



Negative Events and Biochemical Markers: Test for the Moderating Effect of Humor

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Abstract

Brazil is known to have a humorous population, but could this humor moderate the relation between negative events and blood markers? This study was designed to conceptually replicate the work of Martin and Dobbin (1988) to investigate whether the sense of humor moderates the immunosuppressive effect of stress. Ninety participants (81% female and 19% male) from a community service project provided by a university laboratory filled out questionnaires of negative events and humor styles. For the immunosuppressive markers, fluid was drawn to describe serum levels of cortisol, glycemia, and neutrophils-lymphocytes ratio (NLR). The results, in general, did not corroborate the effects found by the authors. None of the four humor measures were directed related to negative events or immunosuppressive markers. Although self-deception humor moderates NLR and negative events, showing that it could be used and be protective when there are no or few negative events, but increasing the number of negative events, the use of this humor style is detrimental. The relationship between stressor events and biochemical markers was not moderated by styles of humor for a Brazilian underserved population sample.

Keywords Mood · Humor style · Immunity · Cortisol

Brazil is known to have a happy humorous population (Teixeira & Teixeira, 2014). The relationship between humor, health, and stress are in proverbs and popular sayings in Brazil as “he who sings gets rid of bad things”, “laugh, it passes”; however, their relationship is still little explored despite the long tradition of studying stress and health (Rogers, 2016). Brazil is one of the happiest countries in South

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America to live (behind Chile) (Helliwell et al., 2018) and among the 30 happiest in the world. It is also a country that has increased its life expectancy relative to other countries (Marli, 2017), which is probably related to the humor of the population. Of the BRICS countries, Brazil is the one with the best indexes of results in social progress (Porter et al., 2017). Although the moderating relationship of humor between stress and immunological markers in Brazil seems logical, the authors were unable to locate any studies with this framework. Thus, the present article aims to investigate the moderating relation of humor styles between negative life events and immunosuppressive markers. The conceptual proposal of this study is not new; it comes from the study of Martin and Dobbin (1988).

Martin and Dobbin (1988) proposed a study on how the moderating effect of the sense of humor would affect the immunosuppressive effects of stress. The authors found moderating relationships between hassles, situational humor, and allergy markers (immunoglobulin A) in Canada, however, did not move in other directions with the measures used (of hassles, humor, or markers). Martin (Martin et al., 2003) and other authors gave support to the relationship between stress and immunosuppressive markers (Cacioppo et al., 2015; Sapolsky et al., 2000; Shelby & Kathryn, 2001; Yehuda, 2000), between coping strategies and immunosuppressive markers or even well-being (Herbert & Cohen, 1993a, 1993b; Jemmott & Locke, 1984; Martin et al., 2003).

Overall, although the study results suggest that different facets related to stress, hassles, negative events in life, perceived stress etc. are related to a decrease in the immune system and an increase in cortisol and glycemia (e.g. Christie and Schrater, 2015; Pandrc et al., 2017; Pulopulos & Kozusznik, 2018; Staufenbiel, et al., 2013). Recently, some studies included neutrophils and leukocytes ratio to describe inflammation states (e.g. Demir et al., 2015; Pandrc et al., 2017). These studies are limited to the Europe-USA and Canada axis and have often been performed with very specific populations (with a chronic disease or university students). Rarely are studies carried out on populations with more affiliative cultures (i.e. collectivist cultures), with severe structural problems (e.g. loss of employment, having had someone in the family murdered, having been robbed, having had problems with the police or with money) or with the population in general. In other words, Brazil, characterized by a general context of political-economic and financial difficulties, differs from the international literature (Teixeira & Teixeira, 2014), being an interesting and relevant context for this type of research. Thus, the general question of this work is will there be a replication of a moderating relationship for humor between and stress events and the immunological system in the Brazilian context?

The sense of humor can be conceptualized as a cognitive ability, an aesthetic (appreciative) response, habitual pattern of behavior, emotional trait, an attitude or an emotional defense mechanism (Martin et al., 2003), creativity (Murdock and Ganim, 1993), etc. Among the conceptualizations, the most relevant for the immune system is to understand the sense of humor as a coping strategy; however, not all humor styles are healthy or positively affect well-being (Martin et al., 2003). In this case, the sense of humor acts as a moderator in the relationship between the stressor event and the impact on the body.

