Neuroendocrinological and neurophysiological adaptation to stress and relaxation during pregnancy

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IB, who was the main investigator, analyzed and interpreted the data and compiled the manuscript. JH contributed to interpretation of the data. FS and HP were the study supervisors and contributed to analysis and interpretation of the data. NS and AW contributed intellectual content. BD contributed intellectual content, analysis and interpretation of the data. All authors contributed to the final manuscript and read and approved the final manuscript.

Zusammenfassung

Stress in der Schwangerschaft kann die fetale und neonatale Entwicklung nachteilig beeinflussen sowie das Risiko für Übergewicht, Diabetes Typ 2 oder psychiatrische Erkrankungen erhöhen. Darüber hinaus wurde bereits in mehreren Studien gezeigt, dass sowohl ein erhöhter Body-Mass-Index (BMI) als auch erhöhte Entzündungsmarker wie Interleukin-6 nachteilige Auswirkungen auf die Entwicklung des Kindes haben können. Um Stress zu reduzieren, haben Entspannungstechniken in der Schwangerschaft an Bedeutung gewonnen. Aufgrund potenzieller körperlicher Einschränkungen im dritten Trimester der Schwangerschaft sind hier vor allem mentale Techniken besonders weit verbreitet. Es wurde gezeigt, dass insbesondere Musik hören in der Schwangerschaft einen positiven Einfluss auf das Stresslevel der schwangeren Frau haben kann.

Ziel dieser Arbeit ist es zu untersuchen, inwieweit Stress in der Schwangerschaft einen Einfluss auf das mütterliche inflammatorische Milieu und das fetale autonome Nervensystem hat. Zusätzlich soll untersucht werden, welche Entspannungstechnik den größten Entspannungseffekt, gemessen am mütterlichen autonomen Nervensystem, hat.

Im Rahmen dieser Arbeit wurden drei verschiedene Studien durchgeführt:

Die erste Studie befasst sich mit zwei mentalen Entspannungstechniken (Musik und Gedankenreise). Das Ziel war es zu untersuchen, welche der beiden Entspannungstechniken verglichen mit einer Kontrollgruppe (ohne Entspannungsstimulation) schwangere Frauen zwischen der 30. und 40. Schwangerschaftswoche am meisten entspannt. Hierbei wurde die mütterliche Herzrate, die mütterliche Hautleitfähigkeit und das allgemeine Stresslevel der Probandinnen gemessen. In dieser Studie konnten wir keinen signifikanten Unterschied hinsichtlich dieser Parameter zwischen den Entspannungsgruppen feststellen. Alle drei Entspannungsgruppen zeigten eine signifikante Verbesserung des mütterlichen Stresslevels basierend auf den Fragebögen und einer Visuellen Analog Skala.

In der zweiten Studie sollte der Effekt einer akuten mentalen Entspannung (Musik hören) auf das mütterliche und fetale autonome Nervensystem im Vergleich zu einer Kontrollgruppe (keine Entspannungsstimulation) untersucht werden. Zudem sollte untersucht werden, inwieweit der chronische Stress der Mutter einen Einfluss auf das fetale autonome Nervensystem hat. Hierbei wurde mithilfe der Fetalen

Magnetenzephalographie (fMEG) die mütterliche und fetale kardiovaskuläre Aktivität aufgezeichnet. Simultan dazu wurde die mütterliche Hautleitfähigkeit gemessen. Um den Einfluss von chronischem Stress zu untersuchen, wurden bei den Probandinnen Haarproben und Blutproben (Cortisol) entnommen. Basierend auf den physiologischen Parametern konnte keine signifikante mütterliche Entspannung zwischen den Gruppen festgestellt werden. Es zeigte sich jedoch ein signifikanter Unterschied zwischen den Gruppen in der Veränderung der fetalen Herzrate über die Zeit. Darüber hinaus war kein signifikanter Einfluss von mütterlichem chronischem Stress (Haarcortisol) auf die Veränderungen der fetalen Herzrate vorhanden. Basierend auf den Fragebögen und dem dadurch erfassten chronischen Stress ergab sich eine signifikante Korrelation mit der Veränderung der fetalen Herzratenvariabilität.

Das Ziel der dritten Studie war es, Auswirkungen von mütterlichem Stress in der Schwangerschaft auf das inflammatorische Milieu sowie die Insulinsensitivität von schwangeren Frauen zu untersuchen. Zwischen den drei Stressgruppen (kein Stress, wenig Stress, milder Stress) konnte kein signifikanter Unterschied im Interleukin-6 Level, als inflammatorischer Marker, gefunden werden. Es zeigte sich jedoch ein signifikanter Zusammenhang zwischen dem BMI vor der Schwangerschaft und dem Interleukin-6 Level. Interleukin-6 und die mütterliche Insulinsensitivität sowie der Cortisolwert der Frauen korrelierten signifikant miteinander. Basierend auf einer Mediationsanalyse wurde gezeigt, dass ein erhöhter BMI vor der Schwangerschaft in Zusammenhang mit mütterlichem Stress in der Schwangerschaft das Interleukin-6 Level signifikant beeinflusst.

Insgesamt konnte basierend auf den drei durchgeführten Studien gezeigt werden, dass milder mütterlicher Stress keinen Einfluss auf das inflammatorische Milieu der Mutter hatte. Jedoch waren ein erhöhter BMI vor der Schwangerschaft und eine niedrige Insulinsensitivität in Kombination mit mildem mütterlichen Stress in der Schwangerschaft verantwortlich für eine Disbalance im inflammatorischen Milieu. Im Hinblick auf den chronischen Stress konnte ein Einfluss auf die fetale Herzratenvariabilität festgestellt werden.

Bezüglich der Entspannung zeigte sich, dass mütterliche einmalige Entspannung einen signifikanten positiven Einfluss auf das mütterliche Wohlbefinden, nicht aber auf das fetale Nervensystem hatte.

Basierend auf den Ergebnissen dieser Studien rückt vor allem die Zeitspanne vor der Schwangerschaft in den Fokus. Die Auswirkungen des erhöhten BMI *vor* der Schwangerschaft sowie stresseindämmende Maßnahmen *in* der Schwangerschaft als beeinflussbare Faktoren sollten in den Mittelpunkt rücken. Durch strukturelle Stress-Screenings zu Beginn der Schwangerschaft könnten Frauen mit hohem chronischen Stress frühzeitig begleitet und durch Stressmanagement-Interventionen unterstützt werden.

Summary

Stress during pregnancy can adversely affect fetal and neonatal development, as well as increase the risk of obesity, type 2 diabetes, or psychiatric disorders in later life. Moreover, several studies have already shown that both an increased body mass index (BMI) and increased inflammatory markers such as Interleukin-6 can have adverse effects on the development of the child. To prevent stress, relaxation techniques in particular have gained importance during pregnancy. Due to potential physical limitations in the third trimester of pregnancy, mental techniques are particularly well suited. In particular, listening to music during pregnancy has been shown to have a positive influence on the stress level of the pregnant woman.

The aim of this work is to investigate the extent to which stress in pregnancy influences the maternal inflammatory milieu and the fetal autonomic nervous system. In addition, it will be investigated which relaxation technique (music, guided imagery or rest) has the greatest possible relaxation effect as measured by the maternal autonomic nervous system.

Three different studies were conducted as part of this work:

The first study deals with two mental relaxation techniques (music and guided imagery). The aim was to investigate which of the two relaxation techniques compared to a control group (no relaxation stimulation) relaxes pregnant women between the 30^{th} and 40^{th} week of pregnancy the most. Here, maternal heart rate, maternal skin conductance and general stress level of the subjects were measured. In this study, no significant difference between the relaxation groups was determined. All three relaxation groups showed a significant improvement in maternal stress level on basis of the questionnaires and a Visual Analog Scale.

In the second study, we compared the effect of acute mental relaxation (listening to music) on the maternal and fetal autonomic nervous system of pregnant women in comparison to a control group (no relaxation stimulation). The extent to which chronic stress of the mother influences the fetal autonomic nervous system was also investigated. Maternal and fetal cardiovascular activity was recorded using fetal magnetoencephalography (fMEG), during which maternal skin conductance level was also measured. To investigate the influence of chronic stress, hair samples and blood

samples were taken from the subjects. We could not detect any significant difference in maternal relaxation between the groups on the basis of the physiological parameters. However, we showed significantly different changes of fetal heart rates. In addition, no significant effect of maternal chronic stress (hair cortisol) on the changes in fetal heart rate were observed. Based on the questionnaires, we investigated a significant correlation between chronic stress and the change of fetal heart rate variability.

The third study investigated the effects of maternal stress in pregnancy on the inflammatory milieu as well as insulin sensitivity in pregnant women. In three stress groups (no stress, low stress, mild stress), we did not detect any significant difference in the level of Interleukin-6, an inflammatory marker, between the groups. However, in our cohort, we found a significant association between pre-pregnancy BMI and Interleukin-6. Interleukin-6 and maternal insulin sensitivity as well as women's cortisol levels correlated significantly with each other. We used mediation analysis to demonstrate that increased pre-pregnancy BMI associated with maternal stress in pregnancy had a significant influence on the Interleukin-6 level.

Overall, on the basis of the three studies performed, we demonstrated that maternal mild stress had no effect on the maternal inflammatory milieu. However, the combination of increased pre-pregnancy BMI, decreased insulin sensitivity and mild stress during pregnancy resulted in a disbalance in the inflammatory milieu. With regard to chronic stress, we detected influence on fetal heart rate variability only.

Focusing on relaxation, we demonstrated that maternal relaxation has a significant positive influence on the well-being of the mother but not on the fetal nervous system.

On the basis of these studies, the phase *prior to* pregnancy in particular comes into focus, both with regard to an increased BMI before pregnancy and stress-reducing measures during pregnancy. Here, especially through structural stress screening at the beginning of pregnancy, women with high chronic stress could be supported at an early stage and stress management interventions could be developed and offered.

Contents

Abbreviations

ACTH	*Adrenocorticotropic hormone*
AIC	*Akaike-Information-Criterium*
ANS	*autonomic nervous system*
BMI	*Body-Mass-Index*
bpm	*beats per minute*
CRH	*corticotrophin-releasing hormone*
DOHaD	*Developmental origins of health and disease*
ECG	*Electrocardiogram*
EDA	*Electrodermal Activity*
ELISA	*Enzyme Linked Immunosobent assay*
FHR	*Fetal heart rate*
FHRV	*Fetal heart rate variability*
fMEG	*Fetal magnetoencephalography*
fMRI	*functional magnetic resonance imaging*
GA	*Gestational Age*
GDM	*Gestational Diabetes Mellitus*
GzLM	*Generalized Linear Model*
HF	*high frequency*
HFn	*high frequency normalized*
HPA	*Hypothalamus-Pituitary-Adrenal Axis*
HR	*Heart Rate*
HRV	*Heart Rate Variability*
IL-6	*Interleukin-6*
LF	*low frequency*
LFn	*low frequency normalized*
MCG	*Magnetocardiogram*
MES	*adverse maternal emotional states*
MHR	*Maternal heart rate*
MWG	*maternal weight gain*
NEFA-ISI	*nonesterified-free-fatty-acids insulin sensitivity index*
NGT	*normal glucose tolerance*
OGTT	*Oral glucose tolerance test*
PDQ	*Prenatal Distress Questionnaire*

PHQ-D	*Patient Health Questionnaire*
POMS	*Profile of Mood States*
Q_{STIM}	*Questionnaire to rate relaxation stimulation*
RIS	*Relaxation Inventory Scale*
RMSSD	*root mean square of successive differences*
SAM	*Sympathetic-Adrenomedullar Axis*
SCL	*Skin conductance level*
SDNN	*Standard deviation of RR intervals*
SSCS	*Short Screening Scale of Chronic Stress*
VAS_{Stress}	*Visual Analog Scale for current stress level*

1 Introduction

1.1 Developmental origins of health and disease (DOHaD)

This work is based on the concept of developmental origins of health and disease (DOHaD), which assumes that external environmental adverse factors shape early human development and thus increase the risk of chronic diseases developed in later life (Hanson & Gluckman, 2014). In general, the framework underpins the importance of environmental factors, particularly during the prenatal phase and its implications on the understanding of the pathogenesis of various diseases.

One of the scientists who developed this theory was Barker, who mainly described a relationship between environmental factors at an early stage of life and higher risk of disease later in life. This concept of 'fetal origins of adult disease' is also known as Barkers' hypothesis (Barker, 1995). Barker and colleagues first linked poor birth outcomes e.g., low birthweight with a higher prevalence of hypertension, coronary heart disease and other diseases in adulthood (Hanson & Gluckman, 2014). Barker's hypothesis was, in fact, actually based on the fact that mothers who are pregnant in a poorer environment e.g., with poverty, overcrowding and areas with high perinatal mortality, often give birth to newborns with lower birth weight. In one of his first studies, he traced 5654 men born between 1911 and 1930 and investigated their birth weight and whether or not they were breast fed (Barker, Osmond, Winter, Margetts, & Simmonds, 1989). Men with normal birth weight and those who were breastfed for the first year of life were reported to have a lower death rate from heart disease or stroke in adulthood (Barker et al., 1989; Barker, 1990). This and many other epidemiological studies ensured wide acceptance of what later became known as the 'DOHaD concept' (Hanson & Gluckman, 2014). In short: "Adaptive effects of relevance to DOHaD involve physiological processes of developmental plasticity and broadly include the categories of immediately adaptive and predictive or anticipatory adaptive responses." (Hanson & Gluckman, 2014, p. 1031).

1.2 Pregnancy as a sensitive period

Much research in recent years has focused on the psychological and physical well-being of pregnant women. Highlighted major issues that may have long-lasting effects on the offspring, are maternal overweight or obesity, maternal stress, excessive maternal weight gain, preterm birth, gestational diabetes, or the use of any medication, toxins, and endocrine disruptors during pregnancy (Hanson & Gluckman, 2014). Pregnancy is characterized by different personal expectations, hormonal changes and a new coordination of the professional and social environment, which frequently entails financial or health concerns (Adams et al., 2016; Hetherington, McDonald, Williamson, & Tough, 2020; Ozbay et al., 2007). Intense emotional states are therefore often associated with this period of life. One concern of expectant mothers is the well-being of their fetus and the impact of their own lifestyle and behaviors on fetal development, including how their own emotional state may affect the fetus (Adams et al., 2016). Research pertaining to the maternal emotional state during pregnancy and its influence on fetal and neonatal development has largely focused on psychological concepts and triggers of various maternal emotional states.

The placenta – the maternal-fetal window

Within the DOHaD concept, it is therefore clear that the fetus is influenced by maternal factors. Thus, the placenta is sensitive to fetal demands but is also conditioned by maternal factors (Burton & Jauniaux, 2015; Hanson & Gluckman, 2014). Furthermore, the placenta is affected by the caloric intake of the mother (Chen et al., 2013), and the transport of glucose through the placental tissues respectively (Illsley & Baumann, 2020). The placenta also has protective properties. These are mainly regulated by 11ß-hydroxysteroid dehydrogenase type 1 which controls fetal exposure to glucocorticoids like cortisol (Lindsay, Lindsay, Waddell, & Seckl, 1996). While high cortisol values are often initiated by high stress, developmental research is therefore now placing more emphasis on the role of maternal stress during pregnancy (van den Bergh et al., 2020).

