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Low-dose aspirin reduces cardiovascular baseline risk, reactivity, and depressed mood in acutely bereaved

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Dedicated to My Parents,
Klara and Walter Karl,
For Their Love
and for Giving Me the Utmost Support Throughout My Whole Life.

Danke!

Table of Contents

Abbreviations

CES-D	Center for Epidemiologic Studies Depression Scale
COX	cyclooxygenase
EKG	electrocardiogram
ELISA	enzyme-linked immunosorbent assay
HF	high-frequency components of heart rate variability
HRV	heart rate variability
ICG-R	Inventory for Complicated Grief – Revised
IL-6	interleukin-6
K_2EDTA	di-potassium ethylenediaminetetraacetic acid
LF	low-frequency components of heart rate variability
M	mean
NFkB	nuclear factor kappa B
PANAS	Positive and Negative Affect Schedule
PGE2	prostaglandin E2
PSS	Perceived Stress Scale
RSA	respiratory sinus arrhythmia
SD	standard deviation
STAI	State and Trait Anxiety Inventory
STAXI-2	State-Trait Anger Expression Inventory - 2
TNF-α	tumor necrosis factor alpha
UCLA	University of California, Los Angeles
vWF	von Willebrand factor
YSL	Yearning in Situations of Loss

Introduction

Increased risk of mortality in bereavement

Bereavement is defined as the situation of having recently lost a significant person in one's life through death (Stroebe et al. 2007). It is a very stressful experience, and in fact, it is rated as the most stressful life event by the Social Readjustment Rating Scale, a scale of stressful life events (Holmes and Rahe 1967). Spousal bereavement has been the main focus of past research because it typically constitutes the loss of a significant person, it is more common than the death of a child and usually has a greater personal impact than the death of a parent in adulthood (Stroebe et al. 2007). Studies have shown that bereavement has an impact on the surviving spouse beyond the psychological grief reaction. Bereavement increases mortality, with odds ratios for all-cause mortality in the first months of bereavement as high as 1.87 (Moon et al. 2014) compared to a nonbereaved population. This phenomenon, termed the widowhood effect, has been known for at least 60 years (Young et al. 1963). It has been documented all over the world, in men as well as in women, and throughout all age groups (Martikainen and Valkonen 1996; Stroebe et al. 2007; Moon et al. 2011; Shah et al. 2013a; Moon et al. 2014; Seifter et al. 2014). Relative risk is likely to be higher in men than in women (Martikainen and Valkonen 1996; Stroebe et al. 2007; Moon et al. 2011), and higher (Martikainen and Valkonen 1996; Stroebe et al. 2007) or the same (Moon et al. 2011) in younger compared to older bereaved subjects. The cause of bereavement seems to be important for its impact on mortality and an unexpected death of a loved one might pose a higher risk (Barry et al. 2002; Buckley et al. 2009b; Shah et al. 2013b), even though some authors question this relationship (Carr et al. 2001; Stroebe et al. 2007).

Increased cardiovascular risk in bereavement

One cause for the increase in mortality following bereavement is an increase in cardiovascular risk (Martikainen and Valkonen 1996; Elwert and Christakis 2008; Rostila et al. 2013; Carey et al. 2014), with a 21.1-fold increase in acute myocardial infarction risk in the first 24 hours following bereavement (Mostofsky et al. 2012). Most studies find the risk normalizes after the acute phase of bereavement, 6 to 12 months after the death of the significant person,

supporting the hypothesis that the mechanism behind the increase in cardiovascular risk might be psychophysiological (Moon et al. 2011) and thus temporary in nature.

Risk factors elevated during acute bereavement

A *Cardiovascular Risk in Bereavement* Study recently advanced the understanding of cardiac risk factors associated with acute bereavement by examining probable factors contributing to the elevated cardiovascular risk. Buckley and colleagues documented an increased hemodynamic burden, altered autonomic function, increased inflammatory parameters, a prothrombotic state, and elevated cortisol levels in addition to the expected increases in measures of anxiety, anger and depression (Buckley et al. 2009a; Buckley et al. 2011; Buckley et al. 2012a; Buckley et al. 2012b).

Early bereavement was shown to be associated with increased 24-hour heart rate (O'Connor et al. 2002; Buckley et al. 2011; Buckley et al. 2012a) and a decrease in heart rate variability (HRV; Buckley, Stannard, et al., 2012), which are both independent predictors of an increased risk for cardiovascular disease (Thayer and Lane 2007; Kizilbash et al. 2008; Lahiri et al. 2008; Tardif 2009). Systolic blood pressure measured over a period of 24 hours, daytime systolic blood pressure and daytime systolic load were higher in acutely bereaved compared to nonbereaved. Within the bereavement group, 24-hour systolic blood pressure, daytime systolic blood pressure, and daytime diastolic blood pressure were higher during the time of acute bereavement than after 6 months (Buckley et al. 2011). Like increased heart rate and decreased HRV, elevated blood pressure is also a known predictor of cardiovascular disease, albeit this correlation might be less strong for diastolic blood pressure (Schillaci et al. 2009; Habib et al. 2015). Inflammatory changes included a higher neutrophil count in acutely bereaved compared to nonbereaved, and lower neutrophil, monocyte and eosinophil counts within the bereavement group after 6 months compared to the acute phase after bereavement. Increases in von Willebrand factor (vWF) antigen and platelet/granulocyte aggregates in the acutely bereaved caused a prothrombotic state. This resolved within the bereavement group after 6 months, evidenced by drops in the platelet count as well as platelet/monocyte granulocytes and vWF after 6 months (Buckley et al. 2012b).

Risk factors elevated during mental stress

Mental stress incurred by bereavement is hypothesized to be the psychological cause of increased cardiovascular risk during that period. Research investigating cardiovascular risk markers during experimental manipulation of mental stress in samples from nonbereaved participants suggests putative mechanisms for the widowhood effect.

Regardless of the underlying cause, mental stress is associated with an increase in sympathetic nervous system activity (Hickam et al. 1948; Anderson et al. 1991), leading to an increase in heart rate and blood pressure, which in turn increases the oxygen demand of the heart (Deanfield et al. 1984), making it more prone to ischemia. Stress also influences autonomic cardiac regulation, evidenced by influences of mental stress on HRV (Lucini et al. 2005; Allen et al. 2007). HRV can be used as a tool to estimate the relation between sympathetic and vagal influences on the heart. It can be analyzed in time and frequency domain measures, and frequency domain measures of HRV can be roughly split into high- (HF) and low-frequency (LF) components. Vagal changes are conveyed via acetylcholine and are brief, because the sinus node is rich in acetylcholine esterase, limiting the time of action of acetylcholine. This makes vagal activity the major contributor to HF components, whereas LF components represent sympathetic or combined activity. The LF/HF ratio can be used as a marker for sympathovagal balance (Huikuri et al. 1999). Variations in heart rate caused by respiration are termed respiratory sinus arrhythmia (RSA). Those variations are in the frequency range of HF components, and thus RSA is mainly used as a measure of vagal influence to the heart. An abnormal 24-hour HRV could be a prognostic parameter for subsequent death in subjects with and without structural heart disease. A low HRV is associated with arrhythmic death, acute myocardial infarction, the progression of atherosclerosis, and death due to heart failure (Huikuri et al. 1999).

Studies in nonbereaved participants have shown that mental stress induces a state of hypercoagulability. Stress is accompanied by increased platelet activity (Levine et al. 1985) and chronic psychological stress leads to an increase in procoagulant molecules and an impairment of fibrinolytic capacity (von Känel et al. 2001); taken together, these biological responses can lead to gradual fibrin

deposition in atherosclerotic plaques and promote the progression of atherosclerosis and coronary artery disease. A marker used to reveal a state of hypercoagulability by detecting early elevated coagulation activity is the level of D-dimers, a fibrin degradation byproduct (von Känel et al. 2001; von Känel and Dimsdale 2003). The level of D-dimers has been described as a prethrombotic marker because it is more sensitive than usual coagulation tests and can detect early elevated coagulation activity without overt thrombosis (von Känel et al. 2001). The aforementioned vWF, a serum protein which is involved in hemostasis, can also be increased by mental arousal (von Känel et al. 2001), and increased plasma levels of vWF may increase the risk of thrombosis (Franchini and Lippi 2006). D-dimers, as well as vWF antigen, are predictive of coronary artery syndromes (Lowe et al. 1998; Thogersen et al. 1998). P-selectin is a protein expressed on the membrane of activated platelets. Increased levels of its membrane bound as well as its soluble form in the plasma can be used as a marker for platelet activation (Blann et al. 2003). It has been linked to increased levels of symptoms of depression and anxiety in caregivers (Aschbacher et al. 2008). Increased levels of P-selectin have been associated with an increased cardiovascular risk (Blann et al. 2003). Hypercoagulability could be boosted by elevated serum cortisol levels which have been reported following acute stress (von Känel et al. 2001; Kudielka et al. 2007) and also in acute bereavement (Buckley et al. 2009b). Cortisol increases the sensitivity of coronary arteries to catecholamines (Reis 1960), which are released after sympathetic activation, and cause vasoconstriction and thus an increase in blood pressure. Corticosteroids have procoagulant properties and patients with Cushing's disease, a condition characterized by hypercortisolism, show an increase in factor VIII and vWF, and a defective fibrinolytic potential (von Känel et al. 2001).

Psychological stress and inflammation responses are closely linked. Acute and chronic mental stress have repeatedly been shown to cause an increase in inflammatory markers including interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α), two pro-inflammatory cytokines (Brydon, Edwards, Mohamed-Ali, & Steptoe, 2004; Sorenson, Janusek, & Mathews, 2013; Steptoe, Willemsen, Owen, Flower, & Mohamed-Ali, 2001; Von Känel, Kudielka, Preckel, Hanebuth, & Fischer, 2006; Von Känel, Dimsdale, et al., 2006). The expression of IL-6 is mediated by nuclear factor kappa B (NFkB; Epstein, Barnes, & Karin, 1997), which has been

shown to be activated in peripheral blood mononuclear cells by acute psychosocial stress (Bierhaus et al. 2003). IL-6 synthesis is also activated by prostaglandin E2 (PGE2; Noguchi, Maeda, Ruwanpura, & Ishikawa, 2005; Williams & Shacter, 1997). Psychosocial stress can increase PGE2 plasma levels in rats (Morimoto et al. 1991). Increased levels of IL-6 have been linked to an increased cardiovascular risk (Ridker et al. 2000b) and IL-6 also plays a role in creating a procoagulant milieu (Hartman and Frishman 2014). For TNF-α an association with cardiovascular events is less clear, with some studies correlating high levels to the recurrence of adverse cardiovascular events (Ridker et al. 2000a; Sorenson et al. 2013), while others do not find an effect (Goebel et al. 2000).

Effects of aspirin

Aspirin is well known for its dose-independent anti-platelet effect (Furuno et al. 2011) counteracting thrombus formation in the arterial branch of circulation, which is where most blood clots causing acute cardiovascular events happen (Warkentin 2012). This effect is mediated through the irreversible inhibition of cyclooxygenases (COX-1 and COX-2) and the following inhibition of thromboxane A2. Aspirin also increases flow-mediated dilation in the brachial artery, which is a measure for endothelial function (Flammer et al. 2012). Endothelial *dys*function is one of the hallmarks of atherosclerosis (Davignon 2004), and thus cardiovascular disease. Flow-mediated dilation was found to be increased at 81 mg and 162 mg of aspirin and decreased at higher doses, suggesting a more beneficial effect of lower doses (Furuno et al. 2011). The effect of aspirin on endothelial function could be mediated by the effect of aspirin on nitric oxide formation (Monobe et al. 2001).

Furthermore, there are studies suggesting that even low-dose aspirin has an anti-inflammatory effect. Low-dose aspirin was shown to lower levels of circulating TNF-α (Shackelford et al. 1997; Goldstein et al. 2006). Von Känel and colleagues found that a five-day treatment with 100 mg of aspirin led to a less pronounced increase and lower absolute level of IL-6 after a Trier Social Stress Test (von Känel et al. 2008a). A study in diabetic patients also found that aspirin lowers levels of IL-6 and C-reactive protein (Hovens et al. 2008). A potential mechanism through which aspirin mitigates the effect on IL-6 is by inhibiting NFkB (Kopp and Ghosh 1994), which is responsible for the expression of IL-6. Another

mechanism could be the inhibition of COX-2, which is responsible for PGE2 synthesis (Caughey et al. 2001), leading to a reduction in PGE2-induced IL-6 synthesis activation. Aspirin further exerts its anti-inflammatory properties by increasing the formation of nitric oxide, which inhibits leukocyte endothelium interactions (Paul-Clark 2004; Hetzel et al. 2013). Lastly, another important mechanism mediating the anti-inflammatory properties of aspirin is the induction of aspirin-triggered lipoxins. Lipoxins are mediators thought to play a crucial role in the resolution of inflammation, and aspirin – even in a low dose – can trigger epimeric forms of these lipoxins (Maderna and Godson 2009).

Elevated inflammatory parameters do not only play a role in bereavement. As of recently, activation of inflammatory pathways is seen as a key factor in some forms of depression, and at least part of the action of classical antidepressant medications can be explained by their effect on the immune system (Berk et al. 2013). In an open-label clinical study, administration of 160 mg of aspirin was able to shorten the onset of action of fluoxetine (Rahola 2012). A study in patients undergoing coronary angiography found aspirin use to be associated with less depression, anxiety, and worry reported by the patient (Ketterer et al. 1996). Non-adherence to aspirin was associated with more depressive symptoms in survivors of acute coronary syndrome (Rieckmann et al. 2011).

There are also some less known and less established effects of aspirin. A study of De Meersman and colleagues found effects of aspirin on HRV. In their study participants ingested a relatively high dose of aspirin (975 mg per day) over a 2.5-day period. They found the high-frequency components of HRV, likely representing vagal tone, increased in participants taking aspirin (De Meersman et al. 2000). In another study, aspirin was found to influence resting cortisol levels, which were lower in participants taking aspirin. In terms of reactivity, however, aspirin had no effect on changes in cortisol levels (von Känel et al. 2008b). Another potential benefit of aspirin can be inferred from the research of Hermida and colleagues. They used low-dose aspirin administered at bedtime in untreated mildly hypertensive patients and found a highly significant reduction of blood pressure (Hermida et al. 2003; Hermida et al. 2005b; Hermida et al. 2005a; Hermida et al. 2009). A potential mechanism for this could be an aspirin-induced inhibition of angiotensin II (Hermida et al. 2003; Hermida et al. 2005b; Hermida et al. 2005a; Hermida et al. 2009).