The literature indicates two major categories of humor, one positive and one detrimental to health. Positive humor is defined from how much one is feeling enthusiastic, active, benign, or benevolent for the purpose of stimulating relationships or the individual (Martin et al., 2003; Schermer et al., 2019; Segabinazi et al., 2012). Negative humor, on the other hand, is formed by aversive emotions, by self-defeat, and can be detrimental to the person or to their interpersonal relationships (Martin et al., 2003; Schermer et al., 2019).

Forgas (2013) argues that a positive mode is a relaxed state, and when individuals feel that way, the brain is informed that there is no danger, alert, or threat. Negative humor, on the other hand, is a cautious state; without flight or fight, with no adrenaline involved, however, it signals wakefulness. Forgas (2013) described negative humor as an important trait for situations that require visual detailing and accurate memories, such as eye-witness testimonies. He proposed that people who have negative humor are more likely to take care of themselves compared with those who have positive humor; however, the negative humor style can be detrimental to health, generating a possible balance.

The humor style, in addition to being characterized as positive–negative, presents another orthogonal axis: affiliative–intrapyschic, that is, relationship oriented and oriented toward the self or the individual. Therefore, Martin et al. (2003) conceptually proposed four humor quadrants 2 (positive and negative) \times 2 (oriented to relations or to the self) composing 4 profiles: affiliative (positive with relationship orientation), self-enhancing (positive and self-oriented), aggressive (negative and relationship-oriented), and self-defeating (negative and self-orientated). Affiliative humor is defined as the tendency to share humor with others, tell jokes and funny stories, entertain others, make others laugh, and enjoy laughing along with others. Self-enhancing humor is the tendency to maintain a humorous perspective on life, even when alone, to use humor to deal with stress and to cheer oneself up with humor. Aggressive humor is the tendency to use humor to devalue or manipulate others and to use ridicule, offend, and expresses humor even when inappropriate. Self-defeating humor is understood as the tendency to amuse others at one's own expense, to use self-deprecation, and to use humor to conceal true feelings about oneself and others. This proposition was found support in 28 countries (Schermer et al., 2019).

Humor styles seem to be a stable feature, tending to be broader and less rooted to external circumstances when compared with an emotion and can be understood as a reaction to the emotion or subjectively experienced emotion (Forgas, 2013; Martin et al., 2003). Therefore, due to their stability, they are close to a personality trait. In addition, according to the proposal of Martin et al. (2003), the humor questionnaire was constructed to describe humor reactions, having less response bias with this (Martin & Lefcourt, 1984; Schermer, 2019).

Studies on the effect of positive humor on the immune system are scarce. Dickerson and Kemeny (2004) found that the induction of positive humor increased lymphocyte proliferation and the opposite occurred with the induction of negative humor. The use of sense of humor reduces the impact of stressful experiences (Martin & Lefcourt, 1984) and enhances the immune cells (Martin & Dobbin, 1988). However, in a review by Martin (2001), studies reported little or no relationship between

humor and the immune system (e.g., Puig-Perez et al., 2018); some were contradictory and, according to the author, a study with different styles of humor needs to be performed, since optimism or laughter alone seem to have no effect on the immune system.

Thus, the aim of this work was to conceptually replicate (Petty, 2018) the model of Martin and Dobbin (1988). Conceptually the proposal was to work with the moderation of humor between stress and the immune system; however, it differs in theoretical and methodological terms. One of the differences is the investigation of four humor styles (2×2 : affiliative-intrapsychic; positive-detrimental), rather than situational humor and coping and liking humor, since the only type of humor in the original study that increased the immune system was situational humor. Originally the conceptualization of immunosuppression used a marker of allergy (immunoglobulin A), while in the present study, other markers used in the stress literature are put to the test (cortisol, glycemia, neutrophils-lymphocytes ratio—NLR).

The hassles scale was originally used to measure daily stress events (e.g., conflict with colleagues, losing things, having many things to do); however, for this study, due to the national characteristics, it was used to measure stronger stress events that impact the immune system for a longer period of time (e.g., being raped, a person close dying, losing the job, being robbed). Methodologically, the authors performed a cross-sectional study using questionnaires and blood samples, and this work followed the same original design. The first hypothesis of this study was that the positive humor styles (affiliative and self-enhancing) would have a moderating effect between the effect of stress on cortisol, glycemia, and white cells (NLR), and the second hypothesis was that detrimental humor styles (aggressive and self-defeating) would have an inverse effect. Figure 1 shows the proposed relationship among the studied variables.

Method.