1.3 Stress concept

1.3.1 Theory of stress – concept and definition

Stress is a widely used and important concept in basic and clinical psychology to examine changes in the body and to comprehend the pathophysiology of specific diseases. Stress is essentially an adaptive reaction of the organism that should restore the inner balance – the homeostasis (Godoy, Rossignoli, Delfino-Pereira, Garcia-Cairasco, & Lima Umeoka, 2018). However, a high level of stress over a long period of time can lead to chronic stress and cause allostasis (S. Fisher & J. Reason, 1988). Allostasis is the result of long-term adaptation processes after e.g., chronic stress, stabilizing physiological functions that lie outside the normal response range (Godoy et al., 2018). There are, generally speaking, two different systems involved in the stress response: the Sympathetic-Adreno-Medullary (SAM) axis and the Hypothalamus-Pituitary-Adrenal (HPA) axis (Pinel, 1997). The HPA axis responds differently to chronic and acute stress. This is due to the fact that episodic or acute stress modulates the physiological system over a shorter period of time (Pinel, 1997). Chronic stress significantly promotes the development of chronic diseases (Cohen, Gianaros, & Manuck, 2016). These reaction patterns can affect the immune and endocrine system (Cohen et al., 2016; Wurster & Keller, 1985) and/or the autonomic nervous system (Cohen et al., 2016).

One major breakthrough in recent decades in research on the relationship between stress and various diseases was that stress can have a significant impact on the immune system (Pinel, 1997). Cytokines, together with corticosteroids and catecholamines, are important stress mediators (Black, 2003). Cytokines also play a major role in inflammation processes due to immune reactions. Hence, the event of a stressor is associated with elevated circulatory pro-inflammatory cytokines (Godoy et al., 2018; Steptoe, Hamer, & Chida, 2007).

1.3.2 The stress response – general mechanisms and adaptation

The response of stress is essentially a reaction to an external threat. All stressors, both physical and psychological, lead to the same basic patterns of physiological responses. Stress responses cause the body to be ready to fight or flee ('Fight-or-Flight' response (Walter B. Cannon, 1915)). One of the first scientists to define a stress response model

from a biological point of view was Hans Selye. He defined the stress response by dividing it into three phases: the alarm phase (sympathetic activation), the resistance phase (increased catecholamine release), and the exhaustion phase (increased hormone release can no longer be maintained) (Godoy et al., 2018). This model is called the General Adaptation Syndrome (SELYE, 1950).

The coping model of Lazarus mainly depends on the cognitive evaluation of a specific stressor (Lazarus & Folkman, 1984). In this model, three phases are defined: the primary appraisal, the secondary appraisal and the coping phase. Primary appraisal includes the assessment on the given environmental conditions as well as the individual characteristics of a person (Lazarus & Folkman, 1984). In the secondary appraisal the available resources for a possible solution of the stressing situation are estimated (Lazarus & Folkman, 1984). The third phase then includes the coping phase and the reassessment of the stressor (Lazarus & Folkman, 1984). This may explain why the stress perception differs inter-individually.

A biological mechanism invariably underlies these theoretical models. However, psychological and physiological stressors activate different neuronal networks and circuits in the brain (see Figure 1). Physiological stressors activate the autonomic nervous system more frequently, while psychological stressors activate both, physical and cognitive stress responses (Godoy et al., 2018).

First, the stress response is often accompanied by e.g., an elevated pulse, increased blood pressure, rapid breathing or increased electrodermal activity (Godoy et al., 2018). The SAM axis cause the release of catecholamines such as epinephrine and noradrenaline (Pinel, 1997).

Second, the HPA axis and the release of glucocorticoids is activated. The HPA axis and the glucocorticoids increase e.g., cortisol levels in the blood (Pinel, 1997). Glucocorticoids, like cortisol, play an important role in the body's major stress response system. In pregnancy, the HPA axis is profoundly affected by the production of the corticotrophin-releasing hormone (CRH) from the placenta (Sandman, Wadhwa, Chicz-DeMet, Porto, & Garite, 1999). An increase in CRH activates the cortisol production which, in turn, usually increases progressively during gestation (Buss, Davis et al., 2012).

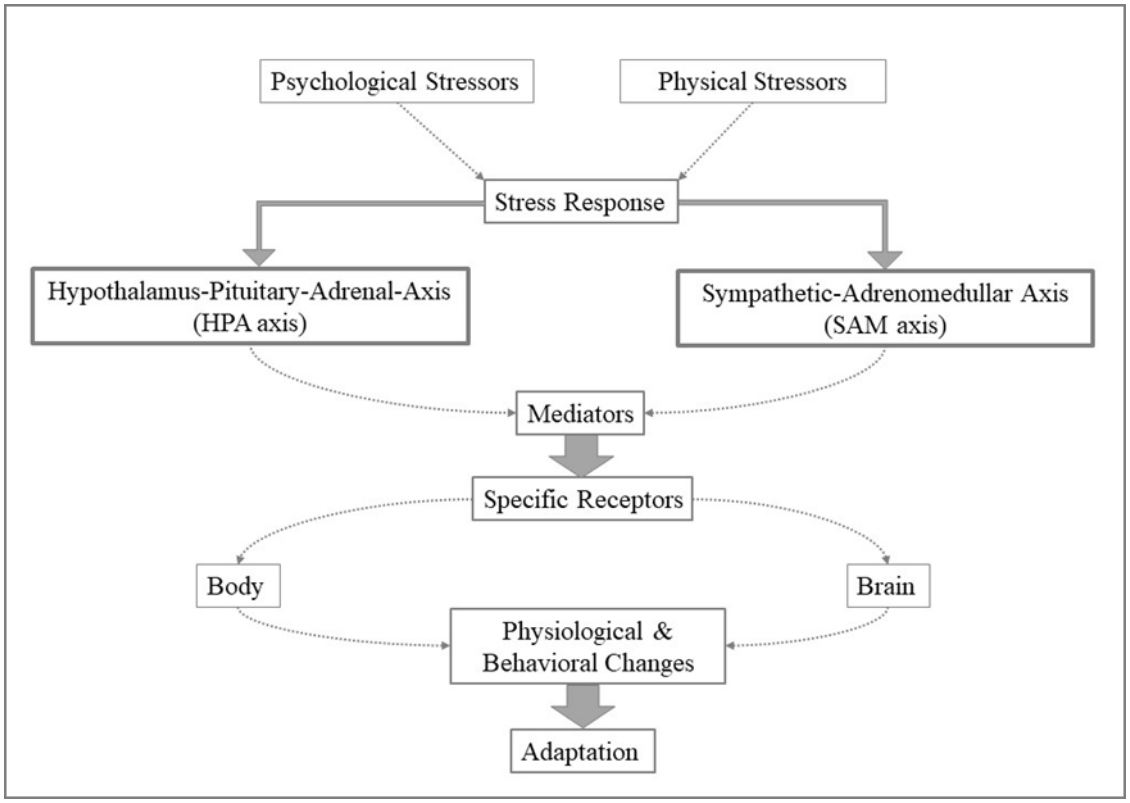


Figure 1. Scheme of the stress response (adapted from Godoy et al., 2018, *Figure 1*).

Once the SAM and HPA systems are activated, they generate a coordinated response to restore homeostasis (Godoy et al., 2018). As a result "[…] the stress response systemically promotes energy mobilization, metabolic changes, activation of the immune system and suppression of the digestive and reproductive systems." (Godoy et al., 2018, p. 3). Collectively, this body-brain activation of mechanisms mediate changes in physiology and behavior that enables further adaptation to stressors (Godoy et al., 2018) (see Figure 1).

Autonomic nervous system

One major key system in response to stress is the autonomic nervous system (ANS). The ANS is typically divided into two main parts: the sympathetic and the parasympathetic nervous system (van den Bergh et al., 2020). In detail, the parasympathetic nervous system plays an important role in modulating the stress response by inhibiting or activating the sympathetic nervous system (Kim, Cheon, Bai, Lee, & Koo, 2018). Both systems are closely connected. The heart rate (HR) can be a measure of stress activity and is controlled by the ANS (Kim et al., 2018).

In addition to the HR, the heart rate variability (HRV) reflects the interval between the successive heartbeats. HRV is an accurate means of investigating the autonomic control of the cardiovascular system (van den Bergh et al., 2020). It is mainly used to predict common psychological and physiological disorders that increase sympathetic activity and lead to autonomic imbalance (Shaffer & Ginsberg, 2017). High HRV is a sign of good health and adaptation, whereas low HRV is often an indicator of an adverse impairment in autonomic control (Böckelmann, 2012). The standard parameters in the analysis of HRV comprise time domain, frequency domain, and non-linear techniques (Böckelmann, 2012). The time and frequency domain parameters, with their formulas and assumed influence of the ANS branches, are summarized and presented in Table 1.

Table 1. Description of assessed heart rate variability (HRV) parameters.

Parameters of Heart Rate Variability (HRV)

	Parameters	Description	Associated autonomic function
Time Domain	HR (bpm)	Heart rate	Sympathetic and parasympathetic
	SDNN (ms)	Standard deviation of RR intervals	Overall HRV, sympathetic and parasympathetic
	RMSSD (ms)	Root mean square of successive differences of RR intervals	Short-term HRV, parasympathetic
Frequency Domain	LFn (normalized)	Low frequency power normalized; Fetal: 0.08 to 0.20 Hz Maternal: 0.04 to 0.15 Hz	Sympathetic and parasympathetic
	HFn (normalized)	High frequency power normalized; Fetal: 0.40 to 1.70 Hz Maternal: 0.15 to 0.40 Hz	Primarily parasympathetic
	LF/HF	Ratio of LF to HF power	Sympathovagal balance

bpm: beats per minute; (from Mat Husin et al., 2020, *Table 2*)

1.4 Stress and relaxation during pregnancy and its implications for mother and fetus

1.4.1 Maternal stress during pregnancy

Over the past few decades, numerous investigations have reported adverse effects of maternal stress during pregnancy on fetal development and offspring (Nazzari et al., 2019; Pawluski, Lonstein, & Fleming, 2017; Scheinost et al., 2017; van den Bergh et al., 2020). However, the literature does not distinguish between the different psychological concepts such as symptoms of anxiety, depressive symptoms and stress per se. As a result, anxiety symptoms or depressive symptoms are equated with stress symptoms and interpreted as such. This then leads to a controversial study situation as to the interpretability and the unambiguousness of the study results with regard to stress in general.

This chapter provides an overview of the current studies on adverse maternal emotional states and the effects on the mother and fetal development as well as the effects on the first years of the child's life. The umbrella term ‘adverse maternal emotional states’

(MES) is therefore used to refer to all symptoms (symptoms of anxiety, depressive symptoms but also maternal stress).

During pregnancy, a wide variety of different factors can impact fetal development and maternal organism. MES during pregnancy can cause alterations in the metabolic, hormonal and inflammatory milieu and in the ANS of both: fetus and mother (see Figure 2).

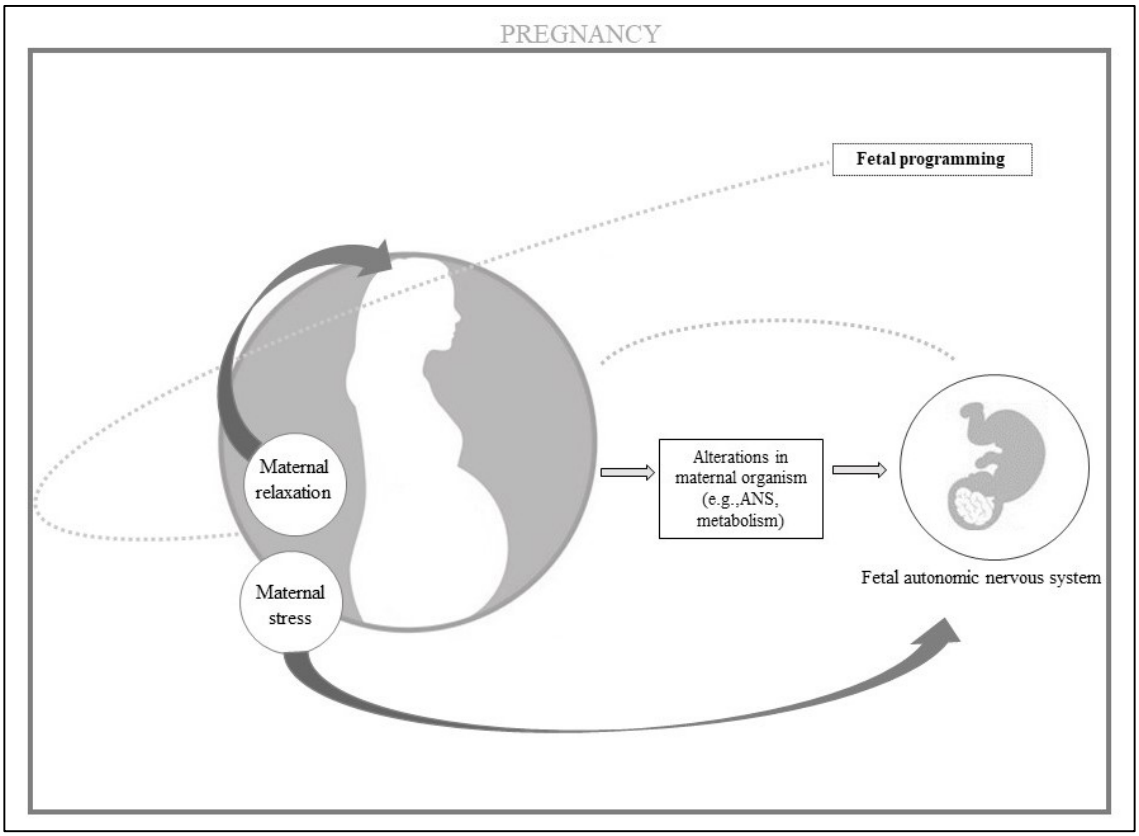


Figure 2. Overview: Maternal stress and relaxation during pregnancy.

Schematic illustration of maternal relaxation and stress and their implications for mother and fetus.

Pregnancy can be characterized by various personal expectations as well as a new orientation towards one's social and professional environment (Hetherington et al., 2020). In addition, hormonal and physical changes occur during pregnancy. Some women also report pregnancy-related concerns regarding the normal course of pregnancy and childbirth, as well as a lack of social support during pregnancy (Adams et al., 2016; Ozbay et al., 2007). Mostly, women reported increased concern with regard to their baby's health, giving birth and possible miscarriage (Ohman, Grunewald, & Waldenström, 2003). All these factors can cause stress or negative moods in the mother

(McLeish & Redshaw, 2017). Besides, an imbalance can cause negative emotions with regard to birth and adverse effects on mother-child bonding postpartum and increases the risk of preterm birth (Radoš, Matijaš, Anđelinović, Čartolovni, & Ayers, 2020; Yonkers et al., 2014). In this context, women who experience 'major life events' such as the death of family member, tend to be more susceptible to preterm birth (Dunkel Schetter & Tanner, 2012).

In general, a healthy pregnancy is characterized as an 'inflammatory state of the body' and both higher cortisol- and inflammatory levels are normal within a certain range (Shelton, Schminkey, & Groer, 2015). Maternal stress over a prolonged period of time therefore not only adversely affects the health of the expectant mother, but may also compromise the development of the offspring. Several authors have defined the adverse effects of severe MES, which include social stress, prenatal distress, depression and anxiety, in several areas of fetal and infant development (Dunkel Schetter & Tanner, 2012; Kingston, Tough, & Whitfield, 2012; Lewis, Austin, & Galbally, 2016; Weinstock, 2005). Accordingly, severe MES levels can lead to adverse effects on the physiological, metabolic and neuronal development of the fetus during gestation with possible long-lasting effects (Entringer, 2013). Maternal stress, depression and anxiety have, for instance, been shown to impact fetal HR, fetal activity, sleep patterns and movement (Kinsella & Monk, 2009). In particular, with respect to fetal development, increased glucocorticoids may have an adverse impact on fetal development in terms of cognitive function (Kapoor, Dunn, Kostaki, Andrews, & Matthews, 2006) and emotion regulation (Buss, Davis et al., 2012). The results of most studies indicate that increased maternal cortisol or Interleukin-6 (IL-6) levels due to high and chronic stress are associated with the emotional and behavioral development across the child's lifespan by affecting cognitive performance (Davis & Sandman, 2010; Graham et al., 2018; Hantsoo, Kornfield, Anguera, & Epperson, 2019; Rudolph et al., 2018).

