoms, metformin use, sample group membership, age, and their four-way interaction. Non-significant interactions were effects.

Results

Table 1 shows descriptive statistics for MIDUS and SALSA study samples. Most notable differences between samples included age, minority status, education, and comorbidity burden. MIDUS participants' average age was 57.32 (*SD*=11.55, range: 35-86). The average age of SALSA participants was 70.64 (*SD*=7.11, range: 60-101). The majority of MIDUS participants were White (78.2%), whereas the SALSA sample consisted entirely of Mexican American participants. Regarding comorbidity burden, approximately one-third of the MIDUS sample (35.7%) reported one or more comorbidities, compared to 52.6% in the SALSA sample. The proportions of diabetic metformin users were similar across MIDUS (pre-diabetic: 24.4%;

diabetic: 74.4%) and SALSA (pre-diabetic: 21.5%; diabetic: 67.7%). MIDUS and SALSA participants were highly similar in their average BMIs (MIDUS: 29.77 (SD=6.63); SALSA: 29.68 (SD=5.91)) and proportions of participants using metformin (MIDUS: 7.2% (n=90); SALSA: 7.4% (n=133). In MIDUS, 15% of participants were diabetic, while 23.5% were diabetic in SALSA. Over half (52.1%) of MIDUS participants were pre-diabetic, compared to approximately one quarter (24.5%) of the sample in SALSA.

Table 1 displays the results of t-tests and chi-square tests, revealing significant differences between the SALSA and MIDUS samples. SALSA participants were significantly older, had higher depression scores, a greater comorbidity burden, a higher prevalence of diabetes, and a higher prevalence of smoking compared to MIDUS participants. Additionally, SALSA individuals demonstrated significantly higher levels across all inflammatory markers in comparison to MIDUS participants. Bivariate correlations among main study variables across both samples are shown in Table 2. As expected, in both MIDUS and SALSA samples, depressive symptoms (CES-D) and all three inflammatory markers were positively correlated, with one exception of TNF- α in MIDUS.

CRP

Results showed that the three-way interaction between depressive symptoms, metformin use, and sample group significantly predicted log-transformed CRP, b=0.036, SE=0.013, p=.005, 95% CI: [0.011, 0.061], $f^2=0.21$. Thus, the interaction between metformin use and depressive symptoms varies based on sample group membership. Sample group significantly moderated the association between depressive symptoms and CRP among people not taking metformin (b=0.008, SE=0.004, p=.037, 95% CI: [0.000, 0.015]) and those taking metformin (b=0.028, SE=0.012, p=.020, 95% CI: [0.004, 0.052]) in both SALSA and MIDUS. Simple slopes were probed to uncover patterns.

MIDUS. Metformin significantly moderated the association between depressive symptoms and log-transformed CRP among those in MIDUS, b=-0.029, SE=0.011, p=.007, 95% CI: [-0.051, -0.008]. That is, among MIDUS individuals not taking metformin, higher depressive symptoms were associated with higher CRP, b=0.01, SE=0.003, p<.001, 95% CI: [0.004, 0.016]. Conversely, the link was not significant among MIDUS metformin users, b=-0.019, SE=0.011, p=.068, 95% CI: [-0.040, 0.001]. Simple slopes are depicted in Figure 1a.

SALSA. Metformin did not moderate the link between depressive symptoms and logtransformed CRP among those in SALSA, p=.304, 95% CI: [-0.006, 0.019]. The main effect of depressive symptoms on CRP was also not significant, p=.002, 95% CI: [-0.002, 0.007], as was the main effect of metformin use on CRP, p=.429, 95% CI: [-0.094, 0.221].

IL-6

Results showed that the three-way interaction between depressive symptoms, metformin use, and sample group predicted log-transformed IL-6, b=0.024, SE=0.012, p=.044, 95% CI: [0.001, 0.048], $f^2=0.17$. Thus, the interaction between metformin use and depressive symptoms varies based on sample group membership. However, sample group did not moderate the association between depressive symptoms and IL-6 among people taking metformin (p=.132, 95% CI: [-0.005, 0.040]) and those not taking metformin (p=.051, 95% CI: [-0.014, 0.000]) in both SALSA and MIDUS. Simple slopes were probed to uncover patterns.