Reactivity during bereavement

Grief and distress caused by bereavement do not have the same intensity throughout the day but come in waves when memories and reminders of the deceased trigger intense grief reactions (Shear et al. 2011). Bereaved individuals show higher physiological reactivity to stressors (Hahn et al. 2014), which could amplify increased cardiovascular risk in bereavement during periods of acute stress. For this reason, measures of reactivity and recovery during periods of intense distress may differ from baseline shifts in cardiovascular functioning in bereavement, making it important to evaluate baseline shifts, reactivity, and recovery. This is especially relevant when investigating an intervention aimed at preventing an increase in risk caused by this distress. In this way, stress caused by grief is similar to stress caused by anger, which also tends to come in outbursts rather than being continuously present. There are promising reports on the use of aspirin in the prevention of an increased cardiovascular risk following anger, providing evidence that aspirin can also prevent changes in reactivity rather than just baseline. In a study by Mittleman and colleagues aspirin lowered the risk of the onset of acute myocardial infarction in patients who reported anger preceding an acute myocardial infarction (Mittleman et al. 1995). Further evidence for the potential of aspirin to affect reactivity comes from the aforementioned study of the effect of aspirin on IL-6 (von Känel et al. 2008a).

Several techniques have been used in past research to simulate physiological reactions that usually occur in normal everyday life in a laboratory setting. One of these established techniques is the anger recall task (Prkachin et al. 2001). A newer, less established technique is the separation recall task, an interview that was developed in close accordance with the anger recall interview. The reactions to the separation recall task were found to be comparable to the well-established mental arithmetic task (Ehrenthal et al. 2011).

Aims and hypotheses

Despite data showing incontrovertible evidence of the widowhood effect, no studies as of now have attempted primary prevention of increased cardiovascular risk during acute bereavement. Considering the discussed parameters potentially contributing to an increased cardiovascular risk during acute bereavement, low-dose aspirin is a good candidate for a preventive medical intervention. In addition,

because aspirin is inexpensive, widely available and does not require a prescription, the public health feasibility of aspirin use in acute bereavement is very high, if it is demonstrated to be efficacious and acceptable to patients. Other studies have already shown the feasibility of short-term prevention using aspirin in the context of periods of heightened cardiovascular risk in healthy participants and subjects with increased cardiovascular risk (Shaw et al. 2009; Tofler et al. 2013).

The aim of the present study was to determine the feasibility and efficacy of low-dose aspirin compared to placebo in reducing markers of cardiovascular risk in bereaved compared to nonbereaved participants. The hypotheses were: 1) that the use of low-dose aspirin taken over the course of 5 days would be feasible among acutely bereaved people, and effective in lowering the elevated baseline of cardiovascular risk markers in acutely bereaved; 2) that bereaved participants would show a greater increase in measures of cardiovascular risk in response to a specific stressor than nonbereaved participants and that aspirin would reduce the reactivity in response to the stressor; and 3) that aspirin would ameliorate depressed mood in bereaved participants.

Methods

Participants

The present study was a randomized, double-blind, placebo-controlled pilot trial. The study sample included 10 bereaved participants and 12 nonbereaved control participants. Participants were eligible to participate in the study if they were between 30 and 85 years old and were not currently using aspirin. Bereaved participants were recruited from the community within 30.00 days (SD = 14.67) of the death of their spouse through two methods. First, letters were mailed to widow(er)s of recently deceased individuals, who had posted an obituary in a local newspaper or online. This has previously been described as a productive and acceptable recruitment method (Schlernitzauer et al. 1998). Second, partners of patients from inpatient hospice care units were recruited. The healthy control group, who had not experienced the death of a loved one, or the terminal diagnosis of their partner, in the six months prior to the study, was recruited through online advertisements and via e-mail newsletters. Recruitment and data collection spanned a limited time frame of six months.

Participants were excluded for current use of aspirin. Furthermore, participants were excluded for immunologic diseases, if they reported infections or injuries in the two weeks prior to the first assessment, or for the use of medication where aspirin use is contraindicated. All participants spoke English. Participants received monetary compensation for their participation in the study. They received $25 for the completion of the first laboratory visit and $50 for the completion of the second laboratory visit. An Institutional Review Board of the University of Arizona and of the Tucson Medical Center approved the study, and all participants gave their informed consent.

Procedures

The study consisted of two laboratory visits (Figure 1). To assess the effects of bereavement and the effects of low-dose aspirin on physiological parameters, participants had their blood pressure and electrocardiogram (EKG) measured, and blood drawn at both visits. To assess the effects of psychological stress on physiological parameters, these measures were taken before and after a stress task at the second laboratory visit.

Participants were screened over the phone for current use of aspirin and for age. If they were eligible to participate in the study, the first laboratory visit was scheduled. This appointment took place between 7 and 50 days (M=30.00, SD=14.67) after the death of the spouse. Participants were asked to refrain from strenuous exercise, caffeine intake or eating a heavy meal in the 3 to 4 hours prior to the laboratory visits. All visits were scheduled in the afternoon, to minimize the influence of circadian biological rhythms in biomarkers. After providing informed consent, the participants' medical history was assessed. Then, baseline levels of selected cardiovascular risk factors were assessed: blood pressure, heart rate and HRV assessed through a single lead EKG, plasma levels of IL-6 and TNF-α as inflammatory parameters, plasma levels of vWF, D-dimer, and P-selectin as prothrombotic parameters, and cortisol from saliva, as well as depressive symptoms and symptoms of complicated grief. At the end of the first assessment, participants were scheduled for a second laboratory visit at least five days after the first visit, and were randomized to receive either aspirin or placebo. Twelve participants were randomized to receive aspirin, and 10 participants were randomized to receive placebo. Of the participants randomized to receive aspirin, 5 were bereaved and 7 were nonbereaved control participants. Of the participants randomized to receive placebo, 5 were bereaved and 5 were nonbereaved control participants. Both participants and investigators were blinded to the treatment group. Participants were given an envelope containing five tablets and instructed to take one tablet at bedtime on the five days prior to the second laboratory visit. The timing of medication ingestion was chosen for a potential effect on blood pressure (Hermida et al. 2003; Hermida et al. 2005a; Hermida et al. 2005b; Hermida et al. 2009) and because of lower risk of gastric lesions when using aspirin in the evening (Moore and Goo 1987). Participants were contacted via telephone in the evening of each of the five days to remind them about taking the medication and to ask about possible side effects.

The second laboratory visit took place between 5 and 33 days (M=10.55, SD=7.51) after the first laboratory visit and between 14 and 64 days (M=38.00, SD=16.87) after the death of the spouse. At the second laboratory visit, participants' social background was assessed, and bereaved participants additionally answered questions about the relationship with their deceased spouse and their bereavement experience. Questionnaires were used to assess

symptoms of complicated grief, stress, loneliness, attachment style, sleep quality, social support, yearning, state anger, and state anxiety. Then, the same baseline measures from blood, saliva, blood pressure and EKG were taken, in order to assess the effect of the study medication on the baseline of these measures and to detect a baseline shift. Depressive symptoms and symptoms of complicated grief were assessed again to detect a baseline shift in those measures as well. Participants rested in the laboratory for 20 minutes before all sample collections and study procedures.

During the second laboratory visit, reactivity was assessed by means of a separation recall task (Ehrenthal et al. 2011). In this interview, participants were asked to immerse themselves in a situation in which they felt alone and abandoned. The objective was to elicit similar physiological and psychological effects to those that participants experienced when they were in that specific situation. The interview was originally developed in German. For use in the present study, it was translated into English and checked for accuracy through back-translation. Nonbereaved control participants were asked to immerse themselves in a situation in which they felt very alone and abandoned, and had wished *someone* had been there for them. To mimic pangs of grief as accurately as possible, while at the same time trying to keep conditions as equal as possible between bereaved and control participants, the separation recall was minimally modified for bereaved participants. Bereaved participants were instructed to recall a situation after their recent loss, in which they felt very alone and abandoned and had wished *their loved one* had been there for them. After choosing a situation, participants were asked to rank the stressfulness of their situation on a scale of *0* to *10*, with *0* meaning *not stressful at all* and *10* meaning *extremely stressful*. If the situation was rated at less than 7 on that scale, participants were asked to think of a different, more stressful situation to ensure that it was sufficient to elicit a detectable reaction. Participants then talked about the situation for at least 5, but no longer than 10 minutes. Whenever necessary, a set of standardized prompts was used to keep participants talking and thereby immersed in the situation. Participants were seated throughout the separation recall task. After the task, participants were asked to rate the stressfulness of the situation again, and the same measures from blood, saliva, blood pressure and EKG were repeated. Additionally, participants were asked to fill out questionnaires on state anxiety,

state anger and affect immediately before and after the separation recall task. The EKG was recorded continuously, therefore assessing heart rate before, during, and after the task.

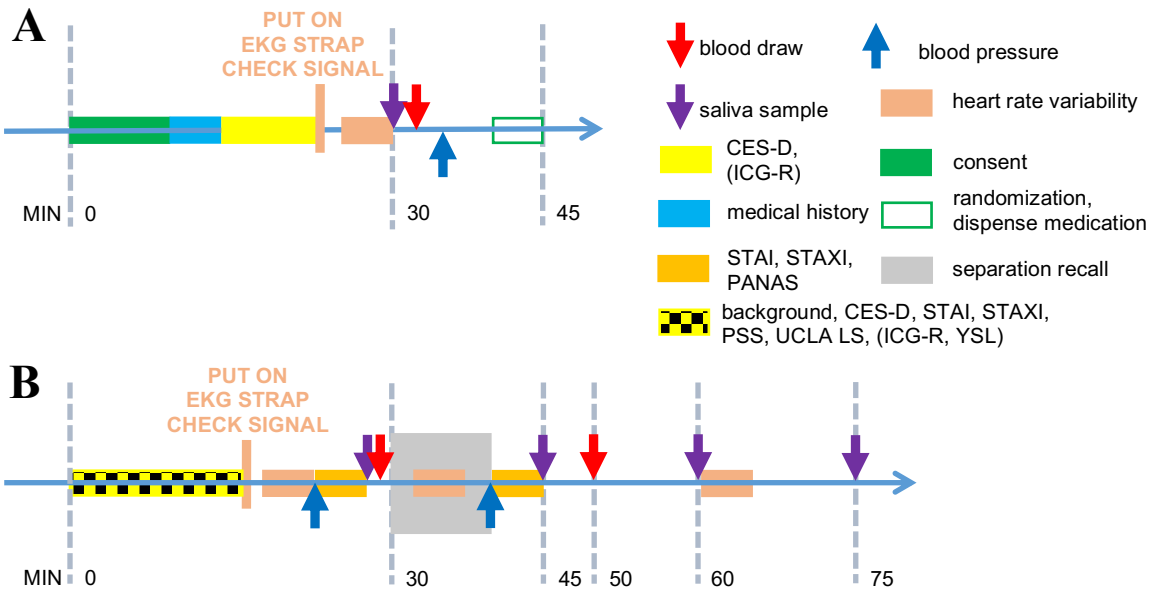

Figure 1. Time scales for the first (A) and second laboratory visit (B) for the study comparing the effects of 5 days of low-dose aspirin versus placebo on cardiovascular and psychological markers in bereaved versus nonbereaved participants. It was conducted in Tucson, AZ between March and September 2014. MIN = minute; CES-D = Center for Epidemiologic Studies Depression Scale; ICG-R = Inventory for Complicated Grief – Revised; STAI = State and Trait Anxiety Inventory; STAXI = State-Trait Anger Expression Inventory – 2; PANAS = Positive and Negative Affect Schedule; UCLA LS = UCLA loneliness scale; PSS = Perceived Stress Scale; YSL = Yearning in Situations of Loss Scale.

Plasma biomarker analysis

Blood sampling was performed in a seated position with a 21-gauge butterfly needle. A tourniquet was used to initiate blood flow but was removed as soon as possible to avoid platelet activation. Blood was drawn into one 6 mL tube spray-coated with K_2EDTA and into three 2.7 mL tubes containing 3.2 % sodium citrate. The tubes were immediately stored in a cooler and centrifuged within the first hour after the blood draw. Plasma was separated and immediately frozen at -80°C. Enzyme-linked immunosorbent assay (ELISA) based immunoassays were used to quantify IL-6, TNF-α (R&D Systems, Minneapolis, USA), vWF (Eagle Biosciences, Inc., Nausha, USA), D-dimer, and P-selectin (RayBiotech, Norcross, USA). Plasma samples were diluted prior to analysis according to manufacturer instructions for TNF-α, vWF, D-dimer, and P-selectin. For IL-6, samples with levels above the linear range were further diluted 1:10 and repeated. Assays were linear

over the following concentration ranges; IL-6: 0.09 – 4.9 pg/mL, TNF-α: 0.0 – 816.4 pg/mL, vWF: 2.0 – 36.5 µg/mL, D-dimer: 0.8 – 19.7 pg/mL, and P-selectin: 0.03 – 4.3 ng/mL. For each assay, baseline and post-intervention samples of the same individual were analyzed in the same batch and each sample was analyzed in duplicate. The assay values were log transformed prior to statistical analysis to resolve the skewed nature of the data.

Salivary cortisol analysis

Saliva was collected for cortisol analysis using Salivettes (Sarstedt Inc., Newton, USA). Salivary cortisol has been shown to accurately reflect free serum cortisol levels (Duplessis et al. 2010). Participants were seated while providing the saliva samples. After collection, saliva was transported in a cooler before being frozen at -80°C until analysis. At the study's end, all salivary cortisol samples were assessed by high sensitivity ELISA in the laboratory of Clemens Kirschbaum (Dresden, Germany). The lower detection limit was 0.09 nmol/L. The mean intra-assay coefficient was 5 %. The mean inter-assay coefficient was 8 %. All samples from a participant were analyzed in duplicate in the same assay to minimize variability. The assay values were log transformed prior to statistical analysis to resolve the skewed nature of cortisol data.

Blood pressure measurement

Blood pressure was measured using Microlife Upper Arm Automatic Digital Blood Pressure Monitor, model BP3GT1-6X (Microlife USA, Inc., Clearwater, USA). For the measurement, participants were instructed to sit comfortably and relax, with their legs uncrossed, thereby complying with recommendations from the American Heart Association for blood pressure measurement (Pickering et al. 2005). Blood pressure was measured on the same arm throughout all assessments.