Moreover, psychological stressors increase maternal and fetal cortisol and inflammatory parameters during pregnancy (van den Bergh et al., 2020). For example, fetal motor activity, which is an indicator for prenatal development, has been associated with maternal cortisol levels (DiPietro, Kivlighan, Costigan, & Laudenslager, 2009), which highlights the relation between maternal cortisol values and fetal development. One recent study demonstrated that both prenatal maternal symptoms of anxiety and depressive symptoms and fluctuations in maternal inflammation in late pregnancy are

associated with changes in fetal growth and higher cortisol values immediately after a newborn's heel-stick test, which is a stressor (Nazzari et al., 2019). This implies that maternal antenatal cortisol values are associated with changes in neonatal stress reactivity. This study confirms other recent reports of an association between maternal increased emotional states, offspring physiological stress reactivity, and cortisol levels (Entringer et al., 2010; Leff Gelman et al., 2019; Valsamakis et al., 2017). Furthermore, prenatal maternal anxiety symptoms are associated with increased maternal serum cortisol levels and lower insulin sensitivity during pregnancy (Valsamakis et al., 2017). A study by Bleker and colleagues (Bleker, Roseboom, Vrijkotte, Reynolds, & Rooij, 2017) reported an association between maternal serum cortisol levels during pregnancy and biological factors such as maternal age, pre-pregnancy BMI, gestational diabetes, parity and ethnicity and lifestyle factors (smoking, alcohol consumption, socioeconomic status, employment and physical activity).

In some cases, a link was also observed between inflammatory markers such as IL-6 and changes in pregnancy (Amirian, Mahani, & Abdi, 2020). IL-6 belongs to the group of cytokines with important functions in immune response, hematopoiesis, and inflammation (Jones, 2005). During pregnancy, IL-6 is important for maternal immunomodulation prior to embryo implantation and for placental development (Santhanam et al., 1991). Together with other components of the immune system, IL-6 plays an important role in maintaining pregnancy by controlling the inflammatory environment (Munoz-Suano, Hamilton, & Betz, 2011). A derailment in serum IL-6 levels can therefore have far-reaching effects on pregnancy outcome and increase potential risk factors for disease in later life (Afshari et al., 2005; Prins, Gomez-Lopez, & Robertson, 2012). Fluctuations in cytokine levels can influence the course of neurological development (Valsamakis et al., 2017). When inflammation is increased due to prenatal stress, the fetal HPA axis may be altered by the adverse intrauterine environment (Challis et al., 2001). This adverse intrauterine environment is attributed to an increase in intracellular 11β-hydroxysteroid dehydrogenase which prevents maternal cortisol from crossing into the fetus and which is known as the placental barrier (Challis et al., 2001).

A number of studies have shown that elevated IL-6 levels are associated with pregnancy-related diseases and may be predictive of gestational diabetes mellitus (GDM) (Amirian et al., 2020) and obesity (Prins et al., 2012; Sardar et al., 2015). The prevalence of GDM is estimated to be 12.6% in Europe and 15% worldwide (Guariguata, Linnenkamp,

Beagley, Whiting, & Cho, 2014). Risk factors include e.g., high BMI prior to pregnancy (Torloni et al., 2009). Another study reported an association between IL-6 with GDM, but independent of obesity (Morisset et al., 2011). The results of the majority of studies indicate that MES increases IL-6 in late pregnancy and reduces maternal insulin sensitivity (Coussons-Read, Okun, Schmitt, & Giese, 2005; Leff Gelman et al., 2019; Valsamakis et al., 2017).

Generally speaking, risk factors for elevated IL-6 levels during pregnancy also include stressful life events (Finy & Christian, 2018). In particular, MES can affect fetal neurodevelopment and neonatal development (DiPietro, 2012; Kinsella & Monk, 2009). For instance, the study by Coussons-Read et al. (2005) provided evidence of an association between psychosocial stress and an increase in serum levels of pro-inflammatory cytokines in late pregnancy. They included 52 pregnant women and assessed maternal psychosocial stress using the Denver Maternal Health Assessment, which primarily enquires about stress and social support. Blood samples were used as inflammatory markers, to determine the concentrations of Interleukin-1B, C-reactive protein, Interleukin-10 and IL-6. In sum, increased psychosocial stress was reported as being associated with increased production of IL-6 and C-reactive protein (Coussons-Read et al., 2005; Coussons-Read, Okun, & Nettles, 2007). It is generally accepted that increased IL-6 levels are associated with a high inflammatory state in the organism (Black, 2003). It is well known that MES with IL-6 as a potential mediator has negative effects on neurodevelopment in the offspring. Hence, maternal and neonatal cortisol and IL-6 levels must be taken into consideration when examining the influence of MES.

In particular, this relation has also been reported with respect to changes in the fetal ANS: The fetal ANS responds rapidly by adapting to environmental changes: Higher fetal heart rate variability (FHRV) and lower fetal heart rate (FHR) have been used as indicators of fetal well-being and ANS development. Elevated maternal corticotrophin-releasing hormone are positively associated with FHR (Sandman et al., 1999). This may have adverse potential effects on the regulation of the HPA axis. Furthermore, DiPietro et al. (DiPietro, Costigan, & Gurewitsch, 2003) found higher FHRV of fetuses under greater maternal psychological distress.

1.4.2 Maternal relaxation during pregnancy

To avoid or compensate for possible negative effects of maternal stress, preventive measures such as relaxation techniques are beneficial during pregnancy (Bauer et al., 2020; Bauer et al., 2021). To date, most studies have suggested that non-pharmacological methods such as different maternal relaxation techniques, e.g., yoga, singing etc., during pregnancy can reduce MES and improve maternal well-being (Bauer et al., 2020; Bauer et al., 2021; Fink, Urech, Cavelti, & Alder, 2012; Persico et al., 2017). This chapter will mainly focus on mental-based relaxation techniques and their effects on the mother and fetal development.

a. Maternal mental-based relaxation techniques

"[…] A significant number of studies (DiPietro, Costigan, Nelson, Gurewitsch, & Laudenslager, 2008; Nwebube, Glover, & Stewart, 2017; Partanen, Kujala, Tervaniemi, & Huotilainen, 2013; Teixeira, Martin, Prendiville, & Glover, 2005; Wulff et al., 2021) have shown that relaxation strategies during pregnancy not only positively influence the maternal ANS but also reduce symptoms of maternal anxiety and depression." (Bauer et al., 2021, p. 2). In addition, relaxation techniques play a key role in the reduction of adverse perception of maternal pain during labor (Janke, 1999) and increase the sense of attachment to the fetus (Carolan, Barry, Gamble, Turner, & Mascareñas, 2012). For instance, in a questionnaire-based study by Nwebube and colleagues (2017), pregnant women who received music for relaxation over a longer period (12 weeks), were found to have lower anxiety and less depressive symptoms than the control group.

In the literature, significant decreases in maternal systolic and diastolic blood pressure, HR (García González et al., 2017), and uterine contractions (Gebuza, Zaleska, Kaźmierczak, Mieczkowska, & Gierszewska, 2018) after relaxation intervention were reported. For instance, DiPietro and colleagues (2008) administered guided imagery and music for relaxation and significant changes in maternal heart rate (MHR) and skin conductance level (SCL) were shown. In addition, lower maternal salivary cortisol levels and oxytocin were reported after prenatal music listening and active song intervention (Wulff et al., 2021). Consistent with this study, Ventura and colleagues (2012) examined cortisol and anxiety levels after a relaxation intervention in pregnant women (N=154) awaiting amniocentesis, which can be a stressful event during pregnancy. The women

had been listening to music, sitting and reading magazines, or simply sitting in the waiting room for 30 minutes. Anxiety levels were determined via State-Trait-Anxiety Inventory before and after relaxation intervention. They identified greater decreases in cortisol and anxiety in the music group after relaxation than the reading and the waiting group.

A study by Urech et al. (2010) distinguished between active and passive relaxation techniques using progressive muscle relaxation intervention and guided imagery for active relaxation. For passive relaxation, the control condition required that the women sit quietly without falling asleep. A significant decrease in MHR was observed after the progressive muscle relaxation intervention and after receiving guided imagery in comparison to the control group. Furthermore, samples of adrenocorticotropic-hormone (ACTH), norepinephrine and epinephrine were collected before and after relaxation and blood pressure was measured. On the basis of these samples, the authors reported a significant reduction in HPA axis and SAM activity and a decrease in state anxiety symptoms following active relaxation interventions (Urech et al., 2010). Additionally, a decrease in hormonal levels was also detected after passive relaxation. Active and passive relaxation interventions were considered equivalent on the basis of objective parameters, but active relaxation differed significantly in subjective reports of self-relaxation (Urech et al., 2010).

So far, several studies have reported significant effects of different relaxation types on fetal and neonatal development (Beddoe & Lee, 2008; Grigoriadis et al., 2018). For example, the study by DiPietro and colleagues (2008) reported a reduced FHR and increased FHRV after a combination of different relaxation intervention such as music and guided imagery in 32nd week of gestation.

There is also evidence that fetuses had higher long-term variations in FHR during and after a relaxation intervention than a control condition (Fink et al., 2011). This was investigated by using progressive muscle relaxation and guided imagery for relaxation in 33 pregnant women during late pregnancy. A significant time effect for fetal behavioral state was observed, with fetuses becoming more active (Fink et al., 2011).

In terms of neonatal development, playing music in neonatal intensive care unit would appear to enhance high-level cognitive brain networks in preterm infants (Lordier et al., 2019). As a relaxation intervention, preterm infants listened to music for eight minutes five times per week. Additionally, they underwent functional magnetic resonance

imaging (fMRI) to enable the scientists to analyze the functional connectivity in the infants. Interestingly, a higher coupling between brain networks compared to the full-term neonates was detected.

1.4.3 Research question and goals

The specific focus of this work is to investigate maternal and fetal responses to stress and relaxation during pregnancy. In detail, the aim is to assess maternal hormonal, metabolic and psychological changes as well as changes in the maternal and fetal ANS during late pregnancy. First, we aim to gain knowledge about maternal responses to different relaxation techniques during pregnancy. This will entail comparing different mental-based active/passive relaxation interventions in pregnant women. Therefore, study 1 addresses the question as to what kind of mental-based active or passive relaxation techniques lead to greater relaxation in pregnant women.

The second goal is to determine the impact of acute relaxation and chronic stress on fetal ANS with fetal magnetoencephalography (fMEG). We aim to investigate the extent to which a specific maternal relaxation technique could lead to changes in fetal and maternal ANS and hormonal responses. At the same time, our objective is to investigate the effects on chronic maternal stress on the fetal ANS. Additionally, we want to determine women's chronic stress via questionnaires and via hair cortisol to assess any potentially adverse effects on FHR and FHRV.

Third, in study 3, we examine psychological maternal stress during pregnancy and its effects on the hormonal and metabolic milieu of pregnant women. To this end, we aim to gain insight into the impact of maternal stress on IL-6 concentrations with regard to maternal pre-pregnancy BMI and maternal insulin sensitivity in a larger sample in late pregnancy. Here, we would particularly like to investigate the interplay of different parameters of stress with respect to IL-6 levels. This approach will provide new insight into the interplay between metabolic, psychological and hormonal players in late pregnancy. In the following, the study designs, materials and methods and results of all three performed studies are described.

The detailed hypotheses of each study are presented separately in the individual chapters.

2 Study 1: Acute relaxation during pregnancy and its impact on maternal electrodermal and cardiovascular activity and self-reported stress levels

This study and parts of this chapter 2 presented are already published as "Acute relaxation during pregnancy leads to a reduction in maternal electrodermal activity and self-reported stress levels. *I Bauer, J Hartkopf, A-K Wikström, NK Schaal, H Preissl, B Derntl, F Schleger. BMC Pregnancy and Childbirth, 2021"*.

2.1 Study design and research question

In this cross-sectional study (see Figure 3), we investigated the impact of maternal relaxation on maternal well-being and maternal ANS. We used a pseudo-randomized study design. The following hypotheses were defined:

"(1) We [assumed] that acute relaxation, with either music, guided imagery or resting, leads to a decrease in physiological stress levels, e.g., decreased maternal heart rate and [skin conductance level].

(2) We expected the effects of the three interventions to differ, with a significantly stronger effect on physiological parameters during the relaxation for guided imagery (active relaxation) than music or during resting (passive relaxation).

(3) The subjective effects of the three relaxation conditions, which were also of interest to us, were assumed to decrease after the intervention.

In an exploratory analysis, we also investigated whether gestational age influenced any of the stress ratings assessed." (Bauer et al., 2021, pp. 2–3).

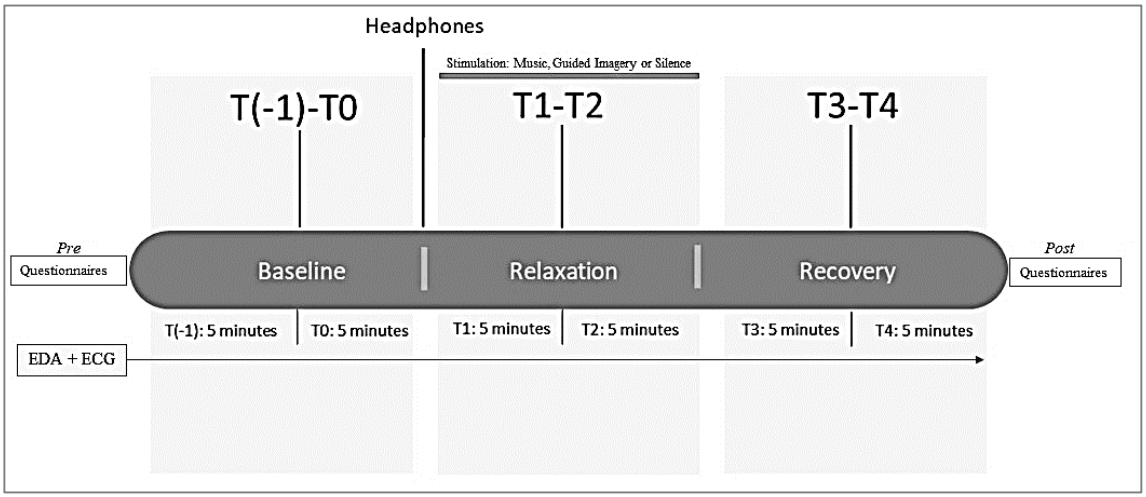


Figure 3. Study 1 design.

EDA: Electrodermal Activity; ECG: Electrocardiogram; T (-1): Pre-Baseline measurement; T0: Baseline measurement, after baseline measurement headphones were placed; T1-T2: Relaxation phase where stimulation or silence (resting) occurs depending on the group; T3-T4: Recovery phase: no stimulation. Pre-Questionnaires/Post-Questionnaires: before and after the measurement, women were asked to fill out different questionnaires by paper-pencil.

We included following parameters (see Table 2):

Table 2. Study 1: Parameters.