Participants

We established the sample size based on *g**power test 3.1 version; the chosen statistical test was linear multiple regression, two tails, effect size 0.15, error probability 0.15, power 0.95 with two predictors; and the total sample size was 89. This study included a total of 90 users of a community service 82% female ($n = 73$) with mean age of 43.75 years ($SD = 16.04$), 80.5% of the sample had completed the 2nd year of schooling, and 50.10% had no fixed work, while 54.3% did not practice regular physical activity (it should be mentioned that 55.6% of these reported up to 3 h per week, therefore, below what is expected to be out of the sedentary range). The participant selection criteria were to speak the Portuguese language, to be over 18 or a minor with the signature of the person responsible, to have fasted for 8 h, to be available to rest after the collection of the cortisol hormone performed in the community service project provided by a university laboratory, and to have signed the consent form.

The exclusion criteria that were applied after the data collection were that the participants should be in good health, not taking antibiotics, not HIV positive, not having cancer or pregnant, with fasting glycemia below 126 mg/dL (based on the guidelines of the Brazilian Diabetes Society, 2018), and not presenting any other type of injury that could interfere with the examinations. The total number of participants was 108, which was reduced to 90 valid participants with the application of these exclusion criteria.

The work of Martin and Dobbin (1988) included 40 university students, while, in the present study, the participants were from the population and did not have conditions to attend a private health system (in the case of Brazil, the majority of the population), resorting to this type of service because of its speed and availability, with them usually being workers (formal and informal) that are doing routine health exams. Due to this characteristic of the sample, the study design was cross-sectional, considering the difficulty of accessing the participants again.

Instruments

Negative Events.

To access the negative events in life, a questionnaire based on that of Norbeck (1984) was adapted. The original questionnaire, although interesting, was too complex for the population. In a pre-test with 6 participants, they had great difficulty in understanding the full scale that sought to measure both the presence and the impact of a stressor event; however, given the type of response (dichotomized) performed by the participants (if the stressor event was present was always evaluated as highly stressful), the adaptation was chosen. In this way, the participants were asked whether any event on a dichotomous scale had happened to them in the previous year: assault, death of close relative (child, partner, or of the primary circle—each one item), presence of serious illness (in the participant or a close family member), divorce, unemployment, poor evaluation at work, fighting at work, problems with the police, problems with money, and victim of violence (rape, physical assault) and others. The scale, formed by the sum of the negative events reported, is similar to other studies using scales of negative events (Cramer et al., 2012; Hamaker et al., 2018).

Humor Styles

The humor style instrument is composed of 32 items, with anchored scales of agreement from 1 to 7 where responses with greater value on the scale indicate greater agreement. The instrument was translated and back-translated from the original in English proposed by Martin et al. (2003). The analysis of the adaptation of the instrument is reported in the data analysis session.

Blood Markers

A total of 108 samples were drawn through three vacuum tubes with separator gel (except for the complete blood count), containing 4ML in each tube, for the following biological reagents: Baseline plasma cortisol (30 min rest prior to examination), Fasting glycemia and Complete blood count, the equipment used were INTEGRA 400 plus, Cobas e 411, and XS-1000.

Data Collection Procedure

The participant when being called had to wait from 30 min–2 h (due to waiting for the cortisol examination) and meanwhile answered the questionnaires with the help of the researchers. As with the Martin and Dobbin (1988) study, we collected data at the same day.

It was observed that in order to answer all the original questions, the participants took up to 1 h–45 min (average of 30 min). There were cases of difficulties in understanding what was being asked, since most of the participants had up to high school education and the researchers decided to read the instrument for the participants in cases of illiteracy or for those who claimed to “have forgotten their glasses,” with no differences observed between the means of these participants ($n = 8$).

Ethical Care.

The present work was accepted by the ethics committee under CAAE number: 51340715.2.0000.0023, on 05/05/2016, and informed consent was obtained from all individual participants included in the study. The datasets generated during the current study are available from the corresponding author on reasonable request.

Data Processing and Analysis

The data analysis started with a descriptive analysis of the data in which the normality of the data was verified, and any typing errors corrected. The data of the fluid material were supplied by the laboratory as examination worksheets and included in the table. They were standardized when the analyses required this (Z score). The normality of the data was verified. There was no gender difference in cortisol, glycemia, and NLR ($F(1, 89) = 2.84, p = 0.10$; $F(1, 89) = 0.20, p = 0.66$; $F(1, 89) = 0.71, p = 0.40$, respectively).