MIDUS. Metformin also significantly moderated the association between depressive symptoms and log-transformed IL-6 among those in MIDUS, b=0.21, SE=0.010, p=.046, 95% CI: [.000, .041]. Simple slope analyses revealed the same trend: among MIDUS individuals not taking metformin, higher depressive symptoms were associated with higher IL-6, b=0.011, SE=0.003, p<.001, 95% CI: [0.005, 0.017]. Conversely, the link was not significant among MIDUS metformin users, b=-0.010, SE=0.010, p=.329, 95% CI: [-0.029, 0.010]. Simple slopes are depicted in Figure 2a.

SALSA. Metformin did not moderate the link between depressive symptoms and logtransformed IL-6 among those in SALSA, p=.557, 95% CI: [-0.016, 0.008]. The main effect of depressive symptoms on IL-6 was also not significant, p=.188, 95% CI: [-0.004, 0.019], as was the main effect of metformin use on IL-6, p=.632, 95% CI: [-0.113, 0.185].

TNF-α

Results showed that the three-way interaction between depressive symptoms, metformin use, and sample group was not significant for log-transformed TNF- α , *b*=0.001, SE=0.013, *p*=.931, 95% CI: [-0.025, 0.027], *f*²=0.08. Metformin did not moderate the link between depressive symptoms and log-transformed TNF- α among those in MIDUS (*p*=.518, 95% CI: [-0.015, 0.030]) and SALSA (*p*=.369, 95% CI: [-0.007, 0.020]). Upon dropping the triple interaction term, two-way interactions and main effects remained nonsignificant (*p*s>.106).

Supplemental Analyses

Secondary Covariates. Supplementary analyses showed the robustness of results after adding secondary covariates of smoking, steroid use, diabetes status, and minority status. There were no differences in the findings as a function of adding the secondary covariates. There was still a significant triple interaction between metformin use, depressive symptoms, and sample group membership for CRP (b=0.036, SE=0.013, p=.004) and IL-6 (b=0.024, SE=0.012, p=.043), but not TNF- α (p=.983). Metformin use significantly moderated the link between depressive symptoms and CRP (b=0.03, SE=0.011, p=.006) and IL-6 (b=0.021, SE=0.010, p=.040), respectively. Following the same pattern as the primary results, metformin did not moderate the link between depressive symptoms and TNF- α (p=.525).

Age Analyses. Supplemental analyses showed that the four-way interaction between depressive symptoms, metformin use, sample group, and age was not significant for log-transformed CRP (p=.457), IL-6 (p=.849), or TNF- α (p=.269). The non-significant four-way interaction term was removed in each inflammatory marker model, and the lower-order interactions were examined. There were no significant lower-order age effects for TNF- α (ps>.108).

The three-way interaction between age, metformin use, and depression did not significantly predict CRP (p=.928) or IL-6 (p=.921), indicating that age did not function as a significant moderator in both models. Moreover, the sample moderating effect, indicated by the only significant three-way interaction between sample group, metformin use, and depression, remained unchanged for both CRP (p=.003) and IL-6 (p=.019).

After the removal of the non-significant three-way interactions within the IL-6 model, a significant two-way interaction effect was observed between age and metformin use, b=0.018, SE=0.007, p=.012, 95% CI: [0.004, 0.032], $f^2=0.18$. Simple slope analyses revealed that among individuals not taking metformin, advancing age was associated with increased IL-6 (b=0.021, SE=0.002, p<.001). Conversely, age was not significant among participants taking metformin (b=0.003, SE=0.007, p=.688). This pattern was not shown in the CRP model, as the two-way interaction between age and metformin use was not significant (p=.138).

Within the IL-6 model, a significant two-way interaction effect between sample group and age was detected, b=0.013, SE=0.004, p<.001, 95% CI: [0.006, 0.020], $f^2=0.18$. Older adults had higher IL-6 among both samples, but this effect was stronger in SALSA (b=0.027, SE=0.003, p<.001) compared to MIDUS (b=0.014, SE=0.002, p<.001). This two-way interaction was not significant in the CRP model (p=.669).

Discussion

In a national sample of midlife and older Americans, metformin buffered the link between depressive symptoms, a well-known behavioral risk factor, and inflammation, a key hallmark of biological aging, in two inflammatory markers—CRP and IL-6. However, in a large sample of older Mexican Americans, metformin usage did not exhibit a protective role. Furthermore, the significance of the three-way interaction underscores the importance of considering the sample group in understanding the relationship between depression, metformin use, and inflammatory levels. The specific characteristics or circumstances of the sample groups (e.g., different demographic profiles or health conditions) may contribute to the observed interaction effects. On the one hand, these findings uncover the potential for metformin to disrupt the risky tie between depressive symptoms and systemic inflammation, and thus, may further bolster its promise for healthy aging. On the other hand, the null results among older Mexican Americans raise questions about whether people in historically underserved communities and those of ethnic minority groups will reap the potential benefit.