Physiological parameters	**Psychological parameters**
• Electrodermal Activity (Skin conductance level) • Electrocardiogram	• TICS • POMS • PDQ • VAS_{Stress} • Q_{Stim}

TICS: Trier Inventory of Chronic Stress; POMS: Profile of Mood States; PDQ: Prenatal Distress Questionnaire; VAS_{Stress}: Visual Analog Scale for current stress level; Q_{Stim}: Questionnaire to rate relaxation stimulation.

2.2 Psychological parameters - Maternal subjective stress and relaxation

To assess subjective stress and relaxation, we included the following questionnaires for the analysis:

2.2.1 Visual Analog Scale (VAS_{Stress}) – Current stress level

The Visual Analog Scale contained the question "How stressed do you feel at the moment?". The scale ranged from '0' meaning 'I am not stressed at all' to '10' meaning 'I am totally stressed' (see Appendix A17). Women were instructed to rate their current

stress level. To analyze the VAS_{Stress}, we measured the distance from zero to the set mark in cm to gain the pre (before the measurement) and post values (after the measurement).

2.2.2 Prenatal Distress Questionnaire (PDQ) – Pregnancy-related distress

"The Prenatal Distress Questionnaire (PDQ) uses 12 items to assess pregnancy-related concerns as well as concerns with regard to birth (A M. Yali and M. Lobel, 1999) [(see Appendix A13)]. The questionnaire included five response categories (0=never to 4=always). PDQ scores ranged from 0 to 48. The Cronbach's alpha for the overall PDQ score was consistently reported to lie between 0.80 and 0.81 (Alderdice et al., 2013) and test-retest reliability was reported to be r = 0.75 (Pluess, Bolten, Pirke, & Hellhammer, 2010). The PDQ has been found to have good convergent validity, since it is significantly correlated with general stress measures (State Trait Anxiety Inventory – State Scale, Life Event Stress and Perceived Stress Scale) (Alderdice et al., 2013)." (Bauer et al., 2021, p. 4). To evaluate the questionnaire, all scale scores were summed to a total score.

2.2.3 Trier Inventory of Chronic Stress (TICS) – Chronic stress

"For the assessment of chronic stress, we used the Trier Inventory of Chronic Stress (TICS) questionnaire (Schulz, Schlotz, & Becker, 2004) [(see Appendix A19)]. An evaluation period of the last three months is required. This questionnaire is based on an interaction-related stress concept (Richter & Hacker, 1998) according to which, stress arises in and through the active confrontation of a person with the demands of their environment. The questionnaire includes 57 items which from 9 different subscales: work and social overload, pressure to succeed, dissatisfaction with work, excessive demands at work, lack of social recognition, social tensions, social isolation and chronic concerns. Participants answer using a 5-point Likert scale response format (0=never to 4=always). In addition, its short screening scale for chronic stress (SSCS), which uses 12 items to record chronic stress in a non-specific and global manner, was used for the analysis. The SSCS score includes items of five different types of stress: chronic concern, work and social overload, lack of social recognition and excessive demands at work.

The internal consistencies (Cronbach's alpha) of the scales range from .84 to .91 (M = .87). The Rasch reliabilities range from .78 to .89 (M = .83). The procedure has good

profile reliability (.72). Numerous results on construct validity (factor analyses, correlations with stress questionnaires, personality traits, partnership behavior, social support, sleep quality, physical and psychological complaints, cortisol release) are available for the TICS. Furthermore, the TICS profiles of different study groups endorse for the validity of the procedure (Schulz et al., 2004).” (Bauer et al., 2021, p. 4). To evaluate the questionnaire, all items were summed according to the questionnaire guidelines.

2.2.4 Profile of Mood States (POMS) – Current mood

“In the present study, the German short form of the Profile of Mood States (POMS) with 35 items and 7-point Likert scale response categories (0=not at all to 6=very strong) was used (Albani et al., 2005) [(see Appendix A14)]. The 35 items form the four subscales of depression/anxiety, fatigue, vigor and hostility. In the POMS, the participants were asked to evaluate their state of mood over the last 24 hours. The POMS appears to be an internally consistent instrument and the Cronbach’s Alpha ranged from 0.89 to 0.95. There are also indications of convergent validity of POMS-scales with two questions: ‘1. Over the past two weeks, have you felt down, depressed, or helpless?’ and ‘2. Over the past two weeks, have you felt little interest or pleasure in doing things?’ (Albani et al., 2005).” (Bauer et al., 2021, p. 4). For the analysis, we summarized for each of the four subscales the relevant items.

2.2.5 Questionnaire about the relaxation stimulation (Q_{STIM})

We asked the participants after the relaxation intervention, how they liked the stimulation (music or guided imagery). They were asked to answer the question “How did you like the relaxation stimulation?”. Participants could rate between 1=’very good’, 2=’good’ and 3=’not good’ (see Appendix A18).

2.3 Neurophysiological, hormonal and metabolic parameters

2.3.1 Maternal Electrodermal Activity

"Electrodermal activity (EDA, quantified by skin conductance level (SCL)) was measured by administering a constant 0.5 Volt root-mean square 35 Hz AC excitation signal and detecting the current flow (MP36R Research System, BIOPAC (USA)). SCL was monitored from two disposable pre-gelled electrodes (EL507, Biopac Systems, Inc., CA). These were placed on the distal phalanxes of the index and middle finger of the non-dominant hand. Electrodes were fixed in position with adhesive tape. Before the measurement, all women took off their shoes to avoid any sudden spikes in the SCL trace due to rubber-soled shoes (JJ Braithwaite, DG Watson, R Jones, M Rowe, 2013). […] SCL was scaled from 0 to 25 microsiemens. Data quantification continued offline using Biopac software (Acqknowledge5 (CA, USA)). Artifacts were substituted with linear interpolation based on the values at the left and right edges if necessary and with a median smoothing with 50 samples to delete movement artifacts (Posada-Quintero & Chon, 2020). All values were baseline corrected for the analysis and the complete data set was divided into 5-minute sections, via which the mean value was then calculated." (Bauer et al., 2021, p. 5).

2.3.2 Maternal Autonomic Nervous System

Electrocardiogram (ECG)

"Maternal […] cardiovascular activity [was] recorded with a four-channel data acquisition and analysis device (MP36R Research System, BIOPAC (USA)). Data were recorded at a sampling rate of 2000 Hz. ECG was recorded from three disposable electrodes (EL503, Biopac Systems, Inc., CA) which were placed on the forearms of the participants. Data quantification was processed offline using Matlab R2018a (The MathWorks, Natick, MA). ECG data underwent R-peak detection (in house software), manual editing for artifacts and interbeat interval computation. The time-domain parameters included root mean square of successive differences (RMSSD) and the heart rate (beats per minute (bpm))." (Bauer et al., 2021, p. 4).

The analyses, including pre-processing of RR time series and short-term HRV analysis in the time domains, were developed and performed by in-house routines in MATLAB (Mathworks, Inc., Natic, MA, USA).

All values were baseline corrected for the analysis and the complete data set was divided into 5-minute sections, via which the mean value was then calculated.

2.4 Study population and procedure

"This cross-sectional study was conducted between February 2018 and September 2019. The Ethics committee of the Medical Faculty of the University of Tuebingen, Germany, approved the study (748/2017BO1). Participants were informed about the course of the study and gave their written informed consent prior to participation.

Pregnant healthy women were recruited by electronic communication e.g., e-mail and phone. Eligibility was restricted to women over 18 years of age, German speaking, singleton, uncomplicated pregnancy and gestational age (GA) between the 30th and 40th weeks of gestation. Exclusion criteria were hearing impairments, acute depression, mental disorders (self-report) or drug/nicotine consumption during pregnancy (self-report). Once initial information about the study had been provided, a single visit was scheduled between 30th and 40th weeks of gestation. A total of 38 women were enrolled. With regard to any previous experience with relaxation, four women reported that they did not participate in any strict routine activities to encourage relaxation. Of those who did, the most commonly reported techniques were yoga and meditation, exercising, daytime naps or reading. However, none reported that they availed themselves of any of the relaxation techniques in question on a daily basis. Three women stated that they also liked to listen to music to help them relax. On average, participants were 30.9 years old, in their 34th week of gestation, and 72% were expecting their first child. Nine of 38 women had already participate in another measurement in our center only shortly before the reported study. In that case, the participants had rested for 30 minutes but without any specific intervention. Two-thirds of the women had a college or a higher education/university degree. […] The participants were alternately assigned to the music group, the guided imagery group or the resting group shortly before the measurement commenced. We scheduled a 1h-visit between 8am and 2pm. On each participant's arrival, the midwife checked fetal vitality with a pinard horn. All women were asked to

fill out a questionnaire (Profile of Mood States, POMS) to assess their current mood (Albani et al., 2005). They also rated their current stress level on a [Visual Analog Scale (VAS_{Stress})] ranging from 0 (not stressed at all) to 10 (highly stressed). The women filled out both paper-pencil questionnaires at our center before and after the intervention. The relaxation intervention took place for each woman individually and personally. The relaxation intervention started once the women had been positioned in a semi-recumbent comfortable armchair in a noise-reduced room. During the intervention, the light in the room was dimmed. The electrodermal activity- and heart rate monitor was positioned out of view of the participants. Women were asked whether the room temperature was comfortable, or whether or not they were cold and whether they felt generally comfortable. In all three groups, we began with a test measurement for three minutes to ensure that the electrodes were in working order. Afterwards, ten minutes of baseline data were collected, during which we gave instructions to remain quietly seated, breathe calmly and to avoid any movements. Subsequently, headphones were given to the women (also in the resting group to ensure comparability between conditions) and the intervention was initiated (only for music group and guided imagery group) for the next 10 minutes. The music ("Find Your Inner Peace", Rostar) was designed specifically for relaxation, using certain tempos for inducing a calm state, but without vocals. The guided imagery text was designed for use during pregnancy by a midwife on the basis of her own professional experience and adapted specifically for this study. To avoid conscious active physical tension, which might cause artifacts in the data, the guided-imagery contained no body-related instructions. For the resting condition, the women were requested to remain seated quietly without moving and to breathe normally. Following the relaxation period, a 10-minute interval served as a recovery measurement (e.g., no specific relaxation intervention). During the entire procedure, our in-house midwife and the study assistant remained in the room with the woman. Once the measurement was complete, the [principal investigator] removed the electrodes and headphones.

Afterwards, all women rated their stress level again on the POMS and [VAS_{Stress}] and indicated how they had experienced the intervention. After their participation, they received a link for additional questionnaires [(TICS, PDQ)] via email assessing chronic stress and pregnancy-related distress [...]. They completed these questionnaires at home, one to five days after their appointment at our center. We used the software Unipark (www.unipark.de) for the computer-based questionnaires. […] Testing was discontinued

for two participants due to technical issues and discomfort of the women. The data analysis is therefore based on the remaining 36 participants." (Bauer et al., 2021, pp. 3–4).

2.5 Statistical analysis

"All statistical analyses described were performed using the software program SPSS (IBM SPSS Statistics 26) and alpha levels was set to $p<.05$.

Data preparation of all dependent variables included tests for normality, homogeneity of variances and examination of outliers. Where not normally distributed, variables were subjected to transformation by natural logarithm and adding a constant, or were ranked prior to the application of the statistical procedures.

Outliers were removed if the values were more than three standard deviations away from the mean value. Excluding these individual data points lead to a sample size of seven to twelve subjects per group […]. As some women were participating in another measurement before the current study and thus were probably more at ease in the clinical environment, we included this variable as covariate in all analyses. Additionally, we also included maternal chronic stress in our analyses because different chronic stress levels can have an impact on maternal baseline values." (Bauer et al., 2021, p. 5).

To determine group differences in group characteristics (maternal age, chronic stress, PDQ), we performed a MANOVA with factor group (music group, guided imagery group, resting group). Overall, data are presented in mean (SEM).

"Group differences in all dependent variables (maternal heart rate, maternal skin conductance level and subjective stress) were evaluated using mixed-effects ANCOVAs with the between-subjects factor group (music, guided imagery, resting) and the within-subject factor time (T1-T4) and the two covariates 'participating in another measurement before at our center' and 'chronic stress'." (Bauer et al., 2021, p. 5). Since putting on the headphones for relaxation constitutes an interruption in our data, we took into account only those segments after the beginning of the intervention (relaxation) phase (T1) until the end of the recovery phase (T4) for data analysis (see Figure 3). The total time of the relaxation phase and the recovery phase was thus 20 minutes. For the analysis of the maternal physiological activity, we used baseline correction by subtracting the mean of

the first five minutes at the beginning (T0) without any intervention from the data measured during the period from T1 to T4.

"To analyze effects of time within relaxation phase and recovery phase separately, we used a repeated measures ANCOVA (rmANCOVA). We used Bonferroni or Dunnett T3 correction for post-hoc analysis. In case of non-normal distribution of the data, Wilcoxon signed-rank test were applied.

State and trait questionnaires were analyzed by the standard evaluation procedure for every questionnaire (POMS, PDQ and TICS) and all data were analyzed using MANCOVA with group and/or time as factors (POMS, TICS, [VAS_{Stress}]). […]

Explorative analysis of differences in [VAS_{Stress}] between GA groups (Group 1 (30-34 GA)) and Group 2 (35-40 GA): For the mean delta value included in the analysis, we subtracted the pre-value from the post-value for each woman separately. An [one-way] ANCOVA was used to determine the differences between GA groups. The dependent variable was the mean of [VAS_{Stress}] delta to describe changes in subjective stress pre vs. post intervention." (Bauer et al., 2021, p. 5). To calculate the VAS_{Stress} delta, we subtracted the pre-value from the post-value.

2.6 Results

Group characteristics are shown in Table 3.

Table 3. Study 1: Demographic data of participants.

Group	Gestational Age (GA) (mean) in weeks (SD)	Maternal Age (mean) in years (SD)	Primiparous/Multiparous
All groups (total)	33.56 (3.21)	30.86 (4.30)	26/10
Music Group	34.50 (3.15)	30.08 (3.85)	11/1
Guided Imagery Group	31.83 (3.13)	29.58 (5.09)	8/4
Resting Group	34.33 (2.87)	32.92 (3.34)	7/5

Baseline characteristics of participants in the groups: music (N=12), guided imagery (N=12) and resting (N=12) and all groups in total (N=36). (from Bauer et al., 2021, *Table 1*)

"To determine group differences, we performed a MANOVA with factor group (music group, guided imagery group, resting group) and total score of TICS and PDQ, maternal age and GA. While maternal age did not differ between the groups, $F(2,33) = 2.424$, $p=.122$, or for GA, $F(2,33) = 2.875$, $p=.071$, the total score of chronic stress ([short] screening scale of chronic stress (SSCS)) […] differed significantly, $F(2,33) = 3.808$, $p=.033$, $\eta p2=.187$. Bonferroni post-hoc test revealed a significant difference in SSCS

between the music group and the guided imagery group [(see Appendix 21)]. Pregnancy-related distress (PDQ) did not differ significantly between groups, F (2,33) = 1.221, p=.308. [(see Appendix 20)]."

Maternal cardiovascular response to relaxation procedures

[…] Maternal heart rate: A MANCOVA with factors group (music, guided imagery, resting) and time (T1 to T4) revealed non-significant main effects for time, F (3,94) = 0.109, p=.955, or group, F (2,94) = .317, p=.729, and no significant interaction between group and time, F (6,94) = 1.007, p =.426.

Maternal heart rate variability: For HRV, we used the root mean square of successive differences (RMSSD). A MANCOVA with factors group (music, guided imagery, resting) and time (T1 to T4) revealed no significant main effects of time, F (3,94) = 2.143, p=.100, or group, F (2,94) = 0.624, p=.538, and no significant interaction between group and time, F (6,94) = 1.339, p =.248.

[(For an overview of maternal cardiovascular activity parameters, see Appendix A1 and A3.)]