Electrocardiography

A single lead EKG was acquired with a sampling frequency of 1000 Hz using the Zephyr BioHarness 3.0 (Zephyr Technology Corp., Annapolis, USA). The BioHarness consists of a chest strap and an electronic module that attaches to the strap and logs the data. Data was downloaded using the BioHarness Log

Downloader (V1.0.29.0). The BioHarness is also equipped with an internal clock and all data is logged with a timestamp. The internal clock of the device was synchronized with a computer before each assessment. Signal quality was checked through wireless data transmission to a smartphone app (SenseView, Mobili d.o.o., Ljubljana, Slovenia).

Five-minute EKG periods were used to calculate HRV, which is a time frame that has been recommended by the Task Force of The European Society of Cardiology and The North American Society of Pacing and Electrophysiology (Camm et al. 1996). For baseline measures, participants were instructed to sit relaxed for 5 minutes with their eyes open and without talking. For EKG measurement during the separation recall, the 5-minute period exactly in the middle of the separation recall was used, regardless of the length of the separation recall. The EKG measurement continued for 30 minutes after the separation recall. All participants were in sinus rhythm.

The EKG data was analyzed using QRSTool (Allen et al. 2007), which allows for automated detection of R-spikes in the EKG and generation of an interbeat interval series. The generated interbeat interval series was manually scanned for artifacts and ectopic beats. Ectopic beats were identified by a characteristic too-short-too-long pattern in the interbeat interval series. After the interbeat interval series was obtained, CMetX (Allen et al. 2007) was then used to obtain a measure of RSA.

Questionnaires

The Center for Epidemiologic Studies Depression Scale (CES-D; Radloff, 1977) was used to assess depressive symptoms on the first and second laboratory visit. It is comprised of 20 statements, whose frequency during the last week is rated as either *rarely or none of the time*, *some or a little of the time*, *occasionally or a moderate amount of time*, or *most or all of the time*.

The Inventory for Complicated Grief – Revised (ICG-R; Prigerson et al., 1995) was used to assess indicators of complicated grief on the first and second laboratory visit, such as anger, disbelief, and hallucinations. Complicated grief has been renamed by the Diagnostic and Statistical Manual of Mental Disorders Fifth Edition as “Persistent Complex Bereavement Disorder”. The disorder may result from the death of a person to which the bereaved person had a close relationship

if they exhibit symptoms including persistent longing and yearning, preoccupation with the deceased, disbelief, and difficulty accepting the death. Deficits in work and social functioning may result and the disorder can lead to harmful health behaviors. The symptoms of complicated grief have been found to predict long-term dysfunction. Its prevalence is estimated at 2.4 to 4.8 % (American Psychiatric Association 2013). The ICG-R is comprised of 19 first-person statements reflecting bereavement-related thoughts and behaviors. These statements are rated according to the frequency of their occurrence on a scale ranging from *0* to *4*, where *0* is *never* and *4* is *always*. With a score over 25, an individual is considered to be at a high risk for requiring clinical care.

Affect was measured on the second laboratory visit right before and after the separation recall task using the Positive and Negative Affect Schedule (PANAS; Watson, Clark, & Tellegen, 1988). This scale assesses positive affect and negative affect, which have been found to be highly distinct dimensions that are only weakly correlated. The PANAS consists of 20 adjectives reflecting either positive or negative affect. The items are rated on a scale of *1* to *5* (*very slightly or not at all* to *extremely*), based on how much the participant felt that way within a particular timeframe. In the present study, the PANAS was used to assess the momentary mood state of the participants. Hence, participants were asked to rate the items based on the way they felt *right now* (i.e., at the moment they were filling out the questionnaire).

The State and Trait Anxiety Inventory (STAI; Spielberger, Gorsuch, Lushene, Vagg, & Jacobs, 1983) was used to assess anxiety. It assesses two dimensions of anxiety: state and trait anxiety. State anxiety is situationally-related and can be described as fear or nervousness, whereas trait anxiety is a more consistent form of anxiety that is best described as stress or worry. State anxiety was assessed on the second laboratory visit right before and after the separation recall task. Trait anxiety was also assessed on the second laboratory visit. The 40 items of the scale are first-person statements and participants indicate on a 4-point Likert scale ranging from *not at all* to *very much so* to what extent they agree with these statements. The instructions for the first 20 of the 40 items ask participants to rate the items based on how they feel at this moment, thereby assessing state anxiety, whereas the other 20 ask about how participants generally feel, assessing trait anxiety.

Anger was measured using the State-Trait Anger Expression Inventory - 2 (STAXI-2; Spielberger, 1999), which is a 57-item questionnaire comprised of six scales. These scales measure state anger, trait anger, anger-in (anger is experienced but not expressed), anger-out (anger is experienced and directed outward against other people or objects), anger control - in (anger is controlled by calming down or cooling off), and anger control - out (anger is controlled by preventing the expression of anger towards other people or objects). Items 1 to 15 assess the state anger, items 16 to 25 assess the trait anger, and items 26 to 57 assess the four other scales. Participants endorse the items on a 4-point Likert scale ranging from *not at all* to *very much so*. State anger was measured on the second laboratory visit right before and after the separation recall task. The other measures of anger were also assessed on the second laboratory visit.

Psychological stress was measured on the second laboratory visit using the Perceived Stress Scale (PSS; Cohen, Kamarck, & Mermelstein, 1983). It measures the degree to which situations in one's life are perceived as stressful. In this context, stressful can be defined as the amount to which people feel that the demands of a situation exceed their personal coping abilities. Higher scores on this scale can predict biological markers of stress, such as increased cortisol (Malarkey et al. 1995) and risk for stress-related disease (Cohen et al. 1993). The scale lists a number of feelings and thoughts and participants indicate on a scale of *0* to *4* (*never* to *often*) how often they experienced these feelings and thoughts in the past month.

Loneliness was assessed on the second laboratory visit by means of the UCLA Loneliness Scale (Russell et al. 1978). It consists of 20 items, which are subjective measures of loneliness and social isolation. They are rated on a scale of *1* to *4* (*never* to *always*) based on how often participants experience that feeling.

The Yearning in Situations of Loss Scale (YSL; O'Connor & Sussman, 2014) was used to assess yearning on the second laboratory visit. It measures yearning in bereavement, romantic breakup or homesickness. In this study, the YSL scale specific to bereavement was used. It is comprised of 21 statements that are rated on a scale of *1* to *5* (*never* to *always*), based on how often participants generally feel that way.

Study medication

Tablets containing either 81 mg of aspirin (Aspirin 81 mg Enteric Coated Tablets, Walgreens, Deerfield, IL, USA) or placebo (Standard Homeopathic Company, Los Angeles, CA, USA) were administered. Placebo tablets were composed of lactose and sucrose powder. Both the participants and the investigators were blind to what medication participants were taking.

Data analysis

Statistical analyses were performed using SPSS version 21 (SPSS, Chicago, IL, USA). The study design resulted in four different experimental groups: 1) bereaved participants who received aspirin, 2) bereaved participants who received placebo, 3) nonbereaved control participants who received aspirin, and 4) nonbereaved control participants who received placebo. For the comparison of bereaved participants and nonbereaved control participants, 10 bereaved participants were age-matched with 9 control participants. For all other investigations, all 22 participants were analyzed. Differences between groups were analyzed using analysis of variance for continuous and chi-square test for categorical variables. Differences within one group between different time points were analyzed using paired-samples t-test. Cohen's *d* is reported as a measure of effect size.

For the comparison of the difference in change from one time point to the next within one variable and between experimental groups, change scores were calculated for all variables (e.g. change score for systolic blood pressure from the first to the second laboratory visit = systolic blood pressure at the second laboratory visit – systolic blood pressure at the first laboratory visit). A change score yielded positive values for an increase in the respective variable from one time point to the next, and negative values for a decrease in the respective variable from one time point to the next.

The primary aim of this study was to assess the impact of aspirin on cardiovascular risk in bereavement. For this purpose, a compound cardiovascular risk variable was created. Incorporated in this variable were IL-6 as an inflammatory parameter; vWF, P-selectin, and D-dimers as prothrombotic parameters; heart rate, HRV and blood pressure as hemodynamic parameters; and cortisol as a parameter for stress. Participants' values were ranked from

highest to lowest for HRV and from lowest to highest for all other variables and attributed a rank number ranging from *1* to *10* according to the rank order. Then, an average was calculated over the 8 parameters to yield a cardiovascular risk variable ranging from *1* to *10*, with *1* being the lowest cardiovascular risk and *10* being the highest (see Table 1 for an example).

Table 1. Example of the attribution of rank numbers to variables. The variable *average heart rate during the first laboratory visit* is brought into order from the lowest to the highest value and every value is attributed an individual rank number between *1* and *10*.

participant ID number	average heart rate during first laboratory visit	attributed rank number
139	57.23	1
103	58.49	1.43
112	58.61	1.86
111	60.15	2.29
113	60.47	2.71
101	63.39	3.14
141	65.59	3.57
134	66.75	4
106	68.11	4.43
136	69.91	4.86
109	70.26	5.29
107	71.45	5.71
133	72.02	6.14
138	72.29	6.57
131	73.67	7
135	76.72	7.43
132	79.28	7.86
105	81.83	8.29
108	83.59	8.71
104	85.25	9.14
140	85.95	9.57
110	87.13	10

Table 2. Demographic characteristics of bereaved participants and nonbereaved control participants. For the comparison of bereaved and nonbereaved participants at baseline, 9 nonbereaved participants were age-matched with the 10 bereaved participants. The study compared the effects of 5 days of low-dose aspirin versus placebo on cardiovascular and psychological markers in bereaved versus nonbereaved participants. It was conducted in Tucson, AZ between March and September 2014.

	Bereaved (n=10)		Nonbereaved (n=9)			
	mean	SD / %	mean	SD / %	F / χ^2	*p*
Age	66.60	12.40	57.67	6.38	3.76	0.07
Sex (*female*)	7	70 %	4	44.44 %	1.27	0.26
Race (*white*)	10	100 %	8	88.89 %	1.17	0.28
Ethnicity (*Hispanic*)	1	10 %	2	22.22 %	0.53	0.47
BMI	28.82	5.85	30.14	7.31	0.19	0.67
Currently smoking	0	0 %	1	11.11 %	1.17	0.28
Pack years	16.07	24.09	2.22	6.67	2.77	0.12
Alcohol (*drinks per week*)	6.85	7.67	1.87	2.52	3.44	0.08
Caffeine (*drinks per week*)	14.05	12.63	8.56	6.54	1.37	0.26
Time since death (*days*)	30	14.67				
Time between assessments (*days*)	8.00	4.03	10.11	7.42	0.61	0.45
Time with spouse (*years*)	38.60	19.72				
Preparedness (*yes*)	6	60 %				
Primary caretaker (*yes*)	9	90 %				
Caretaking time (*months*)	33.83	37.03				

SD = standard deviation; BMI = Body Mass Index, calculated as weight (kg) / (height (m))^2; pack years calculated as packs (20 cigarettes) smoked per day times total years smoked

Table 3. Demographic characteristics of participants randomized to receive aspirin and participants randomized to receive placebo. For this comparison, all 22 participants were analyzed. The study compared the effects of 5 days of low-dose aspirin versus placebo on cardiovascular and psychological markers in bereaved versus nonbereaved participants. It was conducted in Tucson, AZ between March and September 2014.

	Aspirin (n=12)		Placebo (n=10)			
	mean	SD / %	mean	SD / %	F / χ^2	*p*
Bereaved	5	41.67 %	5	50 %	0.15	0.70
Age	59.75	15.60	57.20	12.34	0.18	0.68
Sex (*female*)	6	50 %	6	60 %	0.22	0.64
Race (*white*)	10	83.33 %	10	100 %	1.83	0.18
Ethnicity (*Hispanic*)	2	16.67 %	3	30 %	0.55	0.46
BMI	30.89	7.66	25.59	3.01	4.22	0.05
Currently smoking	1	8.33 %	0	0 %	0.87	0.35
Pack years	12.98	23.09	2.51	5.39	1.95	0.18
Alcohol (*drinks per week*)	3.50	4.86	5.85	7.33	0.81	0.38
Caffeine (*drinks per week*)	11.42	8.66	10.45	12.36	0.05	0.83
Time between assessments (*days*)	9.17	7.98	12.20	6.94	0.89	0.36

SD = standard deviation; BMI = Body Mass Index, calculated as weight (kg) / (height (m))^2; pack years calculated as packs (20 cigarettes) smoked per day times total years smoked

Results

Comparison of bereaved and nonbereaved participants at the first laboratory visit

To compare the sample of bereaved participants in the present study to existing studies of bereaved populations in the literature, 9 nonbereaved control participants (average age 57.7 years, 44.4 % female) were age-matched with 10 bereaved participants (average age 66.6 years, 70.0 % female; see also Table 2). Bereaved participants reported more depressive symptoms than nonbereaved control participants (CES-D score 23.80 vs. 8.42, $p<0.001$, $d=1.46$, [Karl et al. 2018]). In physiological measures at baseline, bereaved participants showed higher systolic blood pressure (133.45 vs. 128.44 mmHg, $p=0.48$, $d=0.33$), heart rate (71.94 vs. 70.76 beats per minute, $p=0.79$, $d=0.12$), number of ectopic beats in a 5-minute interval (4.50 vs. 0.67, $p=0.60$, $d=0.60$), TNF-α (log TNF-α 2.05 vs. 1.95, $p=0.68$, $d=0.20$), and cortisol (log cortisol 2.28 vs. 1.99, $p=0.34$, $d=0.46$), and lower heart rate variability (log RSA 4.39 vs. 4.83, $p=0.57$, $d=0.28$), but these differences were not significant (Table 4). Nonbereaved participants showed significantly higher levels of D-dimer (log D-dimer 7.99 vs. 7.48, $p<0.05$, $d=1.24$) and vWF (log vWF 2.92 vs. 2.48, $p<0.05$, $d=1.08$). Also, nonbereaved participants showed non-significantly higher levels of diastolic blood pressure (84.44 vs. 78.80 mmHg, $p=0.33$, $d=0.47$), IL-6 (log IL-6 1.11 vs. 0.54, $p=0.12$, $d=0.77$), P-selectin (log P-selectin 3.91 vs. 3.83, $p=0.54$, $d=0.30$) and of the composite cardiovascular risk variable (5.92 vs. 5.13, $p=0.20$, $d=0.62$).