Consistent with hypotheses, among metformin non-users in the MIDUS study, greater depression was significantly associated with higher CRP and IL-6. This pattern replicates the firmly established association between depressive symptoms and inflammation (12,13). However, also as predicted, these associations were attenuated among metformin users. Metformin did not buffer against the association with TNF- α , in part because the main effect of depressive symptoms was non-significant.

These results that identified metformin as a significant moderator in the MIDUS study widen the scope of potential benefits metformin may carry. To date, metformin has been identified to act on biological aging pathways, inhibiting the mammalian target of rapamycin (mTOR) pathway which leads to a reduction in inflammation and promotes longevity (66). Preclinical studies, including in animals and patient records, have also shown metformin's antiinflammatory effects (7). Rapidly gaining momentum in the geroscience research community, metformin is the focus of ongoing clinical trials to confirm the safety and efficacy of its potential role in altering the pace of biological aging (5). Even beyond these main effects that metformin may have in lowering inflammation, this study's results suggest that metformin may also buffer against key psychosocial sources of inflammation such as depressive symptoms (12), a novel, unintended benefit of this therapeutic.

Critically, the moderating association that emerged in the MIDUS study was not observed in the SALSA sample. Indeed, among older Mexican Americans, the association between depression and inflammatory markers, CRP and IL-6, did not depend on metformin use. MIDUS and SALSA participants were strikingly similar in many ways. These two large samples featured similar average BMIs, numbers of men and women, proportions of metformin users, and proportions of diabetics and pre-diabetics among the metformin users. However, they differed widely on demographic factors, structural factors, and socioeconomic status (SES).

SALSA participants were much older (M=70.64) than MIDUS participants (M=57.36). Older adults often experience chronically elevated low-grade inflammation with increasing age, or inflammaging (8,67). SALSA, a sample that consists entirely of ethnic minorities, also had lower education levels compared to MIDUS participants who were mostly white.

Interestingly, there was a lack of significant associations between CRP and IL-6 with depressive symptoms in the SALSA sample. A prior study using data from another nationally representative sample (National Health and Nutrition Examination Survey, or NHANES) found the association between depression and inflammation was only present in non-Hispanic white individuals, while no significant associations were observed in non-Hispanic black, Mexican American, or other Hispanic individuals (68). Our findings emphasize the importance of further

investigating the role of race/ethnicity as a moderator in depression-inflammation studies, supporting the need for more comprehensive research in this area.

Low SES and being a member of a minority group are considered to be among the social hallmarks of risk, a set of social factors that can "get under the skin" and impact age-related health outcomes, also termed "social hallmarks of aging" (41). The presence of compounding risk factors, including older age, minority status, and low SES, within the SALSA sample may diminish the potential anti-inflammatory benefit of metformin. To gain a deeper understanding of the underlying mechanisms, further investigation in future studies is essential. These investigations should focus on elucidating how these specific compounding risk factors, such as older age, minority status, and low SES, can impact the potential anti-inflammatory effects of metformin in the SALSA population. Groups characterized by social hallmarks of risk are aging quicker and dying younger (69). Although there may be a moderating effect of metformin on depression and inflammation in some populations, the findings of the present study indicate that this pattern of results is weaker and inconsistent across inflammatory indicators. This underscores the significance of taking into account individual and population-based variations as crucial factors for comprehending the interplay between various risk and protective elements in influencing the process of healthy aging (6,41). An implication that emerges from the current findings is the potential challenge of providing gero-therapeutics, such as metformin, to disadvantaged groups. It suggests that while these groups may have the greatest need for such interventions, the presence of social risk factors that contribute to biological aging could potentially diminish the benefits associated with these promising interventions. (41,69).