Relaxation vs. recovery phase:

To further determine whether a relaxation effect sets in during the recovery phase, we analyzed whether there is a difference between the relaxation (delta of mean T1-T2) and the recovery (delta of mean T3-T4) phase in maternal parameters.

A MANCOVA with factors group (music, guided imagery, resting) and change of time (delta) revealed neither a significant main effect of group, F (2,56) = 0.824, p=.444, nor time, F (1,56) = 2.181, p=.145, or group-by-time interaction, F (2,56) = 0.626, p=.538, for maternal heart rate. Similarly, no significant main effect of group, F (2,56) = 0.377, p=.688, or time, F (1,56) = 0.815, p=.371, or group-by-time interaction, F (2,56) = 0.245, p=.784, was found for maternal RMSSD.

Maternal electrodermal response to relaxation procedures

We assumed a significant relaxation effect over time within each group, with decreasing SCL values, and differences in the size of this effect between groups in maternal SCL.

[…] A MANCOVA with factors group (music, guided imagery, resting) and time (T1 to T4) revealed a significant main effect of time, F (3,94) = 18.011, p=.001, ηp2=.365, but no group effect, F (2,94) = 0.075, p=.928. The interaction between group and time was not significant, F (6,94) = 1.192, p=.317. For secondary analysis and to determine time effect for each group separately, a repeated measures ANCOVA with a Greenhouse-Geisser correction revealed no significant main effects over time within the music group, F (3,21) = 1.250, p=.301, the guided imagery group, F (3,24) = 0.122, p=.836, and the resting group, F (3,30) = 0.152, p=.771, separately. Hence, no significant relaxation effect over time could be determined.

[(For an overview of maternal skin conductance level, see Appendix A2 and A4.)]

Relaxation vs. recovery phase:

A MANCOVA with factors group (music, guided imagery, resting) and change over time (delta) revealed no significant main effect of group, F (2,56) = 2.856, p=.066, but a significant main effect of time, F (1,56) = 21.935, p=.001, ηp2=.281, in maternal SCL. The interaction between group and time was not significant, F (2,56) = 0.490, p=.615. A Wilcoxon signed-rank test revealed a significant difference between relaxation and recovery phase in SCL within every group. For women in the music group, the relaxation effect decreased significantly in the recovery phase compared to the relaxation phase, z=-2.666, p=.008, r=0.61, as well as for women in the guided imagery group, z=-2.191, p=.028, r=0.58, and resting group, z=-2.981, p=.003, r=0.61. To explore potential group difference in the long-term impact of the relaxation intervention during the recovery phase, delta SCL values of the recovery phase only, were subjected to a MANCOVA with the factor group […]. The analysis revealed no significant difference between the groups, F (2,27) = 1.700, p=.202. The [interventions] in the recovery phase therefore did not differ significantly from each other.

Maternal subjective response to relaxation procedures

On the basis of our third hypothesis, we assumed that there would be a decrease of stress levels in all groups independent of the type of relaxation administered. The MANCOVA with group as between-subject factor (music, guided imagery, resting) showed no significant main effect of group on delta of mean levels of the [VAS_{Stress}] for subjective stress (Post-Pre), F (2,31) = .583, p=.564.

Albeit we did not observe a significant group effect on [VAS_{Stress}] levels, a Wilcoxon signed-rank test determined a significant time effect within groups for the [VAS_{Stress}] (pre vs. post). Maternal stress levels were significantly reduced in the music group, z=-2.936, p=.003, r=0.84, in the guided imagery group, z=-2.934, p=.003, r=0.84, and in the resting group, z=-3.059, p=.002, r=0.88 [(see Figure 4. Study 1: Visual Analog Scale (VASStress) for all groups.]

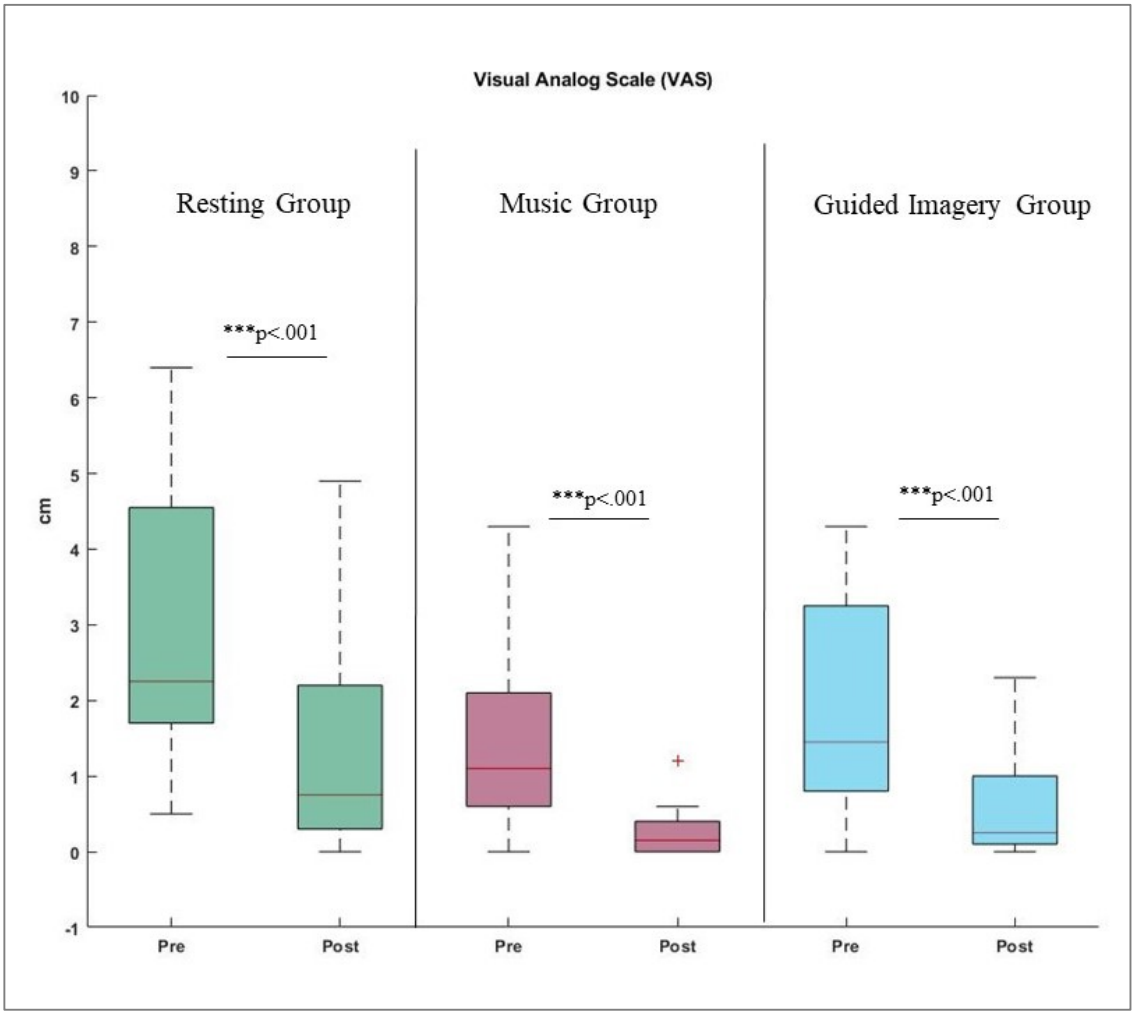


Figure 4. Study 1: Visual Analog Scale (VAS_{Stress}) for all groups.

Resting group (N=12), Music group (N=12), Guided imagery group (N=12). ***on a statistical level of p<.001 significant.

A MANCOVA with group as between-subject factor showed no significant effect in delta of mean POMS (Pre-Post) levels for each subscale, F (2,31) = 0.952, p=.397 (depression/anxiety), F (2, 31) = 2.366, p=.111 (fatigue), F (2,31) = 2.222, p=.125 (vigor), F (2,31) = 1.831, p=.177 (hostility).

With regard to the POMS, rmANCOVA with group as factor showed no significant interaction effect for all four subscales, depression/anxiety: F (1,33) = 2.178, p=.129; fatigue: F (1,33) = 1.826, p=.177; vigor: F (1,33) = 1.074, p=.353; hostility: F (1,33) =2.035, p=.147. However, we found significant main effects over time for depression/anxiety, fatigue and hostility. In particular, the improvement in depression/anxiety was shown for at least two of the three groups, but not for the guided imagery group. [For statistical details, see Table 4. Study 1: Overview of statistical details in Profile of Mood States (POMS).]

Table 4. Study 1: Overview of statistical details in Profile of Mood States (POMS).

	POMS Subscales	*Music Group*	*Guided Imagery Group*	*Resting Group*
Paired t-test	Depression/anxiety	t(11)=2.698, p=.021*, dz=0.73	t(11)=2.007, p=.070	t(11)=4.201, p=.001, dz=1.21
	Hostility	t(11)=1.605, p=.137	t(11)=2.166, p=.053	t(11)=3.895, p=.002, dz=1.12
	Fatigue	t(11)=2.538, p=.028*, dz=0.73	t(11)=-0.192, p=.851	t(11)=0.967, p=.354
	Vigor	t(11)=1.575, p=.143	t(11)=3.178, p=.009*, dz=0.91	t(11)=2.726, p=.020*, dz=0.79

*on a statistical level of p<.05 significant. (from Bauer et al., 2021, *Table 4*)

In addition, we were interested in whether these subjective effects depended on GA:

In an exploratory approach, we divided women into two groups on the basis of their GA (Group 1: 30^{th}-34^{th} gestational week, N=21; Group 2: 35^{th}-40^{th} gestational week, N=15). The difference in the pre-rating values of [VAS_{Stress}] between the groups was not statistically significant, F (1,34) = 1.318, p=.259. In general, women with higher GA (Group 2) had lower values in the pre-rating, meaning that they might have been somewhat less stressed (Mean (SD) Group 1: 2.33 (1.59); Group 2: 1.79 (1.72)) beforehand.

As an explorative approach, an ANOVA showed a significant difference between GA groups in mean [VAS_{Stress}] delta, F (1,34) = 4.971, p=.032, ηp2=.128, e.g., women with higher GA had less change in [VAS_{Stress}] levels (delta mean: 1.02) compared to women with lower GA (delta mean: 1.23). Thus, women earlier in the third trimester (30^{th}-34^{th} gestational week) appear to be able to relax more easily than compared to women with higher GA. However, when we included ‘chronic stress’ and ‘participating in another

measurement before at our center' as covariates, this effect was no longer of significance, F (1,32) = 2.113, p=.156." (Bauer et al., 2021, pp. 5–9).

2.7 Discussion

2.7.1 Maternal relaxation is associated with significantly increased maternal well-being

In study 1, our results showed that maternal relaxation can have a positive effect on maternal well-being and mood independent of intervention.

A study by Chang and colleagues (2008) investigated the effect of a two-week music intervention on stress, anxiety and depression in 236 pregnant women. Their scores in subjective stress perception were significantly lower than those of the control group. The following questionnaires were mainly used: Edinburgh Postnatal Depression Scale, State Scale of the State-Trait-Anxiety Inventory and Perceived Stress Scale. They reported a significant decrease after music intervention in maternal MES. Another study by Tragea and colleagues (Tragea, Chrousos, Alexopoulos, & Darviri, 2014) also showed increased maternal well-being in sixty pregnant women after a six-week stress management program (relaxation breathing and progressive muscle relaxation) compared to the control group, who had received no intervention. An increase in maternal well-being and attachment to their child was described in a previous study (Persico et al., 2017) where pregnant women sang lullabies during four musician-led group sessions for 45 minutes. However, it must be considered that these studies covered a longer period of time, during which the relaxation intervention or stress management was applied regularly.

Our results endorse those of a study by Ventura and colleagues (2012), who also used a one-time relaxation intervention. They compared three different relaxation methods: listening to music, sitting and reading magazines and simply remaining seated in the waiting-room. They study consisted of 154 pregnant women whose anxiety response was measured using the State-Trait-Anxiety Inventory and whose plasma cortisol levels were also recorded. The women were all awaiting amniocentesis, which may be a psychologically stressful procedure (Ventura et al., 2012). This data indicates that women who listened to music for 30 minutes had the greatest decrease in both anxiety and cortisol in comparison to the other groups (Ventura et al., 2012). A decrease in

anxiety levels after listening to music was also observed in a study by Nwebube and colleagues (2017). They included 111 pregnant women and used a 12-week intervention during which women listened to specially composed songs twice a day compared to the control group who had been exposed to daily relaxation only (Nwebube et al., 2017). They asked the women to fill out the State-Trait-Anxiety Inventory to indicate anxiety levels and the Edinburgh Postnatal Depression Scale to indicate depressive levels before and after the 12-week intervention. In comparison to the control group, anxiety levels and depressive scores decreased significantly in the music group. In sum, evidence suggests that relaxation during pregnancy, applied once or regularly, leads to an increased maternal well-being (DiPietro et al., 2008; Nwebube et al., 2017).

Furthermore, a study by Wulff and colleagues (2021) also showed reduced anxiety and stress levels in 172 women in late pregnancy (30^{th}-36^{th} weeks of gestation). Each woman was exposed to one of three different relaxation interventions: singing, music or to no intervention. In detail, stress and anxiety was measured with a Visual Analog Scale and the State-Trait-Anxiety Inventory questionnaire. Singing and listening to music were guided prior to the first time of measurement and the 34^{th} week of gestation (Wulff et al., 2021). The relaxation intervention generally took place in a group (music: up to three other women; singing: up to seven other pregnant women) (Wulff et al., 2021). The participants were then asked to continue the music intervention at home for at least 10-15 minutes on a daily basis (Wulff et al., 2021). The singing group followed the same procedure as the music group, but used singing for relaxation. Furthermore, they used a longitudinal study design and relaxation intervention at home and were not supervised. An earlier study (Urech et al., 2010) reported differences between the various relaxation interventions (guided imagery, progressive muscle relaxation and passive relaxation control group). It detected significantly increased levels of relaxation on the basis of the Visual Analog Scale and State Scale of the State-Trait-Anxiety Inventory in the guided imagery group in comparison to the other two groups. They included 39 pregnant healthy women who had been instructed on how to practice relaxation stimulation at home.

Most of the studies used a longitudinal study design and relaxation intervention at home, both of which were, however, non-controlled. This disparity in comparison to our study design may explain why they found differences between interventions while we did not. Additionally, in study 1 we did not use any body-related relaxation instructions since we wished to avoid any movement during the measurements. An increase in maternal well-

being, albeit not always significant, was observed on the basis of the questionnaires. Some studies reported differences between the intervention types. However, this did not apply to the results in study 1. In addition, there was an indication that GA was associated with subjective ratings, suggesting that women in later stages of their pregnancy (35^{th}-40^{th} weeks of gestation) were less relaxed than women in earlier stages (30^{th}-34^{th} weeks of gestation) according to subjective stress levels. This result could be important in determining the point at which an intervention makes sense and whether it needs to be adjusted during the course of pregnancy. However, it should be mentioned that when we added the covariate 'participating in another measurement' and 'chronic stress', this effect was no longer significant. We would suggest that this result be seen as an indication of an effect of gestational age only and that it should be investigated in more detail in further studies.

2.7.2 Maternal relaxation is associated with decreased maternal heart rate and skin conductance levels independent of relaxation intervention

In addition to the increased sense of well-being, we measured a slight but insignificant decrease in MHR and SCL in study 1.