Table 4. Comparison of bereaved participants and nonbereaved control participants at baseline at the first laboratory visit. The study compared the effects of 5 days of low-dose aspirin versus placebo on cardiovascular and psychological markers in bereaved versus nonbereaved participants. It was conducted in Tucson, AZ between March and September 2014.

Variable	Nonbereaved (n=9)		Bereaved (n=10)				
	mean	SD	mean	SD	F	*p*	*d*
systolic blood pressure	128.44	16.74	133.45	13.68	0.51	0.48	0.33
diastolic blood pressure	84.44	10.65	78.80	13.37	1.02	0.33	0.47
heart rate	70.76	11.05	71.94	7.92	0.07	0.79	0.12
log RSA	4.83	2.13	4.39	1.03	0.34	0.57	0.28
ectopic beats	0.67	1.66	4.50	11.14	1.04	0.32	0.60
log cortisol	1.99	0.66	2.28	0.61	0.95	0.34	0.45
log vWF	2.92	0.37	2.48	0.45	5.37	0.03	1.08
log D-dimers	7.99	0.50	7.48	0.32	7.16	0.02	1.24
log IL-6	1.11	0.69	0.54	0.81	2.74	0.12	0.77
log TNF-α	1.95	0.51	2.05	0.44	0.18	0.68	0.20
log P-selectin	3.91	0.39	3.83	0.19	0.39	0.54	0.30
CV risk	5.92	1.42	5.13	1.13	1.81	0.20	0.62
CES-D	8.78	10.29	23.8	10.29	10.09	0.01	1.46

SD = standard deviation; RSA = respiratory sinus arrhythmia; vWF = von Willebrand factor; IL-6 = interleukin-6; TNF-α = tumor necrosis factor alpha; CV = cardiovascular; CES-D = Center for Epidemiologic Studies Depression Scale

Effect of aspirin on depressive symptoms

Five-day treatment with low-dose aspirin had an impact on depressive symptoms measured with the CES-D (Figure 2). All bereaved participants receiving aspirin reported less depressive symptoms at the second laboratory visit compared to the first visit, while only one of the five bereaved participants receiving placebo reported a decrease in depressive symptoms (Karl et al. 2018). A dichotomous variable yielding a *1* for a *decrease in depressive symptoms from first to second laboratory visit* and a *0* for *no decrease in depressive symptoms from first to second laboratory visit* was significantly different for the aspirin versus the placebo group within bereaved participants (χ^2=6.67, p<0.01, d=3.54, [Karl et al. 2018]).

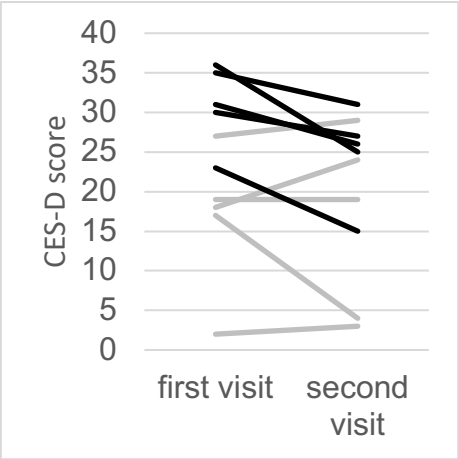

Figure 2. Change from the first to the second laboratory visit in the Center for Epidemiologic Studies Depression Scale (CES-D) only in bereaved participants. Each line represents data from an individual participant. Grey lines represent bereaved participants receiving placebo, black lines represent bereaved participants receiving aspirin. The study compared the effects of 5 days of low-dose aspirin versus placebo on cardiovascular and psychological markers in bereaved versus nonbereaved participants. It was conducted in Tucson, AZ between March and September 2014.

Effect of aspirin on the baseline of physiological markers

Low-dose aspirin administered for five days affected the baseline levels of hemodynamic, prothrombotic, and inflammatory measures, as well as the composite cardiovascular risk variable (Figure 3).

Diastolic blood pressure decreased significantly from the first to the second laboratory visit both in participants taking aspirin and participants taking placebo (aspirin group 84.25 to 77.21 mmHg, $p<0.01$, $d=0.61$; placebo group 81.50 to 78.25 mmHg, $p<0.03$, $d=0.25$). The rank number for diastolic blood pressure decreased non-significantly in participants receiving aspirin (5.93 to 5.46, $p=0.15$, $d=0.18$) and increased non-significantly in participants receiving placebo (4.99 to 5.54, $p=0.08$, $d=0.18$) from the first to the second laboratory visit. However, when comparing the change between groups, the change scores of the rank number for diastolic blood pressure between the aspirin and the placebo group were significantly different (aspirin group -0.46 vs. placebo group +0.56, $p<0.03$, $d=1.05$). The change scores of the absolute values of diastolic blood pressure were not significantly different between the aspirin and placebo group, but there was a trend towards a stronger decrease in the aspirin group (aspirin group -7.04 mmHg vs. placebo group -3.25 mmHg, $p=0.08$, $d=0.82$).

In participants taking aspirin, P-selectin decreased significantly from the first to the second laboratory visit (log P-selectin 4.00 to 3.88, $p<0.05$, $d=0.55$), and did

not change significantly in participants taking placebo (log P-selectin 3.72 to 3.88, p=0.09, d=0.73). Also, the rank number of P-selectin decreased in the aspirin group (6.86 to 5.46, p<0.01, d=0.53) and did not change significantly in the placebo group (3.87 to 5.54, p=0.13, d=0.65). The difference in the change score of P-selectin from the first to the second laboratory visit was significantly different for participants taking aspirin compared to participants taking placebo (log P-selectin aspirin group -0.12 vs. placebo group +0.16, p<0.01, d=1.22), and it was also significantly different for the rank of P-selectin (aspirin group -1.39 vs. placebo group +1.67, p<0.01, d=1.31). However, participants receiving aspirin had significantly higher levels of P-selectin than participants receiving placebo at the first laboratory visit (log P-selectin aspirin group 4.00 vs. placebo group 3.72, p<0.02).

The rank number for IL-6 increased significantly in participants taking placebo (4.56 to 5.82, p<0.02, d=0.43) and did not change significantly in participants taking aspirin (5.99 to 5.21, p=0.08, d=0.30). The difference in the change scores of the IL-6 rank number between the aspirin and the placebo group was significant (aspirin group -0.77 vs. placebo group +1.26, p<0.01, d=1.51). In absolute values IL-6 decreased in the aspirin group (log IL-6 0.84 to 0.69, p=0.30, d=0.22) and increased in the placebo group (log IL-6 0.53 to 0.75, p=0.18, d=0.23), and the change scores were not significantly different (aspirin group -0.15 vs. placebo group +0.22, p=0.09, d=0.80).

The cardiovascular risk variable decreased for participants taking aspirin (6.01 to 5.73, p=0.11, d=0.22) and increased for participants taking placebo (4.89 to 5.24, p=0.14, d=0.27) from the first to the second laboratory visit. These changes were not significant. However, there was a significant difference in the change scores between participants receiving aspirin and participants receiving placebo (aspirin group -0.30 vs. placebo group +0.38, p<0.03, d=1.02). At the first laboratory visit, the aspirin group had a significantly higher cardiovascular risk score than the placebo group (aspirin group 6.01 vs. placebo group 4.89, p<0.04, d=0.97).

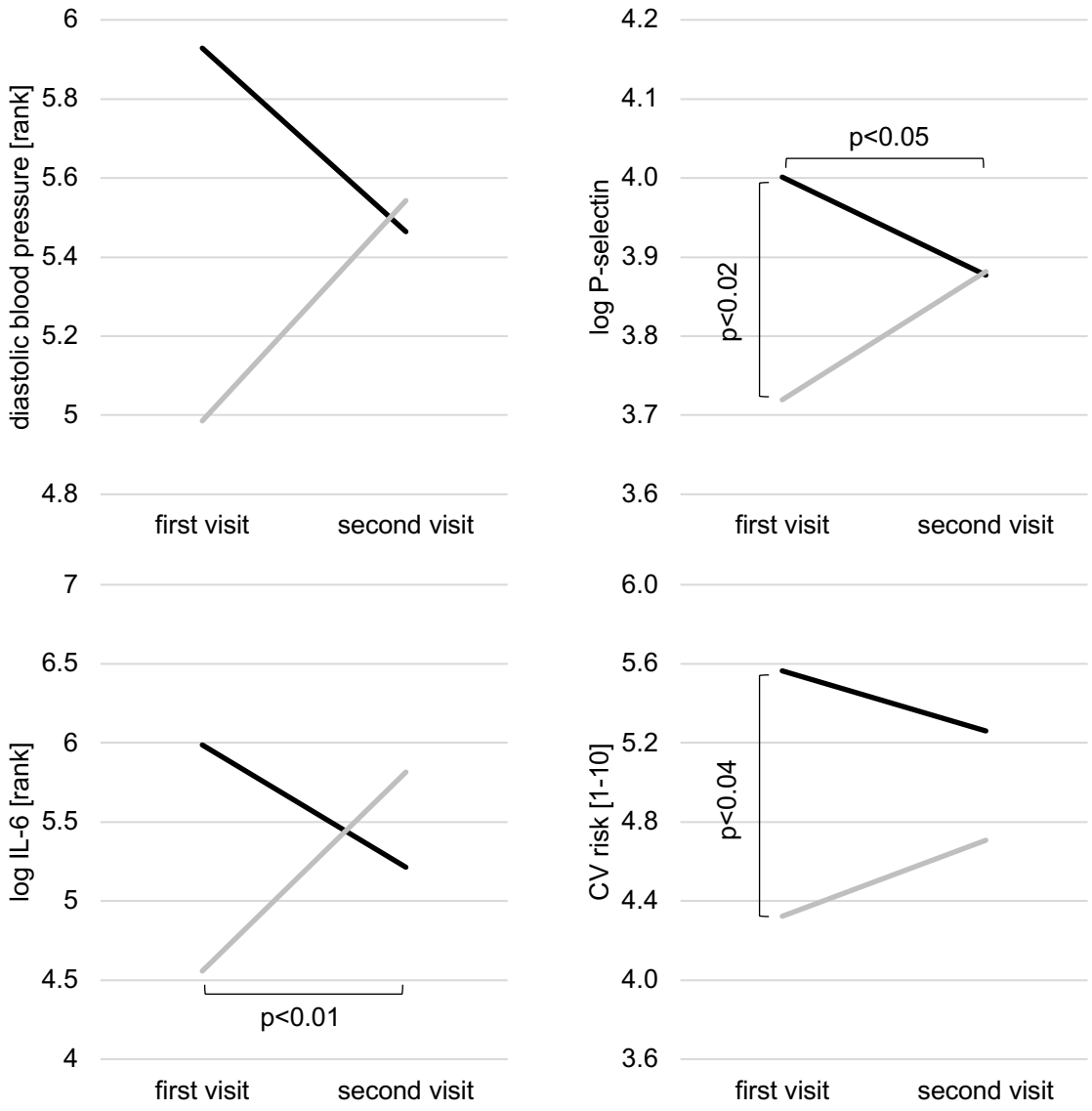

Figure 3. Changes from the first to the second laboratory visit in the rank of diastolic blood pressure, the levels of log transformed P-selectin, the rank of log transformed interleukin-6 (IL-6), and the composite cardiovascular (CV) risk variable. Grey lines represent participants receiving placebo, black lines represent participants receiving aspirin, irrespective of bereavement status. Statistically significant differences are marked with a bracket and p-value. The study compared the effects of 5 days of low-dose aspirin versus placebo on cardiovascular and psychological markers in bereaved versus nonbereaved participants. It was conducted in Tucson, AZ between March and September 2014.

Effects of the separation recall on all participants

The separation recall elicited changes in hemodynamic measures and anxiety in all participants (Figure 4). Participants rated the stressfulness of the situation they recalled during the separation recall on average at 8.71 (SD=2.17) on a scale of *1 (least)* to *10 (most)* when asked before the separation recall, and on average at 8.14 (SD=2.67) when asked after the separation recall. The ratings

before and after the separation recall were highly correlated (r=0.63, p<0.01) and were not significantly different from each other (T=1.23, p=0.23, d=0.24). Bereavement had no impact on how stressful participants rated the situation they recalled on that scale, as high levels of perceived stressfulness were requested of everyone per the interview protocol. Systolic blood pressure (129.21 to 137.60 mmHg, p<0.01, d=0.63) and diastolic blood pressure (78.36 to 83.07 mmHg, p<0.001, d=0.42) increased significantly from before to after the separation recall (Karl et al. 2018). Heart rate increased significantly during the separation recall (74.12 to 75.79 beats per minute, p<0.05, d=0.18) and decreased significantly when comparing heart rate before to heart rate after the separation recall (74.12 to 69.62 beats per minute, p<0.001, d=0.50, [Karl et al. 2018]). Anxiety measured with the state form of the STAI increased significantly from before to after the separation recall (33.05 to 39.85, p<0.001, d=0.78, [Karl et al. 2018]).

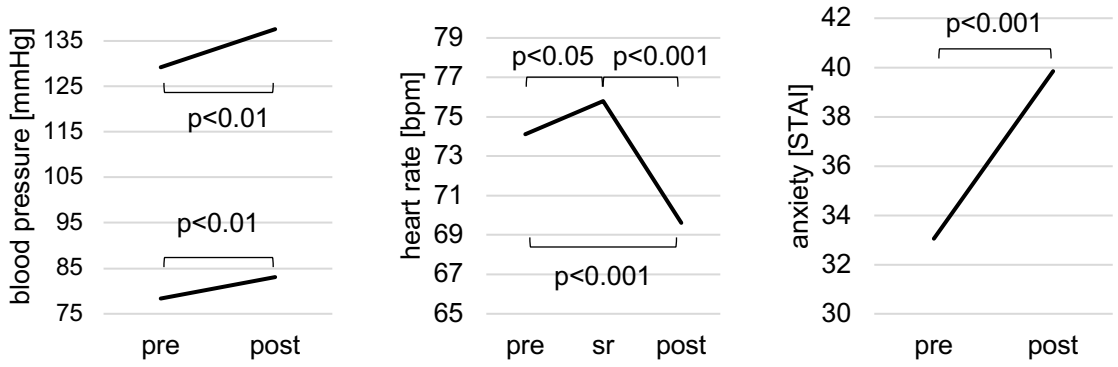

Figure 4. Changes from before (pre) to after (post) the separation recall task (sr) in blood pressure (left graph; upper line systolic blood pressure, lower line diastolic blood pressure), heart rate (middle graph) and anxiety measured through the State and Trait Anxiety Inventory (STAI; right graph). Lines represent averages of all participants. Statistically significant differences are marked with a bracket and p-value. The study compared the effects of 5 days of low-dose aspirin versus placebo on cardiovascular and psychological markers in bereaved versus nonbereaved participants. It was conducted in Tucson, AZ between March and September 2014.
mmHg = millimeters of mercury; bpm = beats per minute

Effects of aspirin on reactivity

In response to the separation recall, participants receiving aspirin recovered faster than participants receiving placebo (Figure 5). Heart rate decreased 2.32 beats per minute in the placebo group and 5.97 beats per minute in the aspirin group from before to after the separation recall, and this difference was significant (p<0.005, d=1.64, [Karl et al. 2018]). HRV decreased non-significantly in the placebo group (log RSA 5.12 vs. 4.95, p=0.23, d=0.14) and increased significantly in the aspirin group (log RSA 4.15 vs. 4.57, p<0.05, d=0.22, [Karl et al. 2018]). The

change scores for HRV from before to after the separation recall were significantly different for participants taking aspirin compared to participants taking placebo (log RSA aspirin group +0.42 vs. placebo group -0.17, p<0.03, d=1.18, [Karl et al. 2018]).