The first analysis of the instrument of Martin et al. (2003) is based on the verification of the reliability of the initial factors as proposed by the Martin et al. (2003) but they do not fit well. A polychoric parallel analysis was then carried out to define the number of factors following Revelle (2018). The result of the analysis suggested 10 factors; however, there was a clear “elbow” for 4 factors that matched the proposed theory. These factors were tested in a confirmatory analysis that presented the following fit indices: Chi Square = 113.34; DF = 84; $X^2/DF = 1.34$; CFI = 0.85; TLI = 0.82; RMSEA = 0.07. Reliability is presented in Table 1. It should be

Table 1 Descriptive analyses and correlations between negative events, humor styles and markers, reliabilities on the diagonal (omegas)

	Reference value	M	SD	1	2	3	4	5	6	7
1 Negative Events	1–22	5.14	3.09	-						
2 Affiliative	1–7	4.75	1.47	.03	(.78)					
3 Self-enhancing	1–7	4.74	1.77	-.12	-.11	(.70)				
4 Self-defeating	1–7	3.51	1.41	-.04	.18	.36*	(.63)			
5 Aggressive	1–7	2.77	2.09	-.08	.06	-.14	-.04	(.72)		
6 ZCortisol	4.3–22.4 g/dL	13.59	7.11	-.24*	.10	-.08	-.05	.15		
7 ZGlycemia	70–99 mg/dl	94.14	10.00	-.17	.00	.18	.16	-.07	-.09	
NLR	-	2.01	1.02	.08	.05	-.08	.06	.13	.11	-.20

* $p < 0.05$; ** $p < 0.01$

NLR neutrophils/lymfocytis ratio

highlighted that being a preliminary version of the construct in Brazil and there are other in course (Schermer et al., 2019), some authors understand that these values, although relatively low, can be considered acceptable (Panayides, 2013; Peterson, 1994).

The exploratory analysis of the data was performed through descriptive statistics for the demographic variables, and to achieve the objectives of the work, correlation regression and moderator analyses were performed using the SPSS packages, Hayes Process procedures, version 3.1, and packages Psych (Revelle, 2018) and Lavaan (Rosseel, 2014).

Results

Table 1 shows the descriptive statistics and correlations between humor styles, negative events, and markers. Note that there was greater variation in relation to the number of negative events. With regard to humor styles, on average, positive styles seem to be more used than negative styles. It should be noted that the values found in the sample do not differ from the expected population patterns; in other words, the sample was not particularly sick or out of some expected pattern.

To test the hypothesis of this work, individual regression analyses were carried out with each marker. As none of the humor styles showed a significant correlation with the biochemical markers, it was decided not to perform regression analysis and moderation.

Table 2 shows that there were no direct or moderating effects between stress and the markers. One result that draws attention, even in the case of relatively marginal effects, is that Negative Events had the opposite effect to what was expected, in that they were related to a reduction of cortisol and glycemia.

The first hypothesis of the work suggested that positive humor styles would moderate the relationship between negative events and markers, with this hypothesis

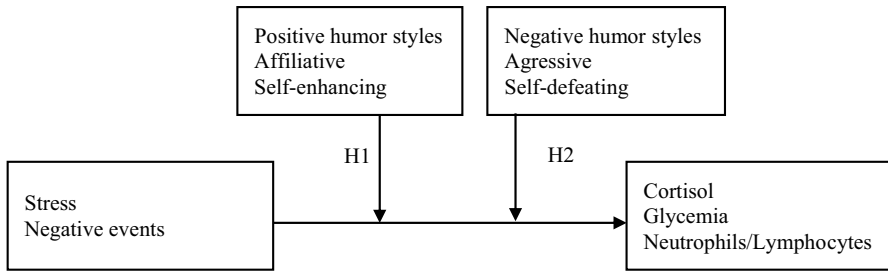


Fig. 1 Moderation model proposed to me tested in the present study

being refuted. Regarding the second hypothesis, which proposed detrimental humor styles would moderate the relationship between negative events and markers, this was refuted in most of the variables. The only exception was the self-defeating humor style, and the moderation graph is in Fig. 2.

Figure 2 suggests low and high levels of negative events had interesting combination of self-defeat style of humor. In this case, the use of self-defeat with low levels of negative events is protective, but the use of self-defeat with high levels of negative events is problematic.