These results are consistent with previous studies in which a continuous decrease in maternal physiological parameters after relaxation interventions in pregnant women was also reported (Chang et al., 2008; DiPietro et al., 2008; Fink et al., 2011; García González et al., 2017). Although, we used a number of different relaxation techniques in study 1, each technique was considered separately to determine possible differences in effectiveness between acute active and acute passive relaxation techniques. In particular, for MHR, we found a non-significant increase in the guided imagery group, despite the fact that the women rated the guided imagery as relaxing. This could be due to the active relaxation instruction. We queried the rating of the relaxation stimulation after the relaxation stimulation itself and observed that women rated the guided imagery as very good (25%) or good (25 %) (see Appendix A8).

This discrepancy between the subjective perception of relaxation and the measurement of objective physiological parameters is also discussed in a study by Urech and colleagues (2010) who postulated that since guided imagery is a comfortable relaxation technique, it might enhance compliance in pregnant women in a long-term relaxation

program. In a short-term relaxation program, the women first have to become familiar with the active relaxation instructions. This acclimatization may take place more readily with music as it offers a passive approach that may be more accessible and more subject to personal taste. However, one earlier study (DiPietro et al., 2008) reported a decreased MHR after relaxation induction in late pregnancy (32nd week of gestation). This slight decrease in MHR was also observed in our study, albeit not to a significant extent.

However, a comparison of the two groups did not show any significant differences in MHR changes. Our results therefore stand in contrast to those of a study by Teixera and colleagues (2005) who reported a greater overall decrease in MHR after active and passive relaxation in 58 women between 28th and 34th weeks of gestation. Here, active relaxation entailed a guided relaxation session with a stress management expert who performed guided imagery (Teixeira et al., 2005). By contrast, passive relaxation stimulation included comfortable, quiet sitting and reading a magazine (Teixeira et al., 2005). This could be explained by the duration of the relaxation intervention, which lasted 45 minutes in both groups. Thus, the relaxation session lasted twice as long as in our study. In addition, the active relaxation group was guided by a professional stress management expert who was present during the entire session. Thus, the women were actively guided. In the case of guided imagination via headphones in our study, it is obviously a different situation, since the women's attention is not guided and controlled via a professional stress management expert.

The fact that we found no differences between the interventions does not coincide with a study by Urech and colleagues (2010), who reported significant differences between mental-based active (guided imagery), body-based active (progressive muscle relaxation), and passive (quiet sitting) relaxation interventions in 39 healthy pregnant women on the basis of subjective ratings and cardiovascular activity (Urech et al., 2010). They took blood and saliva samples before and after a relaxation intervention. MHR decreased during guided imagery and progressive muscle relaxation and was significantly decreased in comparison to the control condition of passive relaxation. This leads to the assumption that women in the active relaxation intervention were more relaxed (Urech et al., 2010).

The fact that the results of study 1 in terms of differences in MHR between groups are less consistent with previous studies may be due to the fact that the relaxation stimulation

in study 1 was relatively short and was applied only once. In addition, previous studies also used a combination of mental-based and body-based relaxation stimulations.

Of note, we observed a significant difference in the change in SCL between the relaxation and recovery phases for all groups, regardless of the intervention. Thus, the relaxation effects are still present in the recovery phase, but less intense than in the relaxation phase.

"To our knowledge, [study 1] is the first of its kind showing a significant decrease in SCL from relaxation to recovery after a short acute relaxation intervention in pregnant women. […] A study by DiPietro and colleagues (2012) […] reported a difference in SCL between the relaxation and recovery phase [as in study 1], showing an increase in the latter. However, in their study a combined relaxation intervention of progressive muscle relaxation audio-recorded, guided imagery and self-selected music was used. The baseline measurement, the following stimulation intervention and the post-relaxation phase (sitting silently) each lasted 18 minutes. In addition, 41% of the participants were given 18-minute pre-baseline (rest). It should be noted that in the study of DiPietro and colleagues, relaxation and recovery was interrupted when the lights were turned on and different questions were answered. This might explain why they reported a significant increase in SCL from baseline to relaxation and from relaxation to recovery." (Bauer et al., 2021, p. 9).

In other words, the fact that no significant differences were observed between interventions underlines the finding that, on both objective and subjective relaxation parameters, quiet, comfortable sitting can, in the short term, be just as effective as an active mental-based relaxation technique in certain situations. In any case, during a 20-minute relaxation and recovery phase, the relaxation effect was shown to increase with time. This result is in line with previous literature which described that an effective music intervention should have a total duration of between 20 and 40 minutes to detect relaxation effects (Chang et al., 2008; Liu, Lee, Yu, & Chen, 2016). "In general, our results show that an acute relaxation intervention during pregnancy without disturbances for 10 minutes can […] lead to relaxation when followed by a recovery phase (also lasting for 10 minutes). All types of interventions used were effective in generating a subjective feeling of relaxation as indicated by a subjective stress ratings post- compared to pre-relaxation intervention." (Bauer et al., 2021, pp. 9–10).

3 Study 2: Acute maternal relaxation and chronic stress and their impact on fetal autonomic nervous system

3.1 Study design and research question

In study 2, the main research question was whether maternal relaxation (music or silence) and maternal chronic stress have an impact on the fetal ANS.

Our hypotheses were:

(1) We expect a relaxation effect for fetus/mother in the music condition (listening to music) compared to fetus/mother in the silence condition (no stimulation) independent of the two time points.

(2) We assume that maternal chronic stress is associated with increased FHR and decreased FHRV.

We used a counterbalanced pseudo-randomized study design (see Figure 5). For an overview of study design, see Table 5. Women were asked to come to two visits to our center (first visit (timepoint): 29-34 GA; second visit (timepoint): 35 GA until term) depending on their GA.

Group A received music stimulation at the first time point and no stimulation (silence) at the second time point. Group B received no stimulation (silence) at the first time point and music stimulation at the second time point. We measured the SCLs and the fetal and maternal cardiovascular activity with fMEG on both visits. On their first visit, we took a blood sample. At the second visit we collected a hair sample from each woman (for overview see Table 5).

For relaxation stimuli, we offered the women a choice of four different types of music: Lounge, Classic, Jazz and Meditation. These were adapted from a study by Schaal (Schaal et al., 2021) (see Appendix A10).

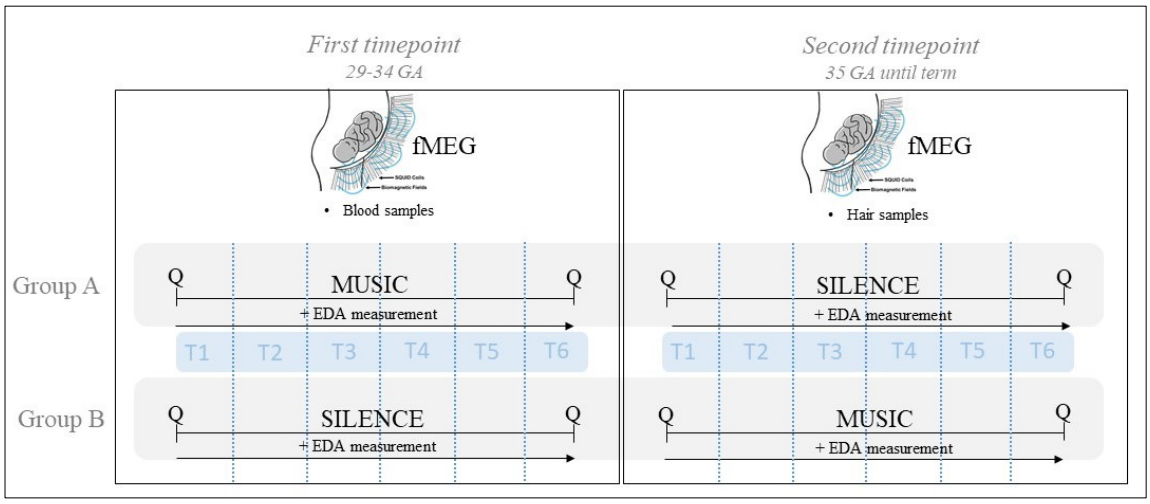


Figure 5. Study 2 design.

Q: questionnaires before and after the measurements; fMEG: fetal magnetoencephalography; EDA: electrodermal activity; Stimulation: music or silence (no stimulation); GA: gestational age. Dashed lines mark the 5-minute sections where mean is calculated for each section (T1-T6) for MCG and SCL.

We included following parameters (see Table 5. Study 2. Parameters.).

Table 5. Study 2. Parameters.

Physiological parameters	**Psychological parameters (Questionnaires)**
• Electrodermal Activity (Skin conductance level)	• TICS
• Fetal magnetoencephalography (Fetal and maternal cardiovascular activity)	• POMS
• Blood samples (cortisol)	• PHQ-D
• Hair samples (cortisol)	• RIS
	• VAS_{Stress}
	• Q_{Stim}

TICS: Trier Inventory of Chronic Stress; POMS: Profile of Mood States; PHQ-D: Patient Health Questionnaire; RIS: Relaxation Inventory Scale; VAS_{Stress}: Visual Analog Scale for current stress level; Q_{Stim}: Questionnaire to rate stimulation.

3.2 Psychological parameters - Maternal subjective stress and relaxation

To assess subjective stress and relaxation, we included the following questionnaires for the analysis:

3.2.1 Relaxation Inventory Scale (RIS) – Maternal relaxation

The Relaxation Inventory Scale used a 5-point Likert scale (1= “I totally agree” to 5= “I totally disagree”) and has 45 items (Crist, Rickard, Prentice-Dunn, & Barker, 1989) which ask about the current state. The RIS was used before and after the measurement in

study 2. The RIS contains of three subscales: Physiological Tension Scale, Physical Assessment Scale and Cognitive Tension Scale (see Appendix A16). Physiological Tension Scale was primarily related to states of tension and non-relaxation. Physical Assessment Scale ask about one's general overall physical state and the Cognitive Tension Scale is related to mental states of worry and anxiety. The reliability coefficients for the Physiological Tension Scale were .87, .97 and .81, respectively. For the Cognitive Tension Scale, the reliability coefficients for days were .95 trials, .99, and days and trials .92. (Crist et al., 1989). For the evaluation, the items of the respective subscales are calculated for each subscale separately.

3.2.2 Patient Health Questionnaire (PHQ-D) – Maternal stress levels

For the maternal psychological investigation, the Patient Health Questionnaire (PHQ-D) was used (Gräfe, Zipfel, Herzog, & Löwe, 2004). The women completed the questionnaire mainly with paper and pencil during the study or at home after their appointment (-/+7 days). The questionnaire consists of 78 items in total with a two to five-level response scale in each case (see Appendix A15). Diagnostic criteria for major depression and other depressive disorders, panic disorder and other anxiety disorders, bulimia nervosa, binge-eating disorder, somatoform disorder and alcohol abuse are asked (Breuer, 2020). The diagnoses major depression, bulimia nervosa and panic disorder are so-called "threshold" disorders, e.g., all diagnostic criteria are queried according to DSM-V (Breuer, 2020). The remaining diagnoses (further depressive or anxiety disorders, alcohol abuse, binge-eating disorders and somatoform disorders) are "subthreshold" disorders, because not all diagnostic criteria are queried (Breuer, 2020). There are both categorical and continuous variables within the PHQ-D. A dimensional evaluation is possible for the somatization module ("PHQ-15" questions 1a-m, 2c, d), the depression module ("PHQ-9" questions 2a-i) and the stress module (questions 12a-j) (Breuer, 2020). For the analysis, the 'Schweregrad Stress' subscale was used to determine the stress level of the women. The subscale 'Schweregrad Stress' contains ten items with the rating 0 ("not impaired"), 1 ("slightly impaired") or 2 ("severely impaired"). In the 'Schweregrad Stress' subscale, the result is a sum of scales between 0-20 (0=no stress, 20=high stress).

Missing values in the PHQ-D questionnaire were treated as described by Kocalevent and colleagues (2013): If ≤ 20% items were missing within a module, the value was supplemented by calculating the average of the other items within this module (Breuer, 2020). In the event of >20% missing items, this module of the questionnaire was removed from the evaluation for the respective respondent (Breuer, 2020). If individual modules were missing, the respondent was excluded for this sub-analysis only, resulting in different numbers of respondents depending on the analysis (Breuer, 2020).

In study 2, we also used TICS, POMS, VAS_{Stress}, Q_{STIM}. (for details, see study 1, section 2.2).

3.3 Neurophysiological, hormonal and metabolic parameters

3.3.1 Maternal and fetal magnetoencephalography (MCG)

We measured maternal and fetal cardiac activity with magnetocardiogram (MCG). The measurement was performed on a dedicated fetal magnetoencephalographic system (SARA 2, VSM Ltd., Port Coquitlam, CA) at the fMEG Centre of the Eberhard Karls University of Tuebingen, Germany. The system is installed in a magnetically shielded room for attenuation of external magnetic fields (Mat Husin, 2020). Continuous communication (visual/auditive) between the operator of the recording and the pregnant mother is possible via an intercom system (Mat Husin, 2020).

Fetal magnetoencephalography is a non-invasive device and technique for recording magnetic fields generated by electrical currents in human tissue (Mat Husin et al., 2020). It enables us to monitor fetal heart and brain activity and maternal heart activity (Preissl, Lowery, & Eswaran, 2004). Biomagnetic signals are recorded from 156 primary squid sensors (superconducting quantum interference devices) and 29 reference sensors arranged in a convex array that covers the whole maternal abdomen (Mat Husin, 2020) (see Figure 6). The sampling rate is 610.352 Hz. During the recording, the pregnant woman rests in an upright position, leaning forward with her abdomen in the convex shell (Mat Husin, 2020). For the localization of the fetus, one localization coil is placed on the mother's abdomen and three localization coils are placed on her left and right side and on her spine (Mat Husin, 2020). Near-field signals which may occur during the fMEG measurement, such as maternal and fetal cardiac activity and muscle activity

(uterine, maternal, fetal) cause interferences with the fetal brain signals and are therefore filtered out during data analysis (Mat Husin, 2020; McCubbin et al., 2006; Preissl, Lowery, & Eswaran, 2005; Vrba et al., 2004). Before and after the measurement, an ultrasound (Ultrasound Logiq 500MD, GE, UK) is performed by a midwife to determine fetal position.

a. Stimulation during fMEG measurement

To investigate neuronal responses of fetuses, there is the opportunity to use auditory and visual stimuli as presented during the fMEG measurement (Sheridan et al., 2010). For study 2, we used maternal relaxation stimulation only. This was assessed via headphones to the mother. No auditory or visual stimulation was presented to the fetus.

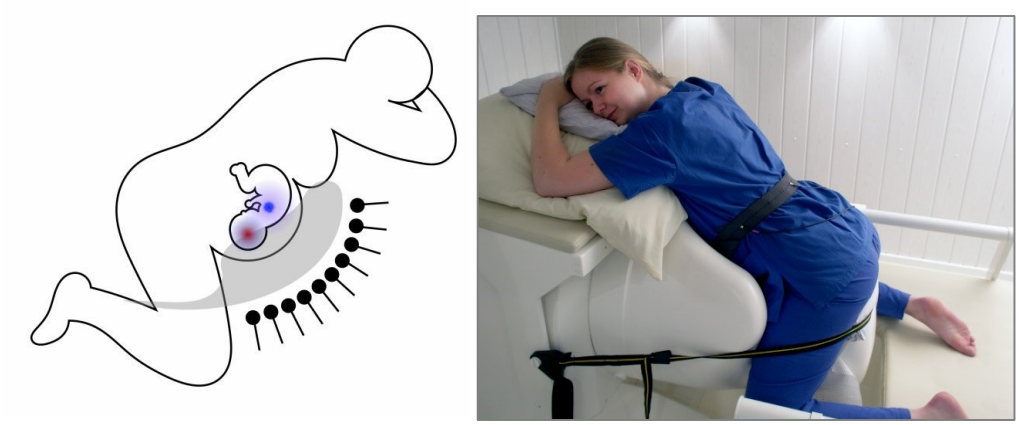


Figure 6. Fetal magnetoencephalography (fMEG).