Supplemental age analyses provided crucial insights into the associations among age, metformin use, sample group, and their interactions within the IL-6 and CRP inflammatory marker models. In the harmonized sample, the three-way interaction between age, metformin use, and depression was not significant, indicating that the magnitude of the buffering effect of age was not significantly greater or smaller for older adults compared to their younger or middle-aged counterparts. However, the sample moderating effect remained, suggesting that sample differences are not attributable to age alone. In both samples, a significant two-way interaction between age and metformin use replicated the well-established association between age and higher IL-6 levels, specifically among those not taking metformin. However, this effect was non-significant among metformin users, suggesting metformin may buffer age-related inflammation, regardless of higher or lower depressive symptoms.

A limitation to consider in the context of this analysis is the uneven age and ethnicity distribution across the sample groups, particularly the overrepresentation of older participants in SALSA. Despite this, the age-related effect on IL-6 levels in both samples supports the presence of inflammaging, aligning with prior research highlighting a strong link between advancing age and heightened IL-6 levels (70–72). These divergent patterns suggest that the MIDUS-SALSA disparities go beyond age, possibly involving ethnicity or the intricate dynamics of being an older person of minority status. Nevertheless, studies with sufficient statistical power to disentangle the effects of age, ethnicity, and their intersection need to further explore this question.

The most important limitation of this study is the cross-sectional nature of the data obtained from the publicly available MIDUS and SALSA datasets (52,54). This study design prevents us from establishing a causal relationship and understanding the dynamic changes that occur over time. Longitudinal follow-up studies are necessary to examine the effects of aging on the associations observed, including the long-term impact of metformin use, the trajectory of inflammatory markers, and the interplay between depressive symptoms and these factors. By incorporating longitudinal data, we can gain a deeper understanding of the complex relationships between metformin use, inflammation, depressive symptoms, and aging processes. Nevertheless, the current study provokes many important and provocative research questions to pursue in the future. Off-label metformin use has exploded in recent years; thus, incorporating more recent waves of data may show an increase in the subsample of metformin users. The current samples did not have enough individuals taking metformin to probe effects of dose and combinations of metformin with other drugs. We also did not have information on medication adherence or barriers to adherence, such as lack of transportation to obtain refills or payment for medication. Other potential social barriers that may impact aging-related health outcomes like inflammation, such as reduced access to healthcare and acculturative stress, should also be considered in future research.

Another notable limitation of this study is the challenge posed by the differences in measurement across both samples, which were derived from two separate studies. These variations in measurement and data collection processes may introduce potential discrepancies and affect the comparability between the cohorts. For example, education, which serves as a proxy for socioeconomic status (SES), was measured differently between the MIDUS and SALSA datasets. In MIDUS, education was measured categorically, whereas in SALSA, it was measured continuously. These discrepancies prevented the inclusion of education as a covariate in the harmonized sample. The inability to include education as a covariate hinders our ability to fully account for the potential confounding effects of SES on the observed associations. Education is a crucial indicator of socioeconomic disparities and plays a significant role in health outcomes. Its omission as a covariate limits our understanding of the influence of SES on the relationships between metformin use, inflammation, depressive symptoms, and aging processes. To evaluate education as a covariate, we performed supplementary analyses within each sample (non-harmonized). These analyses confirmed consistent patterns of metformin's buffering effect on CRP and IL-6, and not TNF-α, in MIDUS. Moreover, no buffering effect was observed in SALSA for all three inflammatory markers (see Supplemental Digital Content, http://links.lww.com/PSYMED/A976). Future research should strive to overcome this limitation by utilizing datasets with consistent measurements of education or by employing other valid measures of SES to capture its impact on the associations under investigation. By including a comprehensive assessment of SES, researchers can better explore the complex interplay between socioeconomic factors, metformin use, inflammation, and depressive symptoms in relation to aging.

This study showed that metformin may have a mitigating role in the link between depression and inflammation in mostly white, highly educated adults. However, the results were mixed, as the benefits did not extend to Latino older adults significantly. The two samples were similar in several ways (BMI, sex demographics, proportion of metformin users, and their diabetes status), except that the group of Latino older adults differed in biological and social hallmarks of risk (older age, minority status, lower education attainment). It is possible that metformin may work differently across varying subgroups, a question needing further exploration in future studies. In sum, the findings of this study point to additional benefits of metformin as a therapeutic agent for aging-related chronic diseases, but also raise important questions about its capacity to promote health equity among diverse population groups.

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FIGURE CAPTIONS

Figure 1. Associations of depressive symptoms with inflammatory marker CRP at different levels of metformin use in the MIDUS and SALSA samples. Parameter estimates (b) are provided for each slope. Estimates in boldface are significant (p<.05).