Discussion

The aim of this study was to conceptually replicate the work of Martin and Dobbin (1988), investigating whether the sense of humor moderates the immunosuppressive effect of stress, with the aim of the work being achieved. For Lessing-Venter (2018), humor and culture are inseparable because humor is a mirror of the culture in which it is produced. Although a conceptual replication of the work was performed, according to the results of this study, in general, it is not possible to state that the relationship between stressor events and biochemical markers is moderated by styles of humor for a Brazilian sample, except for the self-defeating humor style in the NLR.

There are some possible explanations for the differences found in this study compared with the original one. One possible difference is that the original study used an immunoglobulin A biological marker, which differ from the cortisol, glycemia, and NLR rate used here. Although all markers are related to stress, the marker used by them can be more sensitive.

Another possible explanation is due by the style of humor used that was also different from Situational Humor Response Questionnaire in general sense; however, the questionnaire used here derives from that one and was applied by dimension; thus this explanation is less likely. Finally, another difference is on the sample composition. The original study was more balanced in its gender composition (18 males and 22 females), and in the present study we have more female sample that can differ in both variables: style of humor and blood markers. In this sense, we need more studies to make those differences clear.

Table 2 Direct and moderated regression analysis between Negative Events and markers

	R ²	Marker			<i>p</i>
		β	Lower CL	Upper CL	
Z Cortisol					
Negative Events	.04	-.19			.07
Self-enhancing	.00	-.06			.56
Affiliative	.01	.07			.50
Self-defeating	.00	-.05			.64
Aggressive	.02	.13			.23
Negative Events* Self-enhancing	.07	.03	-.01	.07	.12 ⁺
Negative Events * Affiliative	.06	-.03	-.08	.02	.26
Negative Events* Self-defeating	.04	.02	-.04	.07	.51
Negative Events* Aggressive	.06	-.01	-.05	.02	.50
Z Glycemia					
Negative Events	.00	-.05			.64
Self-enhancing	.02	.15			.14
Affiliative	.00	.02			.84
Self-defeating	.00	.04			.72
Aggressive	.02	-.15			.16
Negative Events* Self-enhancing	.03	-.01	-.04	.03	.78
Negative Events* Affiliative	.01	-.03	-.08	.03	.34
Negative Events* Self-defeating	.01	-.01	-.06	.04	.63
Negative Events* Aggressive	.05	-.02	-.06	.01	.17
Z Neutrophils/Lymphocytes ratio					
Negative Events	.01	.08			.43
Self-enhancing	.01	-.07			.48
Affiliative	.00	.03			.76
Self-defeating	.00	.06			.54
Aggressive	.01	.10			.34
Negative Events* Self-enhancing	.01	-.00	-.05	.04	.88
Negative Events* Affiliative	.01	-.02	-.04	.07	.50
Negative Events* Self-defeating	.06	-.06	.01	.11	.04
Negative Events* Aggressive	.02	-.00	-.04	.04	.98

Notes: ⁺p interaction presented

Although it is expected that styles of humor work as a moderator variable between biochemical markers and stress, it could be based on the idea that humor and emotion are close concepts. In fact, they are not. People can feel positive emotions without using styles of humor as a coping strategy, and conversely, people can use different styles of humor and not feel well. Thus, the results found here do not invalidate the idea of laughter and positive emotions on health. The idea is that styles of humor in themselves may not protect you, despite this being a common misconception.