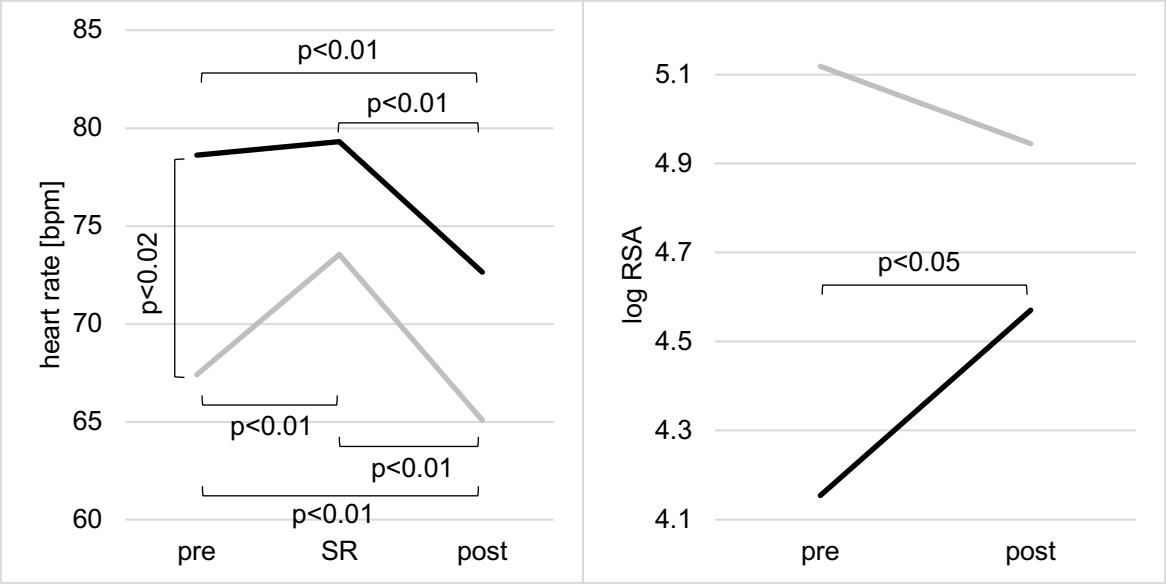

Figure 5. Changes from before (pre) to after (post) the separation recall task (SR) in heart rate (left) measured in beats per minute (bpm) and heart rate variability (right, log RSA). Grey lines represent participants receiving placebo, black lines represent participants receiving aspirin, irrespective of bereavement status. Statistically significant differences are marked with a bracket and p-value. The study compared the effects of 5 days of low-dose aspirin versus placebo on cardiovascular and psychological markers in bereaved versus nonbereaved participants. It was conducted in Tucson, AZ between March and September 2014. Figure first published in (Karl et al. 2018).

Influence of the length of the time period between the death of the significant person and the assessment

The length of the period between the death of the significant person and the first laboratory visit correlated negatively with the scores of the CES-D (r=-0.73, p<0.02) and the ICG-R (r=-0.68, p<0.03) at the first laboratory visit. There was a trend for a correlation between the length of the period between the death of the significant person and the second laboratory visit and the CES-D score at the second laboratory visit (r=-0.60, p=0.07), but not with the score of the ICG-R (r=-0.28, p=0.44).

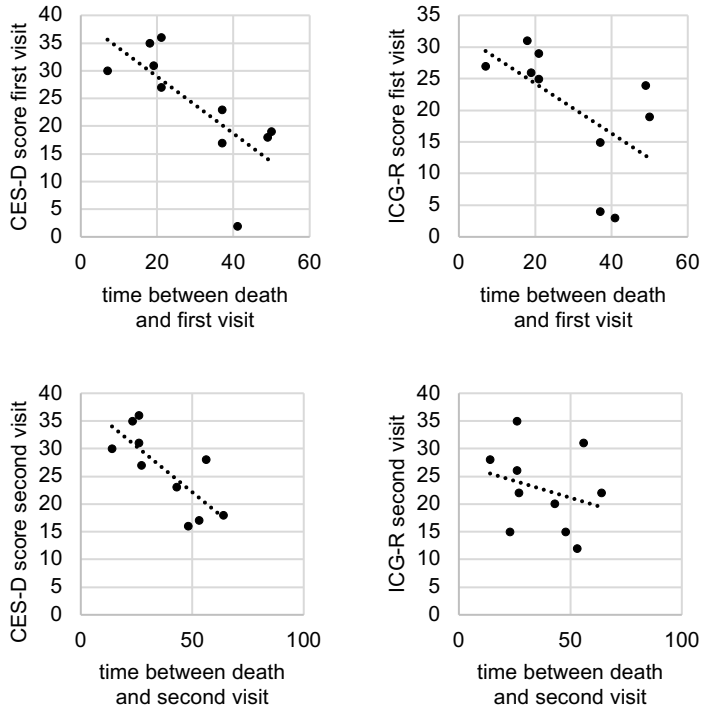

Figure 6. Correlations between the time period from the death of the spouse and the first (upper graphs) or second (lower graphs) laboratory visit in days and the scores of the Center for Epidemiologic Studies Depression Scale (CES-D), and the Inventory for Complicated Grief – Revised (ICG-R). A dashed trend line indicates the correlation. Every dot represents individual data from a bereaved participant. The study compared the effects of 5 days of low-dose aspirin versus placebo on cardiovascular and psychological markers in bereaved versus nonbereaved participants. It was conducted in Tucson, AZ between March and September 2014.

The length of the period between the death of the significant person and the second laboratory visit correlated negatively with the UCLA loneliness scale ($r=-0.79$, $p<0.01$) and Cohen's perceived stress scale ($r=-0.67$, $p<0.04$). Furthermore, it correlated negatively with trait anxiety ($r=-0.72$, $p<0.03$) and state anxiety before ($r=-0.74$, $p<0.02$) and after the separation recall ($r=-0.69$, $p<0.04$). There was a trend for more positive affect before the separation recall with longer time periods after the death of the significant person ($r=0.62$, $p=0.06$).

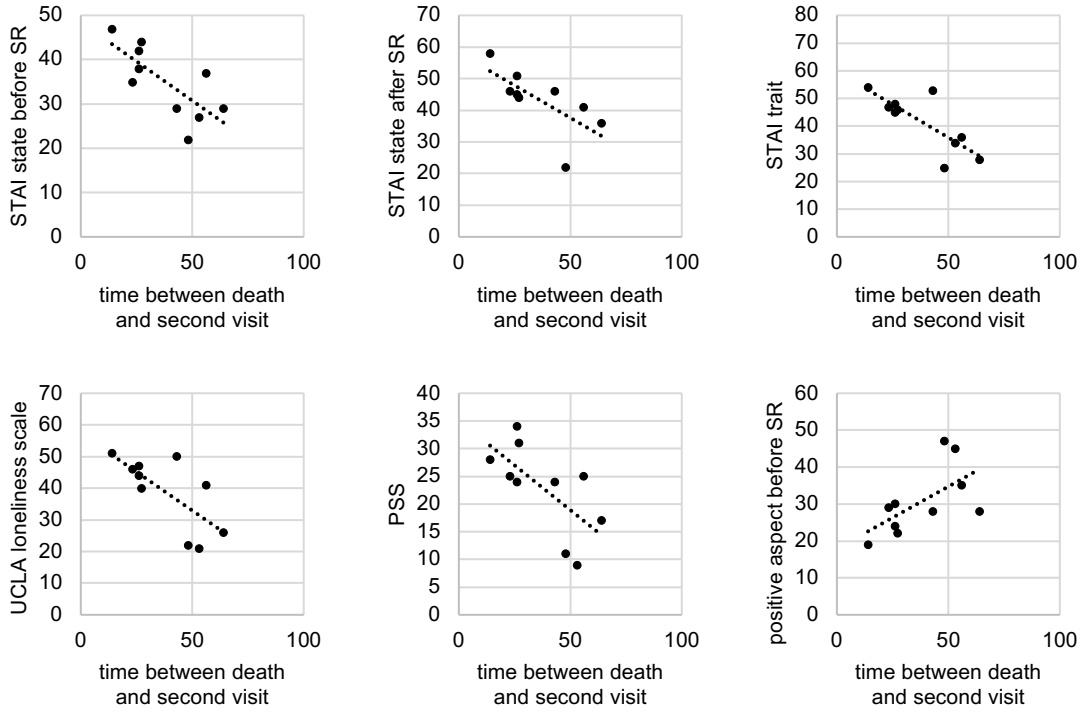

Figure 7. Correlations between the time period from the death of the spouse to the second laboratory visit in days and the scores of the state part of the State and Trait Anxiety Inventory (STAI) before and after the separation recall (SR), the trait part of the STAI, the UCLA loneliness scale, the Perceived Stress Scale (PSS), and positive affect measured through the Positive and Negative Affect Schedule (PANAS) before the SR. A dashed trend line indicates the correlation. Every dot represents individual data from a bereaved participant. The study compared the effects of 5 days of low-dose aspirin versus placebo on cardiovascular and psychological markers in bereaved versus nonbereaved participants. It was conducted in Tucson, AZ between March and September 2014.

The rating of the stressfulness of the situation that bereaved participants gave after the separation recall correlated negatively with the length of the period between the death of the significant person and the second laboratory visit (r=-0.72, p<0.03). However, the rating before the separation recall (r=-0.28, p=0.47) and the average rating (r=-0.57, p=0.11) did not correlate with the time between death and assessment.

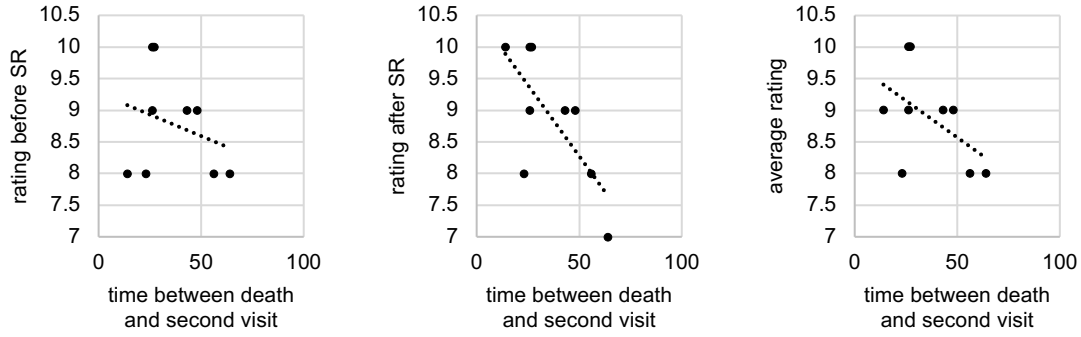

Figure 8. Correlations between the time period from the death of the spouse to the second laboratory visit in days and the scores of the rating of the stressfulness of the situation participants talked about in the separation recall (SR) before and after the separation recall, and the average rating. A dashed trend line indicates the correlation. Every dot represents individual data from a bereaved participant. The study compared the effects of 5 days of low-dose aspirin versus placebo on cardiovascular and psychological markers in bereaved versus nonbereaved participants. It was conducted in Tucson, AZ between March and September 2014.

Influence of the rating of the stressfulness of the separation recall on physiological parameters

The rating of the stressfulness of the situation before the separation recall was positively correlated with the change scores from the first to the second laboratory visit of diastolic blood pressure (r=0.54, p<0.02), of the rank for diastolic blood pressure (r=0.65, p<0.01), and of the rank for systolic blood pressure (r=0.50, p<0.03). There was a trend for correlation with the change score from the first to the second laboratory visit of the absolute values of systolic blood pressure (r=0.42, p=0.06). Furthermore, a higher rating before the separation recall was associated with a greater change score from the first to the second laboratory visit of D-dimers (r=0.70, p<0.01) and of the composite cardiovascular risk variable (r=0.55, p<0.01). Cortisol levels before (r=0.52, p<0.02) and 15 minutes after the separation recall (r=0.46, p<0.05) were positively correlated with the rating after the separation recall. Furthermore, a higher rating after the separation recall was associated with more state anxiety after the separation recall (r=0.54, p<0.02) and less positive affect both before (r=-0.66, p<0.01) and after the separation recall (r=-0.66, p<0.01).