Left side: Schematic illustration of the fMEG technique. SQUID-coils (in black) detect changes in fetal heart rate (blue) and brain (red) activity (adapted from Sippel et al., 2019). Right side: Picture of a pregnant woman resting on the fMEG device during a measurement. Acoustic relaxation stimuli are relayed through headphones (not shown). Picture courtesy of the fMEG Center, University Hospital Tübingen.

b. Data Analysis

“For the analysis of maternal and fetal HRV with fMEG, time differences between consecutive R waves (RR intervals) were acquired as an input signal and extracted from the maternal MCG and fetal MCG. The RR intervals of the maternal and fetal heart signals were extracted using the following methods:

First, the maternal cardiovascular activity was detected and marked with a template matching technique (Vrba et al., 2004) or with adaptive Hilbert transformation (Ulusar et al., 2009).” (Mat Husin, 2020, p. 18). Before we performed the extraction of the

maternal RR intervals, we filtered the data with a high-pass filter (0.5 Hz). The fetal RR data was extracted after application of a band-pass filter (between 1 and 50 Hz). Then, the maternal RR intervals were extracted and the maternal cardiovascular activity was attenuated by signal space projection (Mat Husin, 2020; McCubbin et al., 2006; Vrba et al., 2004). After we removed the maternal cardiovascular activity, we marked the fetal cardiovascular activity in the resulting dataset (Mat Husin, 2020). The fetal RR intervals were obtained by identical methods (Mat Husin, 2020). We then segmented the 30 minutes of data into 5-minute segments and computed the HRV for each segment.

"The analyses, including pre-processing of RR time series and short-term HRV analysis in both the time and frequency domains, were performed by in-house routines in MATLAB (Mathworks, Inc., Natic, MA, USA). The in-house routines were based on existing standard approaches and tailored for use with fetal MEG data on our system." (Mat Husin, 2020, p. 19).

Time domain HRV measures

"Time domain analysis estimates the changes in the HR time series. For time domain measures, we calculated the mean HR in beats per minute […]. The variability within the RR time series was investigated by including […] the root mean square difference of successive RR intervals (RMSSD) in the analysis.

Frequency domain HRV measures

Frequency analysis estimates the fluctuation of HR at different frequencies. In the frequency domain, the power spectral density was estimated using fast Fourier transform (FFT), based on Welch's method. To calculate the FFT, the RR interval time series has to be transformed into an equidistant set of data points. Prior to the analysis, the unevenly sampled RR intervals were resampled with cubic spline interpolation at the resampling frequency of 4 Hz. This 4 Hz sampling frequency is sufficient to satisfy the Nyquist criterion to avoid the aliasing effect. The steps for performing FFT using Welch's method consist of partitioning the time series data into overlapping windows, computing the periodogram for each window separately, and then averaging the periodogram segments to estimate the power spectrum. The segments overlapping by 50% and the Hamming window were applied to each data segment of 512 points in size (length of the Discrete

Fourier Transform) before the computation of the periodogram. Welch's method was implemented using the “pwelch” function in MATLAB.

For maternal HRV, the spectral components of the different frequency bands typically used for adults (Malik, 1996) were evaluated: low frequency (LF: 0.04 to 0.15 Hz), and high frequency (HF: 0.15 to 0.40 Hz). For fetal HRV, the respiratory motion of the fetus is reported to occur at a different frequency range than that of adults.

Therefore, the frequency bands proposed by David and colleagues (2007) were used for HRV analysis in the fetus (LF: 0.08 to 0.2 Hz and HF: 0.4 to 1.7 Hz). The frequency components were expressed in […] normalized (LFn and HFn) measures [as well as the LF/HF ratio for the analysis].” (Mat Husin, 2020, pp. 23–24).

3.3.2 Blood samples

For the blood samples in study 2, a safety multifly needle (Sarstedt (23G)) in the antecubital vein was positioned. Venous blood samples were taken only once after the measurement.

Cortisol was assessed from the blood of the pregnant women. Cortisol levels were analyzed on ADVIA Centaur XP immunoassay system (Siemens AG, Munich, Germany). All blood analyses were carried out at the University Hospital Tuebingen, Germany.

3.3.3 Hair samples

Several thin hair strands were cut as closely as possible to the scalp from the posterior vertex region of the head. For determination of hair cortisol concentration, the first three cm segment from nearest the scalp was used as it presumably reflects the cumulative cortisol secretion of the past three months (Wennig, 2000). Standardized weight of finely cut hair for extraction was 10.0±0.5 mg. Hair wash and cortisol extraction procedures were based on laboratory protocol (Stalder et al., 2012), with minor modifications. In brief, hair samples were washed twice for three minutes using 3 mL isopropanol. For cortisol extraction, 10±.5 mg [or 7.5±.5 mg] whole, finely chopped hairs were incubated in 1.8 mL methanol for 18 h at room temperature. Following incubation, 1.6 mL were

transferred in another glass vial. Next, 1.6 mL of the supernatant was evaporated at 50°C until samples were completely dried. Finally, the samples were resuspended in 225 µL distilled pure water and vortexed for 20 sec.

For cortisol determination, a commercially available cortisol luminescence high sensitivity immunoassay was used (LIA; IBL International, a Tecan Group company, Hamburg, Germany). Inter- and intra-assay coefficients of variation were below 10% for all assays. Hair cortisol values are displayed in pg/mg.

All hair analyses were carried out at the University of Vienna, Austria.

3.4 Study population and procedure

This study was conducted between February 2018 and September 2021. The Ethics committee of the Medical Faculty of the University of Tuebingen, Germany, approved the study (745/2017BO1). Informed consent was obtained from all participants prior to the start of the measurements.

In study 2, pregnant healthy women (N=13) were recruited by electronic communication. We had two drop outs due to illness, and one woman could not attend her second appointment. Consequently, we had N=12 data points for Silence group and N=11 data points for Music Group.

Eligibility was restricted to women over 18 years of age, German speaking, singleton, primiparous, uncomplicated pregnancy and GA between the 29th and 34th weeks of gestation and 35th weeks of gestation until term. Exclusion criteria were hearing impairments, acute depression or other acute psychiatric disorders (self-report) or drug/nicotine consumption during pregnancy (self-report). Once initial information about the study had been provided, the first appointment was scheduled between 29th and 34th weeks of gestation. The second appointment was scheduled between 35 GA and term. Participants were informed about the course of the study and gave their written informed consent prior to participation.

For both visits, we scheduled a one hour visit between 8am and 4pm. The participants were alternately assigned to Group A or Group B shortly before the measurement commenced.

Once the women arrived at the fMEG center, maternal and fetal well-being were assessed and a short interview and a consent discussion was conducted by a midwife. The midwife performed an ultrasound (ultrasound 1). The women were asked to fill out short questionnaires about their current mood (POMS) and they rated their current relaxation level on VAS_{Stress}. We then positioned the women on fMEG and provided the headphones. Then we positioned the electrodes to measure the SCL. Participants were requested to remain quietly in that position and to breathe normally.

We began by measuring spontaneous activity (ten minutes). We then induced the music (lounge, classic, jazz or meditation (see Appendix A10)) or no stimulation, depending on their group, for ten minutes via headphones. Activity was then measured for another ten minutes (recovery). Following removal of the electrodes, the women left the fMEG chamber and rated their relaxation level on the VAS_{Stress} and their current mood (POMS) once again.

After the women had received music stimulation, we requested them to evaluate it and then fulfill Q_{Stim}. We took a blood sample (visit 1 only) and a hair sample (visit 2 only) from each participant. The women were also asked to fill out different questionnaires regarding chronic stress (TICS) and prenatal distress (PDQ) once and to fulfill the RIS before and after the measurement.

3.5 Statistical analysis

We used the same procedure with regard to statistical program, data preparation and outlier removal as in study 1 (see 2.5 Statistical analysis).

To analyze physiological relaxation (MHR, FHR, SCL) and psychological relaxation (VAS_{Stress}, RIS, POMS) we performed rmANCOVA with factor time and interaction effect group*time. We included maternal age as a covariate for all analyses. If interaction effect group*time was significant, we revealed post-hoc test by using paired t-test (VAS_{Stress}, RIS, POMS). In the event of non-normal distribution of the data, Wilcoxon signed-rank test were applied with standardized z (value minus mean/SD). We calculated VAS_{Stress} and POMS delta by subtracting the pre value from the post values. For changes in FHR, SCL and MHR, we calculated delta by subtracting trial 6 from trial 1. All data shown are baseline corrected.

To determine differences in changes in fetal/maternal HR between groups, we performed a MANCOVA with FHR/MHR delta as dependent variable and factor group (SILENCE/MUSIC).

To measure the influence of chronic stress on fetal ANS, we performed a multiple linear regression analysis with FHR as dependent variable and PHQ (Subscale - Schweregrad Stress), hair cortisol or chronic stress (TICS SSCS) as independent variables. We included GA and maternal age as covariates.

With regard to chronic stress, since we are primarily interested in parasympathetic activity and sympathovagal balance, we focused mainly on HR, RMSSD for time domain measures and on HF and LF/HF ratio for frequency domain measures (Kim et al., 2018; Schubert et al., 2009). Additionally, with regard to maternal/fetal relaxation, we describe SDNN and LF for the purpose of completeness.

3.6 Results

The demographic data, questionnaire values and biological data of the participants are provided in Table 6.

Table 6. Study 2: Demographic data, questionnaire values and biological data of participants.

	N	Mean (SD)	Minimum	Maximum
Maternal Age	12	30.58 (3.98)	24	37
Gestational Age (MUSIC Group)	11	33.27 (3.40)	29	38
Gestational Age (SILENCE Group)	12	34.33 (3.39)	30	39
Trier Inventory of Chronic Stress (TICS) – Short Screening Scale of Chronic Stress (SSCS)	11	22.09 (10.44)	3	39
PHQ-D (Subscale: Schweregrad Stress)	11	8.00 (4.94)	1	17
Cortisol (nmol/l)	12	617.17 (129.01)	389	855
Hair cortisol (pg/mg)	11	7.67 (3.48)	1.81	13.69
Fetal sex (female/male) (MUSIC Group)	female:4 male:2			
Fetal sex (female/male) (SILENCE Group)	female:1 male:4			

PHQ-D: Patient Health Questionnaire; N: sample size; SD: standard deviation; TICS: Trier Inventory of Chronic Stress; SSCS: Short Screening Scale of Chronic Stress.

3.6.1 Psychological relaxation

Relaxation Inventory Scale (RIS)

We performed a rmANOVA with time and group as factors, and observed significant effect of time for the subscale Cognitive Tension, F (1,21) = 12.023, p=.002, ηp2=.364, and of group*time F (1,21) = 4.860, p=.039, ηp2=.188.

Paired t-test revealed a significant difference in subscale Cognitive Tension in Music group of factor time, Pre value (3.258 (.065) (Mean (SD)) to Post value (3.400 (.081), t (10) = -6.065, p<.001, but not of Silence group, t (11) = .06275, p=.476. Post-hoc test showed no significant differences between groups.

A rmANOVA for the subscale Physiological Tension showed significant main effects of time F (1,21) = 5.342, p=.031, ηp2=.203 but not of group*time F (1,21) = .054, p=.818. Women in Silence group showed a slight decrease in Physiological Tension, Pre value: 4.134 (.053) (Mean (SD)) to Post value: 4.239 (.037). Same effect was seen in Music group, Pre value: 4.095 (0.55) to Post value (4.181 (.039)).

We found no significant main effect for the subscale Physical Tension of time F (1,21) = 0.199, p=.660 or group*time F (1,21) = 0.683, p=.418. Women in Silence group showed a slight decrease in Physical Tension, Pre value: 4.040 (.096) (Mean (SD)) to Post value: 3.978 (.066). The same effect was seen in Music group, Pre value: 3.960 (.101) to Post value: 3.978 (.069).

Profile of Mood States (POMS)

We performed a rmANOVA with time and group as factors and found a significant effect of time for the subscale POMS Depression/Anxiety, F (1,20) = 6.383, p=.020, ηp2=.242 but not of group*time, F (1,20) = 1.486, p=.237. Women in Silence group had lower values in depression/anxiety after the measurement, Pre: 1.929 (.309) (Mean (SD)) to Post: 1.668 (.213) as well as women in Music group, Pre: 1.811 (.339) to Post: 1.063 (.234).

We found no significant effect of time for the subscale fatigue, F (1,21) = 2.917, p=.102, or for group*time, F (1, 21) = 2.083, p=.164 or for the subscale vigor of factor time, F (1,21) = 0.084, p=.775 or for the interaction group*time, F (1,21) = 0.183, p=.673. Women in Silence group reported a slight increase in vigor from Pre (3.053 (.123))

(Mean (SD)) to Post (3.071 (.206)). For Music group, the same increase from Pre (3.103 (.129)) to Post (3.012 (.2015)) was investigated.

With regard to fatigue, the women in Silence group reported a slight decrease from Pre: 2.376 (.204) to Post: 2.339 (.225), as did the women in Music group; Pre: 2.484 (.213) to Post: 2.053 (.235).

For subscale hostility, the analysis revealed a significant effect of time, $F(1,20) = 8.593$, $p=.008$, $\eta p2=.301$ but not for the interaction group*time, $F(1,20) = 3.226$, $p=.088$. The women in Silence group reported a slight decrease in hostility from Pre (1.500 (.206) (Mean (SD)) to Post (1.344 (.160)). The same slight decrease was seen in Music group from Pre (1.452 (.226) to Post (1.452 (.176)).

Visual Analog Scale (VAS_{Stress})

A rmANOVA showed no significant interaction effect of group*time, $F(1,18) = 1.925$, $p=.182$ but for time, $F(1,18) = 11.522$, $p=.003$, $\eta p2=.390$, in VAS_{Stress}.

Participants in Music Group were more relaxed after the measurement (Post, Median: 0.300) than before (Pre, Median: 1.900). The same effect was observed in Silence group (Pre, Median: 0.850 and Post, Median: 0.250) (see Figure 7).