Figure 2. Associations of depressive symptoms with inflammatory marker IL-6 at different levels of metformin use in the MIDUS and SALSA samples. Parameter estimates (b), standard errors (SE), and p-values are provided for each slope. Estimates in boldface are significant (p<.05).



(A) Metformin Effect Between Depression and CRP in MIDUS





Figure 1. Associations of depressive symptoms with inflammatory marker CRP at different levels of metformin use in the MIDUS and SALSA samples. Parameter estimates (*b*) are provided for each slope. Estimates in **boldface** are significant (p<.05).



(A) Metformin Effect Between Depression and IL-6 in MIDUS





Figure 2. Associations of depressive symptoms with inflammatory marker IL-6 at different levels of metformin use in the MIDUS and SALSA samples. Parameter estimates (*b*), standard errors (SE), and p-values are provided for each slope. Estimates in **boldface** are significant (p<.05).

Table 1

Variables	MIDUS (Mean <u>+</u> SD (range) or %)	SALSA (Mean <u>+</u> <i>SD</i> (range) or %)	t or χ ²
Age	57.36 <u>+</u> 11.56 (35-86)	70.64 <u>+</u> 7.11 (59-101)	<i>t</i> = -36.00*
Sex	56.5% F	58.3% F	χ ² =1.02
Minority Status	21.2%	100%	•
BMI	29.69 <u>+</u> 6.55 (14.99-65.09)	29.68 <u>+</u> 5.91 (15.51-82.72)	<i>t</i> = .013
Comorbidity Burden	.18 <u>+</u> .43 (0-2)	.27 <u>+</u> .50 (0-3)	$t = -5.40^{*}$
Metformin Users	7.2%	7.4%	$\chi^2 = .036$
Diabetes	8.5%	23.4%	χ ² = 110.91**
CESD-D Scores	8.72 <u>+</u> 8.21 (0-54)	10.02 <u>+</u> 10.62 (0-54)	$t = -3.74^*$
Smoking	47.6%	53.9%	χ ² = 1506.39*
Steroid Use	.9%	1.1%	χ ² = .223
Inflammatory Markers			
CRP (pg/mL)	2.59 <u>+</u> 3.01 (.02-19.60)	4.50 <u>+</u> 4.25 (.10-19.90)	$t = -13.68^*$
IL-6 (pg/mL)	1.00 <u>+</u> .70 (.11-5.08)	4.67 <u>+</u> 3.38 (.54-23.64)	<i>t</i> = -41.35*
TNF-α (pg/mL)	2.14 <u>+</u> .67 (.31-5.06)	4.09 <u>+</u> 1.74 (1.10-12.76)	$t = -39.44^*$

Note. Sex = percentage of participants who are female. Minority status = percentage of participants of minority status. Comorbidity burden was indicated by the count of self-reported experience of comorbidities including heart problems, stroke, cancer, and/or liver problems. Diabetes = percentage of participants who are diabetic based on fasting glucose levels. Smoking = current or former smoker. IL-6 = Interlukin-6; CRP = C-reactive protein; TNF- α = tumor necrosis factor-alpha. Independent samples t-tests (df range: 1695-2935) were conducted for continuous variables, whereas chi-square tests of independence (df range: 1-2) were used for categorical variables. No statistical test was conducted for minority status as it was a constant variable in one sample (SALSA).

*p<.001.

		1	2	3	4	5	6	7
1.	CES-D	-	.10**	.09**	.06**	.03	.02	.11**
2.	CRP	.14**	-	.40**	.14**	04	.26**	.04`
3.	IL-6	.10**	.41**	-	.30**	.14**	.10**	.08**
4.	TNF-α	.002	.16**	.26**	-	.17**	.03	.08**
5.	Age	16**	.15**	.15**	.26**	-	14**	.06**
6.	BMI	.11**	.30**	.30**	.16**	05	-	02
7.	Comorbidities	.09**	.18**	.18**	.20**	.31**	.02	-

Correlations among Primary Study Variables in MIDUS and SALSA

Note. Only continuous variables are included. CES-D = Center for Epidemiologic Studies Depression Scale. CRP = C-reactive protein (log). IL-6 = Interleukin-6 (log). TNF- α = Tumor necrosis factor alpha (log). BMI = Body Mass Index. Correlations for MIDUS are below the diagonal and correlations for SALSA are above the diagonal. *p<.05; **p < .01.