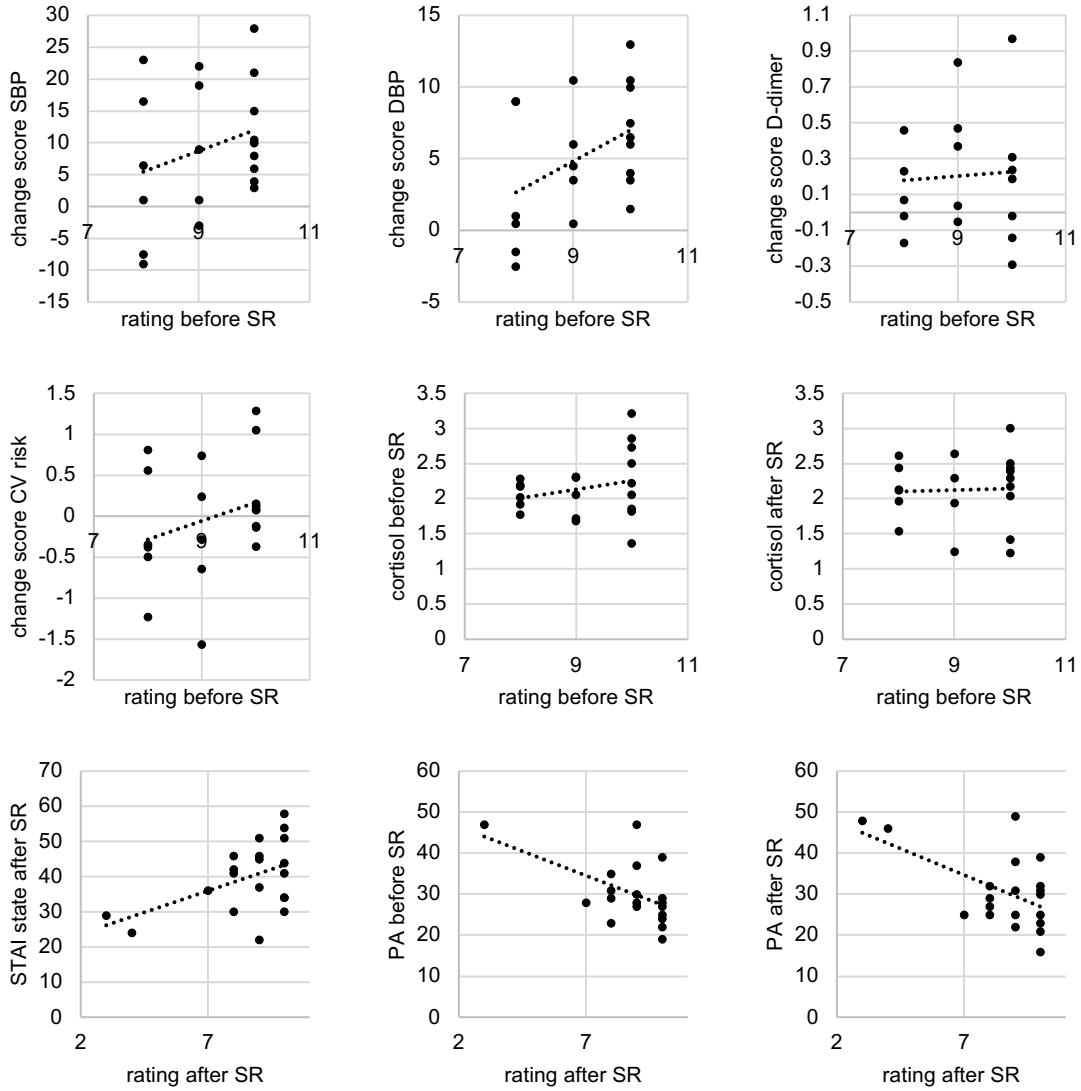

Figure 9. Correlations between the rating of the stressfulness of the situation (from 0 to 10) participants talked about in the separation recall before or after the separation recall (SR) and the change score from the first to the second laboratory visit of systolic blood pressure (SBP) in mmHg, the change score from first to second laboratory visit of diastolic blood pressure (DBP) in mmHg, the change score from first to second laboratory visit of the level of D-dimer, the change score from the first to second laboratory visit of cardiovascular (CV) risk, the level of cortisol before the SR, the level of cortisol after the SR, anxiety measured through the State and Trait Anxiety Inventory (STAI) after the SR, positive affect (PA) before the SR, or PA after the SR. A dashed trend line indicates the correlation. Every dot represents individual data from a participant. The study compared the effects of 5 days of low-dose aspirin versus placebo on cardiovascular and psychological markers in bereaved versus nonbereaved participants. It was conducted in Tucson, AZ between March and September 2014.
mmHg = millimeters of mercury

Influence of the time between assessments

The length of the time period between the first and the second laboratory visit correlated negatively with heart rate at the second laboratory visit before (r=-0.51, p<0.02), during (r=-0.43, p=0.05) and after the separation recall (r=-0.44,

p<0.05). The same applied to the ranks of heart rate before (r=-0.50, p<0.03), during (r=-0.49, p<0.03) and after the separation recall (r=-0.47, p<0.04).

There was a trend for an association between the length of the time period between assessments and the CES-D score at the second laboratory visit (r=-0.40, p=0.07), but the change score of the CES-D scores from the first to the second visit was not associated with the time period between assessments (r=0.06, p=0.80). A similar trend was also observed for Cohen's perceived stress scale (r=-0.39, p=0.07).

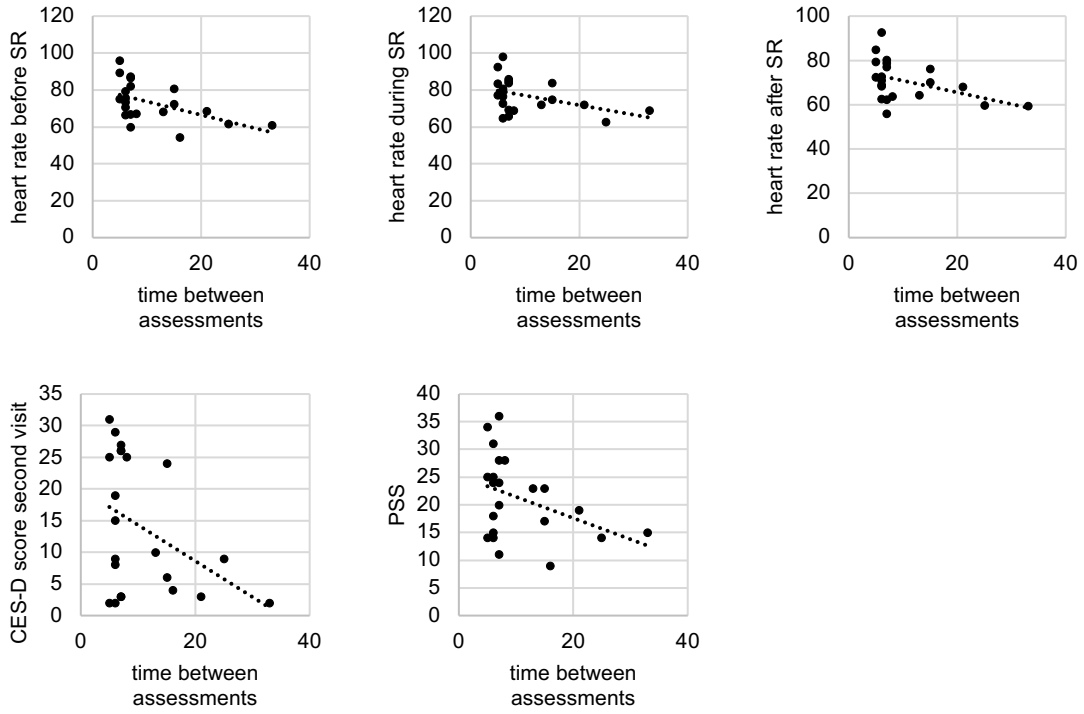

Figure 10. Correlations between the length of the time period from the first to the second laboratory visit in days and heart rate before, during, and after the separation recall (SR), the Center for Epidemiologic Studies Depression Scale (CES-D) score at the second laboratory visit, and the Perceived Stress Scale (PSS). A dashed trend line indicates the correlation. Every dot represents individual data from a participant. The study compared the effects of 5 days of low-dose aspirin versus placebo on cardiovascular and psychological markers in bereaved versus nonbereaved participants. It was conducted in Tucson, AZ between March and September 2014.

Influence of the duration of marriage

In bereaved participants, the duration of marriage correlated positively with heart rate (r=0.75, p<0.04) and cardiovascular risk (r=0.75, p<0.04) at the first laboratory visit. Longer duration of marriage was also associated with more yearning (r=0.75, p<0.04), stress (r=0.82, p<0.02) and trait anxiety (r=0.78, p<0.03), but less state anger after the separation recall (r=-0.93, p<0.01).

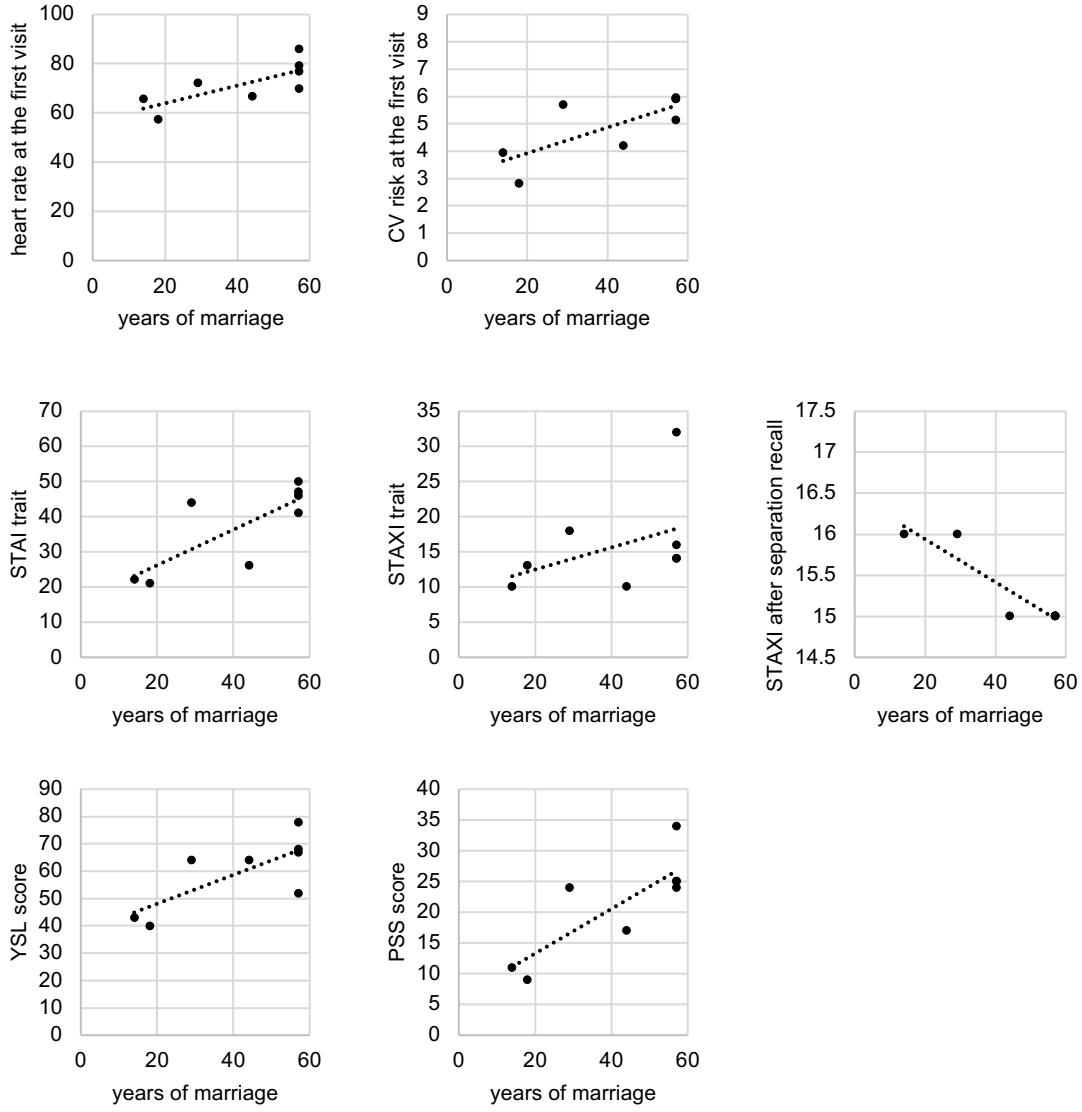

Figure 11. Correlations between the duration of marriage of the bereaved participant and deceased spouse in years and heart rate at the first laboratory visit (in beats per minute), CV risk at the first visit (scale of 1 to 10), trait anxiety measured with the State and Trait Anxiety Inventory (STAI), trait anger measured with the State and Trait Anger Expression Inventory – 2 (STAXI), state anger after the separation recall, the score of the Yearning in Situations of Loss Scale (YSL), and the score of the Perceived Stress Scale (PSS). A dashed trend line indicates the correlation. Every dot represents individual data from a bereaved participant. The study compared the effects of 5 days of low-dose aspirin versus placebo on cardiovascular and psychological markers in bereaved versus nonbereaved participants. It was conducted in Tucson, AZ between March and September 2014.

Influence of complicated grief symptoms

The ICG-R score at the first laboratory visit was positively correlated with the cardiovascular risk variable at the first laboratory visit (r=0.69, p<0.03), and the ICG-R score at the second laboratory visit was positively correlated with the cardiovascular risk variable at the second laboratory visit (r=0.66, p<0.04). The

ICG-R score at the second laboratory visit was further positively correlated with heart rate before (r=0.82, p<0.01), during (r=0.77, p<0.02), and after the separation recall (r=0.72, p<0.02), and also the with the corresponding ranks for heart rate before (r=0.80, p<0.02), during (r=0.79, p<0.02), and after the separation recall (r=0.83, p<0.01). The ICG-R score at the first laboratory visit showed a trend for correlation with heart rate at the first laboratory visit (r=0.60, p=0.07) and the corresponding rank for heart rate at the first laboratory visit (r=0.62, p=0.06). Additionally, the change score of cortisol from the first to the second laboratory visit was positively correlated with the change score of the ICG-R (r=0.68, p<0.04).