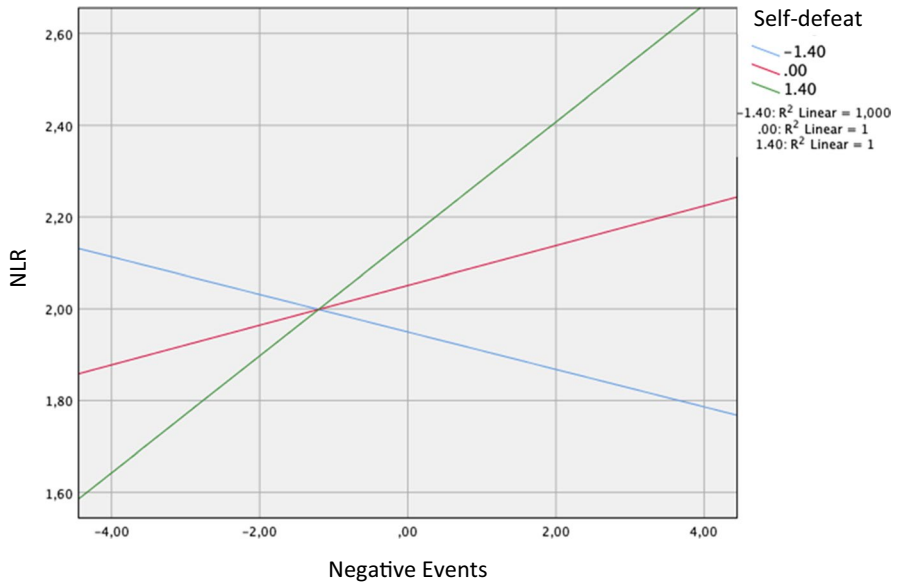


Fig. 2 Self-defeat moderation between negative events and neutrophils/lymphocytes ratio

Self-defeating humor style is the tendency to amuse others at one's own expense, and it is quite common in this sample and shows similar results in other Brazilian samples (Schermer et al., 2019). Apparently, this style of humor has a human cost. When individuals have a well-adjusted life, it seems to be protective, however, when the number of stressors increases, the use of this style of humor can be detrimental. This finding collaborates with Dickerson and Kemeny (2004) proposal and clarifies some possibly contradictory findings between the self-defeat style of humor.

Negative events were also associated with a decrease in cortisol and glycemia. It is assumed to be a marginal effect, however, may be related to a longitudinal effect of the negative event. Many of the cortisol effects measured in the literature occur on the same day (e.g., Pulpulos and Kozusznik, 2018; Puig-Perez et al., 2018) or for short periods and, in this case, could have a different relationship depending on this delta. Longitudinal studies or those proposing dynamic relationships between these constructs are scarce; the dynamic and self-regressive models themselves are still a methodological challenge (Bringmann & Eronen, 2018; Bringmann et al., 2013, 2015; Hamaker et al., 2018). The answer to this kind of question goes beyond the scope of this study.

The present study design conceptually replicated the study of Martin and Dobbin (1988) and presents clear methodological limitations. The first of these is the cross-sectional design, which does not allow conclusions to be drawn regarding the effects (or their absences) and their dynamics. However, as styles of humor are quite stable variable, it allowed us to measure at the same time of other variables. In addition, the scale of negative events cannot estimate or measure the impact of the negative effect on the individual, only their presence.

Stress literature has long argued that stress is due to the interaction between the person and event and not only due to the event or context (Aneshensel, 1992; Coyne & Downey, 1991; Krohne, 2001); however, given the difficulty with the population, it was chosen to make the study feasible and not differ from what has also been carried out in the literature. There are other biological markers that could be included in future studies as glycated hemoglobin¹ and try the immunoglobulin A, biological marker used by Martin and Dobbin original study. There is a sample bias in this study where more females participate in the study and the differences can be due by this. In addition, there are other factors that may affect the relationship between stress and markers and that may contribute to the non-relationship between the variables.

Despite the limitations, it is understood the study also presents contributions. The first is to present the results of a conceptual replication, which is rare in the international and national literature. This approach suggested that the relationship between a stressor context and the markers may be more complex than the one found and that the studies tend to present the results that find significant variables and not present results that are not significant. Another contribution is to present data from a usually neglected group (low-income people) that may have a different stress response when compared with the groups typically studied (Aneshensel, 1992).

There are, however, questions that remain open for future studies: In this difference of social class, is there a relationship mediated or moderated differently between stress and markers? (Aneshensel, 1992). Will there be gender and age difference in the use of humor and with that would the moderators present another pattern? (Pulopulos et al., 2018). Could other psychological variables moderate the relationship between stress-markers? What is the dynamic and longitudinal relationship between psychological variables and blood variables? The answers to these questions could offer critical information in the case of interventions as the development of the relationship between stress and markers can be differentiated and must be treated differently.

This work is a conceptual replication research of the moderated relation between negative events and blood markers by humor styles, which is unusual in psychology and less using biomarkers. It contributes with the literature when our findings show that it was not possible, in general, to state that the relationship between stressor events and biochemical markers was moderated by styles of humor for a Brazilian underserved population sample. Furthermore, it shows that self-defeat humor style could present a double role: for adjusted lives to be protective, for a life with problems, to be detrimental. Also, that the increase of the number of negative events shows a challenging result: it reduced the cortisol levels. These reinforce the importance of replication studies when display contradictory results applied to different samples characteristics.

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Declarations

Conflict of Interest The authors declare that they have no conflict of interest and no fundings were received by the authors to conduct this research.

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