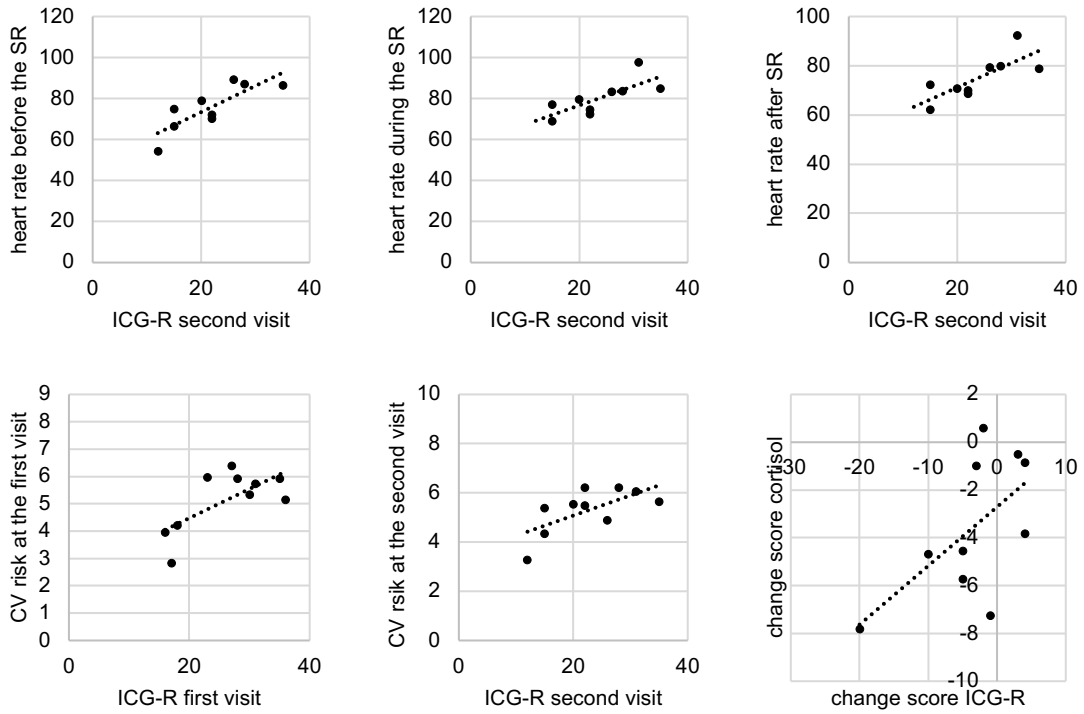

Figure 12. Correlations between the score of the Inventory for Complicated Grief – Revised (ICG-R), at the first or second laboratory visit, or the change score of ICG-R from the first to second laboratory visit, and heart rate before, during, and after the separation recall (SR), cardiovascular (CV) risk at the first and second laboratory visit, and the change score of cortisol from the first to the second laboratory visit. A dashed trend line indicates the correlation. Every dot represents individual data from a bereaved participant. The study compared the effects of 5 days of low-dose aspirin versus placebo on cardiovascular and psychological markers in bereaved versus nonbereaved participants. It was conducted in Tucson, AZ between March and September 2014.

Influence of yearning

The YSL score was positively correlated with heart rate at the second laboratory visit (r=0.67, p<0.05) and the rank of heart rate at the second laboratory

visit (r=0.71, p<0.04). It was also positively correlated with the rank of log RSA at the second laboratory visit (r=0.76, p<0.02) and negatively correlated with the absolute value of log RSA at the second laboratory visit (r=-0.78, p<0.02).

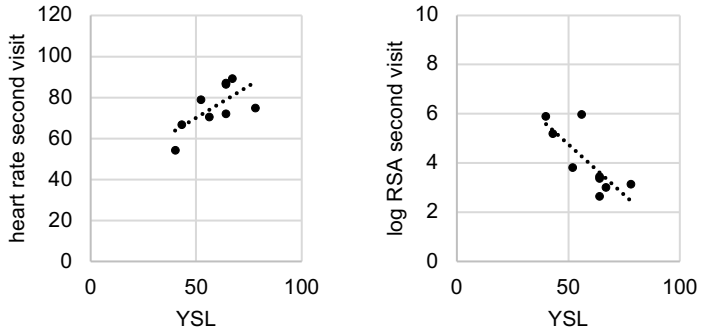

Figure 13. Correlations between the score of the Yearning in Situations of Loss Scale (YSL) and heart rate at the second laboratory visit and respiratory sinus arrhythmia (RSA) at the second laboratory visit. A dashed trend line indicates the correlation. Every dot represents individual data from a bereaved participant. The study compared the effects of 5 days of low-dose aspirin versus placebo on cardiovascular and psychological markers in bereaved versus nonbereaved participants. It was conducted in Tucson, AZ between March and September 2014.

Influence of preparedness for the death of the spouse

Of the ten bereaved participants, six participants indicated that they were prepared for the death of their spouse, while four participants indicated that they were unprepared. Those unprepared for the death of their spouse had significantly higher diastolic blood pressure at the first laboratory visit (unprepared 88.75 vs. prepared 72.17 mmHg, p<0.05, d=1.48). At the second laboratory visit, their diastolic blood pressure was non-significantly higher before (unprepared 80.25 vs. prepared 68.58 mmHg, p=0.13, d=1.07) and significantly higher after the separation recall (unprepared 91.33 vs. prepared 74.75 mmHg, p<0.02, d=2.23). The ranks of diastolic blood pressure were non-significantly higher in those unprepared at the first visit (unprepared 6.68 vs. prepared 3.36, p=0.09, d=1.23) and at the second visit before the separation recall (unprepared 6.25 vs. prepared 3.43, p=0.14, d=1.01), and significantly higher after the separation recall (unprepared 7.90 vs. prepared 3.25, p<0.02, d=2.48). Preparedness did not influence the reactivity of diastolic blood pressure in response to the separation recall (prepared +6.17 vs. unprepared +5.50 mmHg, p=0.86, d=0.11).

Those prepared for the death of their spouse had non-significantly higher levels of TNF-α before the separation recall (log TNF-α prepared 2.31 vs. unprepared 1.84, p=0.08, d=1.38), and significantly higher levels of TNF-α (log TNF-α prepared 2.19 vs. unprepared 1.60, p<0.05, d=3.28) and P-selectin after the separation recall (log P-selectin prepared 3.90 vs. unprepared 3.47, p<0.04, d=3.44). Also, cortisol levels decreased significantly less from before to 15 minutes after the separation recall in bereaved participants prepared for the death of their spouse (log cortisol prepared -0.01 vs. unprepared -0.33, p<0.04, d=2.06).

Discussion

The present pilot study investigated the feasibility and efficacy of aspirin in reducing markers of cardiovascular risk in a bereaved population using a randomized, double-blind, placebo-controlled design. Despite the small sample size, the results show a significant reduction in baseline CV risk markers and attenuated physiological reactivity to stressors suggesting potential benefit for low-dose aspirin use as prevention for increased CV risk in bereaved (Karl et al. 2018). They also show a beneficial effect of aspirin on depressive symptoms (Karl et al. 2018), supporting reports in the literature that point to the involvement of inflammatory pathways in the development of depressive symptoms.

Use of a reactivity task

To my knowledge, the present study was the first to examine reactivity in a bereaved population using a laboratory stress task. It is especially important to include a measure of reactivity when investigating beneficial effects of a medical intervention on cardiovascular risk in bereavement because bereaved individuals show heightened reactivity to daily stressors (Hahn et al. 2014), which could increase cardiovascular risk especially during those times. In this study, the separation recall interview was used as a measure to elicit reactivity in bereaved participants. In its original publication, this interview has been used in healthy participants, who reported a greater amount of sadness and less happiness after the task. Additionally, the authors reported increases in heart rate and blood pressure from baseline to task (Ehrenthal et al. 2011). The present results confirm these physiological findings and additionally show a significant increase in anxiety after the separation recall task (Karl et al. 2018). Anxiety has been found to be increased during acute bereavement (Marshall 2010) and has been linked to increased cardiovascular risk (Mittleman et al. 1995), highlighting the importance of this finding when assessing cardiovascular risk during bereavement. In the original publication, participants ranked the stressfulness of the situation before talking about the situation. This was done to ensure that the situation was stressful enough to produce a sufficient effect. The same was done in the present study, but participants also rated the situation after having talked about it. The ratings before and after the separation recall correlated strongly but were not the same.

Ratings after the separation recall tended to be lower, which might reflect an effect of relief after having talked about the situation and might not reflect actual stress levels during the situation. This view is supported by the fact that a lower stressfulness rating *after* the separation recall was associated with psychological factors such as less anxiety after the separation recall and more positive affect before and after the separation recall. A higher subjective stress rating *before* the separation recall however was associated with physiological risk factors, such as higher change scores for systolic and diastolic blood pressure, a higher change score of D-dimer and the composite cardiovascular risk variable, and higher cortisol levels before and after the separation recall. The latter result is particularly interesting because it might highlight the interdependence between the baseline level of distress that bereavement causes in participants – represented by the correlation with cortisol levels before the task – and the effect an additional stressor has on those participants – represented by the correlation with cortisol levels after the task. Reciprocally, participants with a high baseline stress level would be more likely to give a higher stressfulness rating of the task and this, in turn, is associated with high cortisol levels after the task. Taken together, the correlations of the stressfulness rating with physiological and psychological measures emphasize the importance of measuring the subjective stress level. Most importantly, the present work shows that the separation recall technique can be used safely and effectively in a bereaved population to elicit psychological as well as physiological reactions comparable to what is thought to happen during intense grief reactions. The separation recall could be used in future studies to measure reactivity as an integral part of the grief response and of cardiovascular risk in bereavement.

In the present study, participants receiving aspirin recovered faster from the separation recall task than participants receiving placebo in terms of a stronger decrease in heart rate and an increase in HRV (Karl et al. 2018). This suggests that aspirin influences HRV not only at very high doses (De Meersman et al. 2000) but also at low doses. These results also support findings that aspirin can not only affect baseline measures but also measures of reactivity (Mittleman et al. 1995; von Känel et al. 2008a), and they show this for the first time in a bereaved population. The present work extends existing findings to show that this is not only

true for endpoints of cardiovascular disease, but also for individual cardiovascular risk factors.

Reactivity was not different between bereaved and nonbereaved subjects. Since the separation recall was conducted at the second laboratory visit and therefore after the 5-day-treatment with aspirin or placebo, an unbiased view of the differences between bereaved and nonbereaved participants is only possible in the group that received placebo. When that group is further divided into bereaved and nonbereaved participants, each group consists of only five participants, thus lacking power to detect hypothesized differences between bereaved and nonbereaved participants.

Changes in baseline from the first to the second laboratory visit

There was a beneficial effect of aspirin on the baseline of physiological and psychological measures. Diastolic blood pressure, P-selectin, IL-6, and the surrogate cardiovascular risk variable – which included hemodynamic, prothrombotic and proinflammatory measures – were lower in participants receiving aspirin.

The effect on diastolic blood pressure aligns with results of Hermida and colleagues showing that aspirin administered at bedtime can lower blood pressure (Hermida et al. 2003; Hermida et al. 2005b; Hermida et al. 2005a; Hermida et al. 2009). However, the present results show no effect of aspirin on systolic blood pressure. Diastolic blood pressure is thought to be of lesser predictive value for cardiovascular disease than systolic blood pressure (Schillaci et al. 2009). Hermida and colleagues had found effects of aspirin on both diastolic and systolic blood pressure (Hermida et al. 2003; Hermida et al. 2005b; Hermida et al. 2005a; Hermida et al. 2009). They found effect sizes of 0.63 for systolic blood pressure in a large sample of prehypertensive patients (Hermida et al. 2009). Larger studies in a bereaved sample are needed to determine if this effect could also be achieved in a bereaved population.

The effect on P-selectin suggests that aspirin is counteracting platelet activation. It is not surprising that aspirin has such an effect since this is the main desired effect of low-dose aspirin therapy in cardiovascular disease. This study failed to show differences in levels of P-selectin between bereaved and control participants, but larger studies have shown increased platelet activation during

bereavement (Buckley et al. 2012b). Unfortunately, participants in the aspirin group had higher levels of P-selectin at the initial laboratory visit despite randomization. Differences in baseline were accounted for in the present study by using change scores. An effect of aspirin rather than just convergence of the two groups is probable despite the differences in baseline, since significant changes in levels of P-selectin were only present in the aspirin group.

Furthermore, there was an effect of aspirin on baseline levels of inflammation measured through IL-6. This confirms similar research findings in nonbereaved participants showing that even low-dose aspirin has an anti-inflammatory effect (von Känel et al. 2008a).

No statistically significant differences were observed in the other cardiovascular risk markers, but there was a significant difference between the aspirin and placebo group in the change score of the cardiovascular risk variable. Similar to P-selectin, this result should be interpreted with caution, because cardiovascular risk at the first laboratory visit was significantly higher in participants randomized to receive aspirin. With significant effects of aspirin on some markers of cardiovascular risk included in the surrogate variable, an effect of aspirin is probable.

Effect on depressive symptoms

In the present study, bereaved participants reported significantly more depressive symptoms at the first laboratory visit than nonbereaved controls (Karl et al. 2018). At the second laboratory visit, bereaved participants taking aspirin were more likely to report a decrease in depressive symptoms than bereaved participants taking placebo (Karl et al. 2018). These results are consistent with studies linking the use of aspirin to a decrease in the report of depressive symptoms (Ketterer et al. 1996) and with theories that attribute at least part of the antidepressant effect of selective serotonin reuptake inhibitors to anti-inflammatory properties of these medications (Berk et al. 2013). This evidence gave rise to the theory that stress – with bereavement being one of the most potent stressors – induces inflammation, which in turn creates depressive symptoms (Slavich and Irwin 2014). The importance of this finding, given the objective of this study, is emphasized by results of a recent study by Stahl and colleagues (Stahl et al. 2016). They found evidence that links depressive symptoms in bereavement to

increased mortality. The authors hypothesize that high depressive symptoms might be the mechanism linking bereavement to mortality (Stahl et al. 2016). Further research is needed to determine if the link between mortality and bereavement might actually be inflammation, and if inflammation may contribute to the etiology of depressive symptoms. Supporting this hypothesis is the fact that not only the change in depressive symptoms but also the change in IL-6 rank number from the first to the second laboratory visit was significantly different between the aspirin and placebo group.

Time-dependence of bereavement effects

Most studies find an effect of bereavement on mortality and cardiovascular risk only for a limited time after bereavement. Buckley and colleagues also showed that most of the risk factors they studied normalize after 6 months (Buckley et al. 2009a; Buckley et al. 2011; Buckley et al. 2012a; Buckley et al. 2012b). The present study confirms these results and extends them to show that a negative association between the effects of bereavement and the time since bereavement even exists within 7 weeks after the bereavement event. With more time after bereavement, participants reported lower baseline stress levels. And even though time after bereavement was not associated with how stressful participants rated the situation they talked about in the separation recall *before* talking about it, with more time after bereavement they tended to rate it as less stressful *after* having talked about it. Longer temporal distance to the death of the spouse was also associated with the report of less depressive symptoms, yearning, loneliness, and anxiety, and the report of more positive affect. As stated before, depressive symptoms and anxiety have been linked to increased mortality in bereaved (Mittleman et al. 1995; Stahl et al. 2016). The importance of those psychological consequences of bereavement is stressed by the demonstrated associations between yearning, symptoms of complicated grief, and the rating of the stressfulness of the situation with certain physiological risk factors, even though those risk factors were not directly associated with the time after the bereavement event. More yearning was correlated with higher heart rate and lower heart rate variability; more symptoms of complicated grief were associated with a higher cardiovascular risk variable and higher heart rate; and the change score of the

ICG-R was correlated with the change score of cortisol, likely representing stress levels.

The fact that time after the death of the spouse was associated with psychological measures and the stressfulness rating *after* the separation recall, but not with physiological outcomes, adds to the hypothesis that the rating after the separation recall might be more likely to represent psychological effects of stress, while the rating before the separation recall might be more likely to represent physiological effects of stress.

Influence of preparedness on physiological parameters

Preparedness might be a predictor of psychological as well as physiological outcomes of bereavement, where being unprepared for the death is predictive of a negative outcome (Barry et al. 2002; Shah et al. 2013b), although this question is still controversial (Carr et al. 2001; Stroebe et al. 2007). The present results regarding this question are ambiguous as well. Bereaved participants who were not prepared for the death of their spouse had higher diastolic blood pressure at several time points in the present study. The same could not be observed for systolic blood pressure, which is considered the stronger predictor of cardiovascular risk (Schillaci et al. 2009). Conversely, they had lower levels of TNF-α and P-selectin, and a stronger decrease in cortisol from before to after the separation recall, likely representing reduced reactivity. Larger studies will be needed to clarify those contradictory results.

In existing studies, the impact of preparedness on physiological outcomes was mostly reported as an increase in mortality or morbidity. To my knowledge, none of the studies investigating the effect of preparedness on physiological outcomes took individual risk factors into account. To this end, the present study was the first to link preparedness to changes in individual cardiovascular risk factors.

No effect of preparedness on psychological outcomes was found in the present study. Unpreparedness has previously been found to be a strong predictor of depressive symptoms (Buckley et al. 2009a) and preparedness, in turn, is considered to be protective in terms of psychological consequences of bereavement (Shah et al. 2013b). The studies reporting these connections were

much larger, making it very probable that the present study was lacking power to detect the effect preparedness had on psychological measures.

Feasibility of aspirin use in bereaved

The use of aspirin in secondary prevention of cardiovascular disease is well established (Smith et al. 2011). However, the safety and efficacy of aspirin in primary prevention are still heavily debated. Aspirin therapy contains the risk of internal bleeding, especially in the upper gastrointestinal tract. Using low doses of aspirin can minimize gastrointestinal side effects (Scheiman 2012), as well as administration at bedtime (Moore and Goo 1987) can do so. To be considered a valid option for prevention, the reduction in cardiovascular risk needs to outweigh the risk of bleeding. In people with no history of ischemic heart disease, the cardiovascular risk reduction through daily administration of low-dose aspirin and the increase in upper gastrointestinal bleeding risk cancel each other out approximately (Antithrombotic Trialists' (ATT) Collaboration et al. 2009). However, with an increase in cardiovascular risk factors, this equilibrium might shift in favor of the benefits achieved through the daily administration of low-dose aspirin, especially for a time-limited period. For patients with diabetes for example, who are at an increased risk for adverse cardiovascular events, the American Heart Association and the American Diabetes Association recommend the use of aspirin in primary prevention (Buse et al. 2007). Such an appraisal could also apply to other conditions in which cardiovascular risk is increased, such as the acute phase of bereavement.

Limitations

The major limitation of this study was its small sample size, which is partly because the study was a pilot study and demonstration of feasibility, but in part also because the recruitment of the investigated population is very challenging. Limiting factors for recruitment were mainly refusal to take part in a study shortly after the death of a loved one or existing low-dose aspirin treatment in potential participants otherwise eligible and willing to participate in the study. The necessity to take aspirin during the study was no limiting factor for recruitment. Another limiting factor for the sample size was the short recruitment and data collection time frame of only six months. The small sample size generated further limitations.

Extensive statistical control for confounding was not possible. Confounding was controlled for through the randomized, double-blind study design, as well as through the generation of change scores, to account for differences in baseline levels of certain variables. Differences within one group were analyzed using T-tests, differences between groups were analyzed using analysis of variance. The use of multiple regression models was prohibited by the small sample size. All results presented here should be interpreted considering the small sample size and the subsequent limited statistical validity of the results.

Differences between bereaved participants and nonbereaved control participants that other studies have reported before (Buckley et al. 2011; Buckley et al. 2012a; Buckley et al. 2012c; Buckley et al. 2012b) could not be confirmed in this study. The present results show that systolic blood pressure, heart rate, the number of ectopic beats, TNF-α, and cortisol were higher, and heart rate variability was lower in bereaved participants. This result aligns with previous studies (Buckley et al. 2012c; Buckley et al. 2012a), but did not reach statistical significance. Diastolic blood pressure, IL-6, P-selectin, and the composite cardiovascular risk variable were lower in bereaved participants, and the level of D-dimer and vWF were even significantly lower. These results are unexpected and contradict previous results. As discussed above, this study was neither intended nor designed to confirm or disprove differences between bereaved and nonbereaved participants at baseline, but to investigate the effects of aspirin in this population. The reason for investigating the differences at baseline anyways was to integrate the present sample with samples of previous studies. The differences between bereaved and nonbereaved participants found in other studies were small in effect size. As an example for hemodynamic measures, Buckley and colleagues found the effect size for differences in heart rate between acutely bereaved and nonbereaved control participants in their study was 0.47 (Buckley et al. 2012a). With a power of 0.8, this would call for 57 participants per group, making the current pilot study too small to yield significant results.

Conclusion

The results of the present study indicate that aspirin could have a protective effect on cardiovascular risk in the acute phase of bereavement. Although bereavement is a rare event throughout one person's life, it affects nearly

everyone at some point in their life, creating immense public health effects of increased risk and mortality during the period of acute bereavement. With other studies showing the feasibility of preventive approaches for short-term increases in risk (Shaw et al. 2009; Tofler et al. 2013), the present results warrant a larger investigation to confirm the effects of aspirin and determine the safety of its use in an acutely bereaved population.

Abstract

Objective: The death of a loved one is extremely stressful, and cardiovascular risk increases nearly two-fold in the acute period of bereavement. However, no studies have attempted to intervene to reduce risk during this identifiable period. This pilot study investigated the protective effect of low-dose aspirin on cardiovascular parameters and depressed mood of bereaved participants, compared to nonbereaved healthy controls.

Methods: Ten bereaved participants and 12 nonbereaved control participants had blood pressure, heart rate and heart rate variability (HRV) measured and blood drawn at a first laboratory visit. The visit was within 30 days of the death of their spouse, on average. Participants were randomized to receive low-dose aspirin (81 mg) or placebo, taken for five days. In a second laboratory visit, the same assessments were repeated, as well as a structured separation recall reactivity task (i.e., recalling a time they felt alone or abandoned). Bereaved participants recalled their bereavement experience.

Results: Bereaved participants taking aspirin were more likely to report a decrease in CES-D score from the first to the second laboratory visit than those taking placebo (χ^2=6.67, p<0.01, d=3.54). Levels of P-selectin (-0.12 vs. +0.16, p<0.01, d=1.22) and a composite cardiovascular risk score (-0.30 vs. +0.38, p<0.03, d=1.02) decreased more from the first to the second lab visit in participants taking aspirin. In response to the separation recall, participants taking aspirin recovered faster than those taking placebo: heart rate decreased more in the aspirin group (-2.32 vs. -5.97 beats per minute, p<0.005, d=1.64) and HRV decreased in the placebo group while it increased in the aspirin group (log RSA +0.42 vs. -0.17, p<0.03, d=1.18).

Conclusions: The present pilot study was the first to measure reactivity in a bereaved population using a laboratory stress task. The results suggest that aspirin can reduce baseline cardiovascular risk markers, attenuate physiological reactivity to stressors, and ameliorate depressed mood in acutely bereaved.

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Supplemental material

Table 5. Demographic characteristics of all four experimental groups. The study compared the effects of 5 days of low-dose aspirin versus placebo on cardiovascular and psychological markers in bereaved versus nonbereaved participants. It was conducted in Tucson, AZ between March and September 2014.

	Bereaved, aspirin (n=5)		Bereaved, placebo (n=5)		Control, aspirin (n=7)		Control, placebo (n=5)			
	mean	SD / %	mean	SD / %	Mean	SD / %	mean	SD / %	F / χ^2	*p-value*
Age	70.60	13.45	62.60	11.19	52.00	12.54	51.80	12.01	2.920	0.06
Sex (*female*)	4	80 %	3	60 %	2	29 %	h	60 %	3.331	0.34
Race (*white*)	5	100 %	5	100 %	5	71 %	5	100 %	4.714	0.19
Ethnicity (*Hispanic*)	0	0 %	1	20 %	2	29 %	2	40 %	2.477	0.48
BMI	30.38	7.88	27.26	2.94	31.26	8.10	23.93	2.20	1.577	0.23
Currently smoking	0	0 %	0	0 %	1	14 %	0	0 %	2.245	0.52
Pack years	27.20	30.77	5.00	7.07	2.86	7.56	0.00	0.00	3.315	0.04
Alcohol (*drinks per week*)	5.80	6.65	7.90	9.24	1.86	2.52	3.81	5.01	1.071	0.39
Caffeine (*drinks per week*)	14.80	9.88	13.30	16.12	9.00	7.48	7.60	7.99	0.548	0.66
Time since death (*days*)	20.40	10.76	39.60	11.74					7.268	0.03
Time between assessments (*days*)	6.00	1.00	10.00	5.05	11.43	10.08	14.40	8.41	1.108	0.33
Time with spouse (*years*)	44.60	20.64	32.60	18.96					0.917	0.37
Preparedness (*yes*)	4	80 %	2	40 %					1.667	0.20
Primary caretaker (*yes*)	4	80 %	5	100 %					1.111	0.29
Caretaking time (*months*)	58.50	43.37	14.10	15.38					4.654	0.07

Separation Recall Protocol

Non-bereaved participants

The next thing we will do today is to talk about how your heart reacts to certain memories and feelings.

In the following few moments, I will ask you to recall a certain situation in your life, to visualize this situation as intensely as possible, and to tell me about it.

Please also tell me how you would rate your situation on a scale of 0 to 10, where 0 is not stressful at all and 10 is extremely stressful.

I will then ask you some questions about it.

In a moment, I will tell you exactly what situation you should recall. Do you have any questions about the procedure?

OK. Now I'll ask you to recall a situation in your life in which you felt very alone and abandoned, and in which you wished that someone had been there for you. It could be any situation in your life in which you felt very alone and abandoned, and in which you wished someone had been there for you.

Take your time and mentally immerse yourself in this situation as completely as possible. And whenever you are ready, tell me how you would rate your situation on a scale of 0 to 10. Tell me about it.

Prompts:

In this situation / as you were (use the words of the test person), ...

- How did you feel?
- Some people feel emotions like these even on a physical level. Was that also the case with you?
- Can you still feel that even now?
- While you were going through that painful situation, do you remember what you were focused on? Were you more focused on yourself and your emotions, or were you primarily focused on another person?
- Do you feel like your experience of this painful situation has any *meaning* for your life? Do you feel like you already know what the meaning of this experience will be for you? Do you think you will remember this experience at future times in your life, and, if so, what will you think about it?
- Do you happen to remember what the passage of time felt like during this situation? Did you feel like time slowed down, or sped up? Did you pay attention to the time passing, or not really notice it during this situation? Were you mostly thinking about the past, the present moment, or the future?
- During that situation, did you notice the environment you were in, or the people around you (if there were any)? Did your perception of the environment change at all during the situation? Do you think the environment that you were in affected the way you felt at all?
- What kind of thoughts did you have about yourself, or about other people, in that situation?

- If you feel like you eventually recovered from this stressful experience, can you think of anything that helped you recover? Did another person, or a particular activity, or a particular idea or belief help you feel like you could move on from this situation?

End the separation recall after 5-10 minutes.

Please also tell me how you would rate your situation on a scale of 0 to 10, where 0 is not stressful at all and 10 is extremely stressful.

Thank you very much for your honesty, we have reached the end of this part, next I will take the blood sample.

Bereaved participants

The next thing we will do today is to talk about how your heart reacts to certain memories and feelings.

In the following few moments, I will ask you to recall a certain situation in your life, to visualize this situation as intensely as possible, and to tell me about it.

Please also tell me how you would rate your situation on a scale of 0 to 10, where 0 is not stressful at all and 10 is extremely stressful.

I will then ask you some questions about it.

In a moment, I will tell you exactly what situation you should recall. Do you have any questions about the procedure?

OK. Now I'll ask you to recall a situation since your recent loss in which you felt very alone and abandoned, and in which you wished that your loved one had been there for you. It could be any situation since your recent loss in which you felt very alone and abandoned, and in which you wished that your loved one had been there for you.

Take your time and mentally immerse yourself in this situation as completely as possible. And whenever you are ready, tell me how you would rate your situation on a scale of 0 to 10. Tell me about it.

<u>Prompts:</u>

In this situation / as you were (use the words of the test person), ...

- How did you feel?
- Some people feel emotions like these even on a physical level. Was that also the case with you?
- Can you still feel that even now?
- While you were going through that painful situation, do you remember what you were focused on? Were you more focused on yourself and your emotions, or were you primarily focused on another person, such as your loved one?
- Do you feel like your experience of this painful situation has any *meaning* for your life? Do you feel like you already know what the meaning of this experience will be for you? Do you think you will remember this experience at future times in your life, and, if so, what will you think about it?
- Do you happen to remember what the passage of time felt like during this situation? Did you feel like time slowed down, or sped up? Did you pay attention to the time passing, or

not really notice it during this situation? Were you mostly thinking about the past, the present moment, or the future?

- During that situation, did you notice the environment you were in, or the people around you (if there were any)? Did your perception of the environment change at all during the situation? Do you think the environment that you were in affected the way you felt at all?
- What kind of thoughts did you have about yourself, or about other people, in that situation?
- If you feel like you eventually recovered from this stressful experience, can you think of anything that helped you recover? Did another person, or a particular activity, or a particular idea or belief help you feel like you could move on from this situation?

End the separation recall after 5-10 minutes

Please also tell me how you would rate your situation on a scale of 0 to 10, where 0 is not stressful at all and 10 is extremely stressful.

Thank you very much for your honesty, we have reached the end of this part, next I will take the blood sample.

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