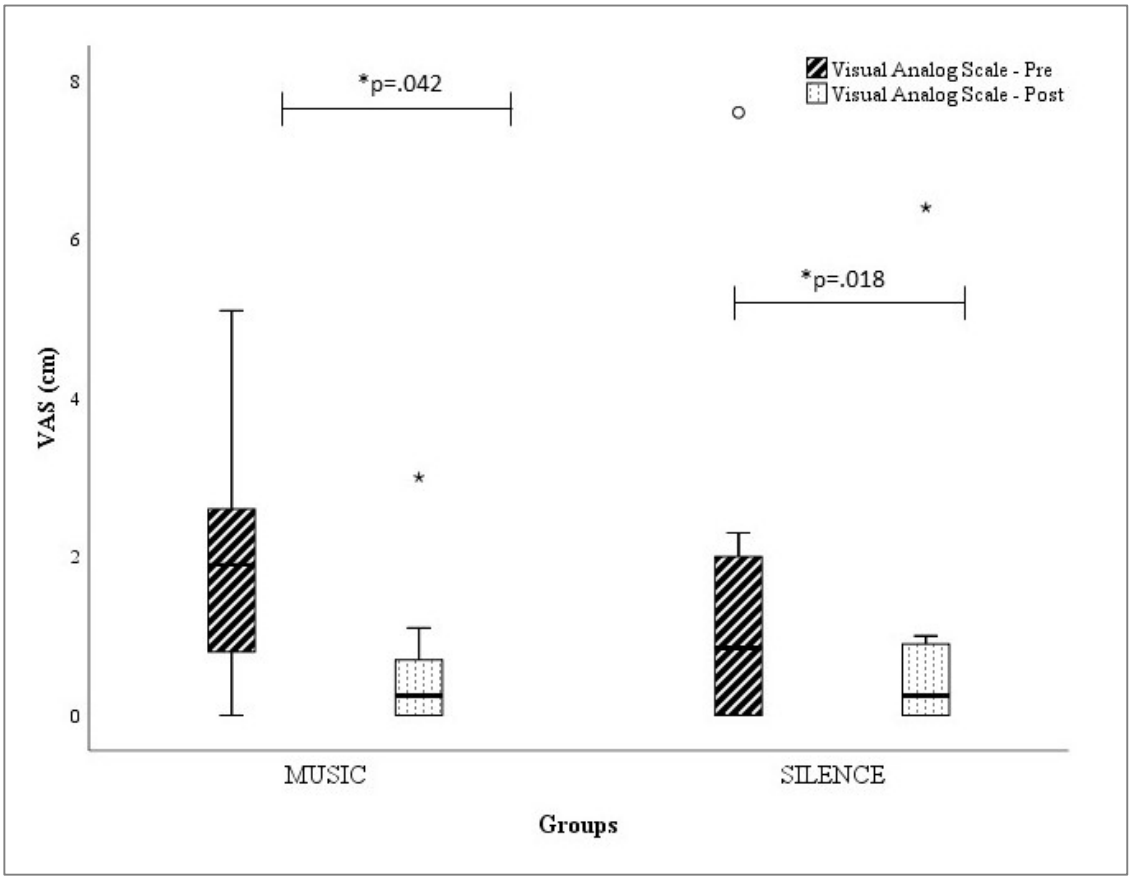


Figure 7. Study 2: Visual Analog Scale (VAS_{Stress}) for MUSIC and SILENCE group (Pre vs. Post).

Visual Analog Scale (in cm) for both groups: MUSIC Group (N=8) and SILENCE Group (N=12); The women were asked to fulfill the VAS_{Stress} before (Pre) and immediately after the measurement (Post); *on a statistical level of p<.05 significant.

Questionnaire (Q_{STIM}) (Relaxation stimulation)

Participants had the opportunity to rate the relaxation stimulation after measurement on a rating scale from 1(‘very good’), 2 (‘good’) to 3 (‘not good’). Six participants rated for ‘very good’, five for ‘good’ and none for ‘not good’ (see Appendix A9).

3.6.2 Physiological relaxation

Maternal skin conductance level

Primarily, to determine whether changes in SCL differ significantly between groups, we used SCL delta as the independent variable and group as the factor. One-way ANCOVA showed no significant differences between groups, F (2,15) = 0.176, p=.681.

Secondly, to determine a possible relaxation effect over time, we performed a rmANCOVA for each group. For Music group, we found no significant effect of time, F (5,35) = 0.392, p=.753 with Greenhouse-Geisser correction. For Silence group, we found a similar non-significant effect of time, F (5,35) = 0.105, p=.990 (see Appendix A12).

Fetal and maternal autonomic nervous system

Time domain HRV measures

Significant difference between groups in changes of FHR:

A MANCOVA with fetal and maternal HR delta revealed a significant effect of factor group in FHR delta, F (2,16) = 15.797, p=.001, ηp2=.497, but not for MHR delta.

Fetuses of the women in Silence group had a significantly higher increase of FHR (6.651 (2.025)) (Mean (SD)) after intervention than fetuses of women in Music group (-4.523 (1.919)) (see Figure 8).

No significant effect of intervention on maternal or fetal RMSSD:

A MANCOVA revealed no significant effect of factor group for fetal/maternal RMSSD delta, F (2,14) = 1.397, p=.257 or for fetal/maternal RMSSD delta, F (2,14) = 0.006, p=.939. Fetuses of the women in Music group showed a lower decrease in RMSSD after intervention (1.084 (2.313)) (Mean (SD)) than fetuses in Silence group (4.891 (2.177)).

To test our assumption that maternal RMSSD increases over time, we performed rmANCOVA with Bonferroni-adjusted post-hoc analysis. This showed no significant differences between time points in Music group, F (5,45) = 3.174, p=.205 and Silence group, F (5,40) = .666, p=.651.

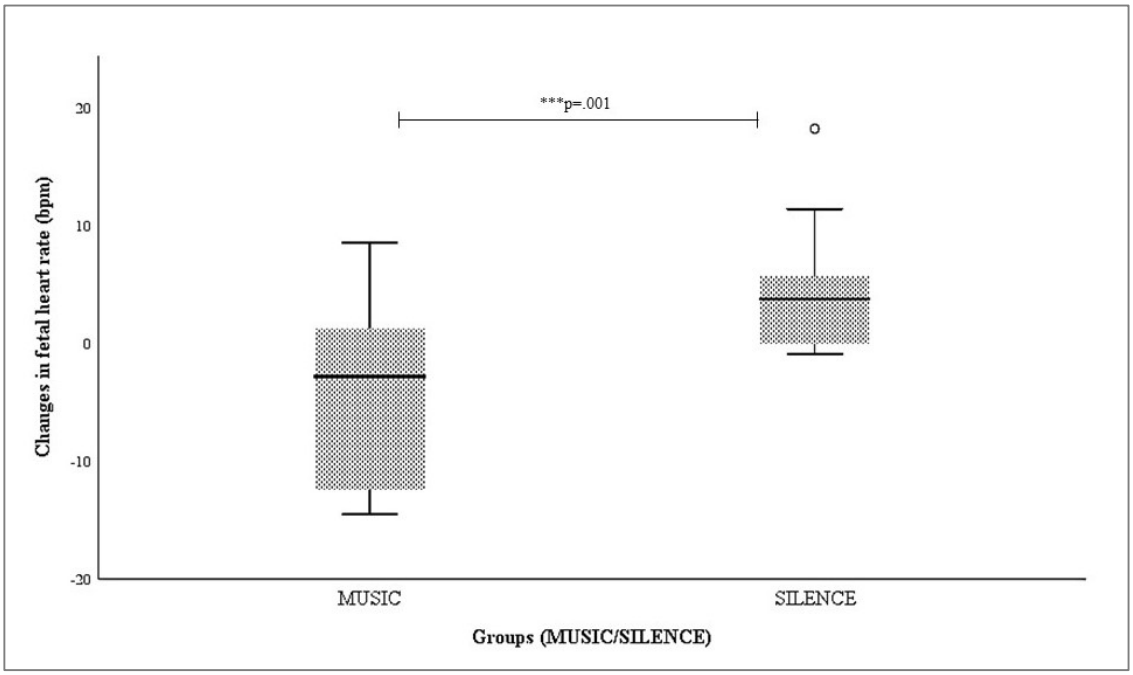


Figure 8. Study 2: Fetal heart rate (FHR) changes in MUSIC and SILENCE groups.

All values of FHR delta (trial 6-trial 1) are baseline corrected. bpm: beats per minute; Music: MUSIC Group (N=9); Silence: SILENCE Group (N=9). ***on a statistical level of p=.001 significant.

Frequency domain HRV measures

No significant effect of intervention on fetal frequency domain HRV parameters LF/HF and HFn:

A MANCOVA revealed no significant effect of factor group for fetal LF/HF ratio delta, F (2,15) = 2.019, p=.167 and for fetal HFn delta, F (2,15) = 0.170, p=.845. Fetuses of the women in Music group showed slightly decreased LF/HF ratio delta after intervention (1.743 (9.611)) (Mean (SD)) compared to Silence group (1.862 (4.644)). The same non-significant difference was observed between groups in HFn with higher HFn delta for Music group (.050 (.187)) than Silence group (-.0292 (.123)) (see Table 7 and Table 8).

Table 7. Study 2: Fetal cardiovascular activity parameters of MUSIC Group.

	MUSIC Group	Trials	N	Mean	Standard error	Standard deviation
Time domain parameters	FHR	T2	10	-3.53	2.683	8.487
		T3	9	-1.81	2.211	6.634
		T4	10	-4.71	1.990	6.293
		T5	8	-5.33	1.538	4.352
		T6	10	-3.95	2.444	7.729
	Fetal SDNN	T2	10	4.81	18.589	58.785
		T3	9	0.49	1.377	4.132
		T4	10	0.67	1.722	5.447
		T5	8	1.27	1.307	3.697
		T6	10	-6.97	10.064	31.825
	Fetal RMSSD	T2	11	-0.07	0.570	1.893
		T3	9	0.62	0.768	2.306
		T4	10	2.05	1.779	5.628
		T5	10	1.62	1.256	3.972
		T6	9	4.26	2.935	8.805
Frequency domain parameters	Fetal LFn	T2	10	-0.07	0.036	0.116
		T3	9	-0.02	0.0447	0.134
		T4	10	-0.08	0.053	0.169
		T5	8	-0.05	0.049	0.138
		T6	10	-0.08	0.065	0.205
	Fetal HFn	T2	10	0.07	0.036	0.116
		T3	9	0.02	0.044	0.134
		T4	10	0.08	0.053	0.169
		T5	8	0.05	0.049	0.138
		T6	10	0.08	0.065	0.205
	Fetal LF/HF	T2	10	-2.17	1.609	5.090
		T3	9	1.97	3.286	9.859
		T4	10	-1.48	1.794	5.673
		T5	8	0.76	1.880	5.319
		T6	9	1.74	3.203	9.611

FHR: fetal heart rate; SDNN: standard deviation of NN interval; RMSSD: root mean square of successive differences; LFn: low frequency normalized; HFn: high frequency normalized; LF/HF: ratio between low frequency and high frequency; Trials: baseline corrected values of each 5-minute trial; N: sample size (the sample size is different between trials because any data sets that were not free from artifacts or disturbances caused by maternal movements had to be excluded).

Table 8. Study 2: Fetal cardiovascular activity parameters of SILENCE Group.

	SILENCE Group	Trials	N	Mean	Standard error	Standard deviation
Time domain parameters	FHR	T2	12	1.55	1.453	5.034
		T3	12	3.55	1.686	5.843
		T4	12	2.65	1.737	6.019
		T5	10	1.35	3.692	11.675
		T6	12	4.44	1.630	5.649
	Fetal SDNN	T2	10	0.24	2.719	8.600
		T3	10	2.65	3.456	10.93
		T4	10	9.98	10.240	32.384
		T5	8	61.03	46.121	130.449
		T6	10	-0.20	3.019	9.549
	Fetal RMSSD	T2	10	0.32	0.447	1.415
		T3	10	1.47	1.231	3.893
		T4	10	0.67	1.080	3.416
		T5	7	-0.47	0.881	2.331
		T6	9	1.49	1.180	3.540
Frequency domain parameters	Fetal LFn	T2	10	0.04	0.057	0.180
		T3	10	0.00	0.037	0.119
		T4	10	0.00	0.026	0.083
		T5	8	0.04	0.051	0.144
		T6	10	0.02	0.039	0.123
	Fetal HFn	T2	10	-0.04	0.057	0.180
		T3	10	-0.00	0.037	0.119
		T4	10	-0.00	0.026	0.083
		T5	8	-0.04	0.051	0.144
		T6	10	-0.02	0.039	0.123
	Fetal LF/HF	T2	10	3.47	1.861	5.888
		T3	10	0.03	0.767	2.427
		T4	10	0.83	1.099	3.475
		T5	8	2.31	1.986	5.617
		T6	10	1.86	1.468	4.644

FHR: fetal heart rate; SDNN: standard deviation of NN interval; RMSSD: root mean square of successive differences; LFn: low frequency normalized; HFn: high frequency normalized; LF/HF: ratio between low frequency and high frequency; Trials: baseline corrected values of each trial which lasted 5 minutes N: sample size (the sample size is different between trials because any data sets that were not free from artifacts or disturbances caused by maternal movements had to be excluded).

3.6.3 Maternal chronic stress and its impact on fetal autonomic nervous system

To investigate if maternal chronic stress has an effect on fetal ANS, we performed a linear regression analysis with dependent variable mean FHR and independent variables

gestational age, maternal age and chronic stress (TICS SSCS) or stress level (PHQ-D - Subscale: Schweregrad Stress).

Time domain HRV measures

No significant effects of chronic stress (TICS SSCS) on changes in FHR:

Multiple linear regression analysis for Music and Silence groups showed no significant effect of independent variable chronic stress (TICS SSCS), maternal age or GA on FHR changes (see Figure 9).

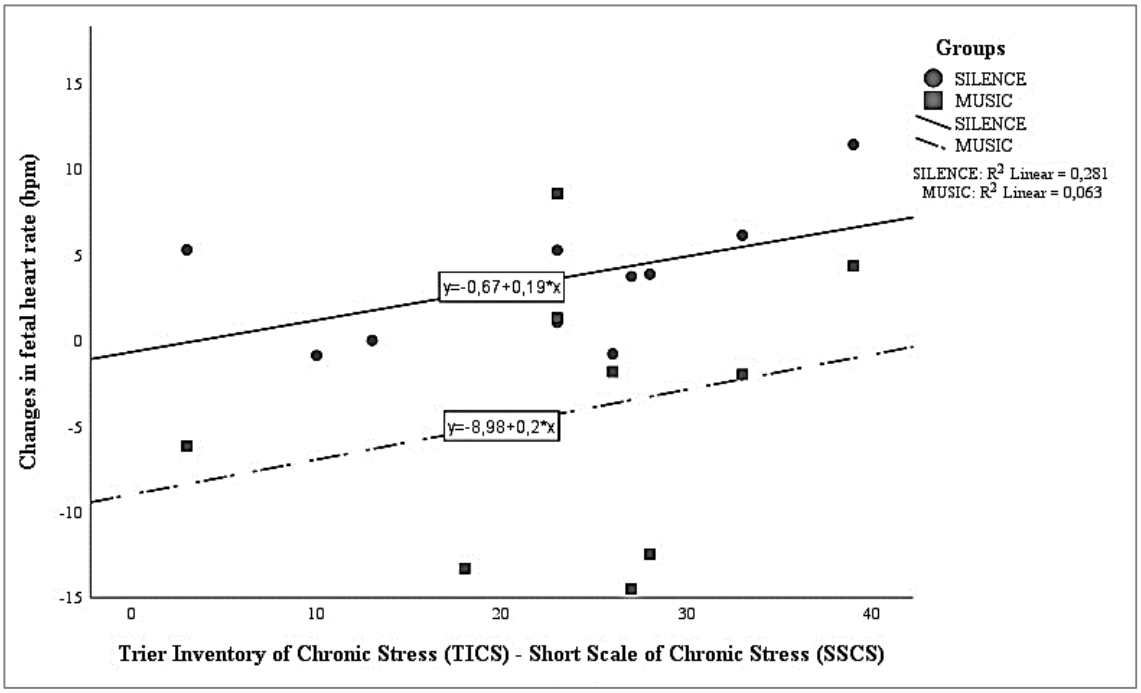


Figure 9. Study 2: Maternal chronic stress levels (TICS SSCS) and their impact on changes in fetal heart rate in MUSIC and SILENCE group.

Dashed line: MUSIC Group: N=9; Solid line: SILENCE Group: N=10; bpm: beats per minute; maternal stress level is based on the chronic stress level (TICS - Short Screening Scale of Chronic Stress (SSCS)).

No significant effects of stress levels (PHQ-D) on changes in FHR:

Multiple linear regression analysis for Music and Silence group showed no significant effect of independent variable stress level (PHQ-D - Subscale: Schweregrad Stress), maternal age or GA on FHR changes (see Figure 10).

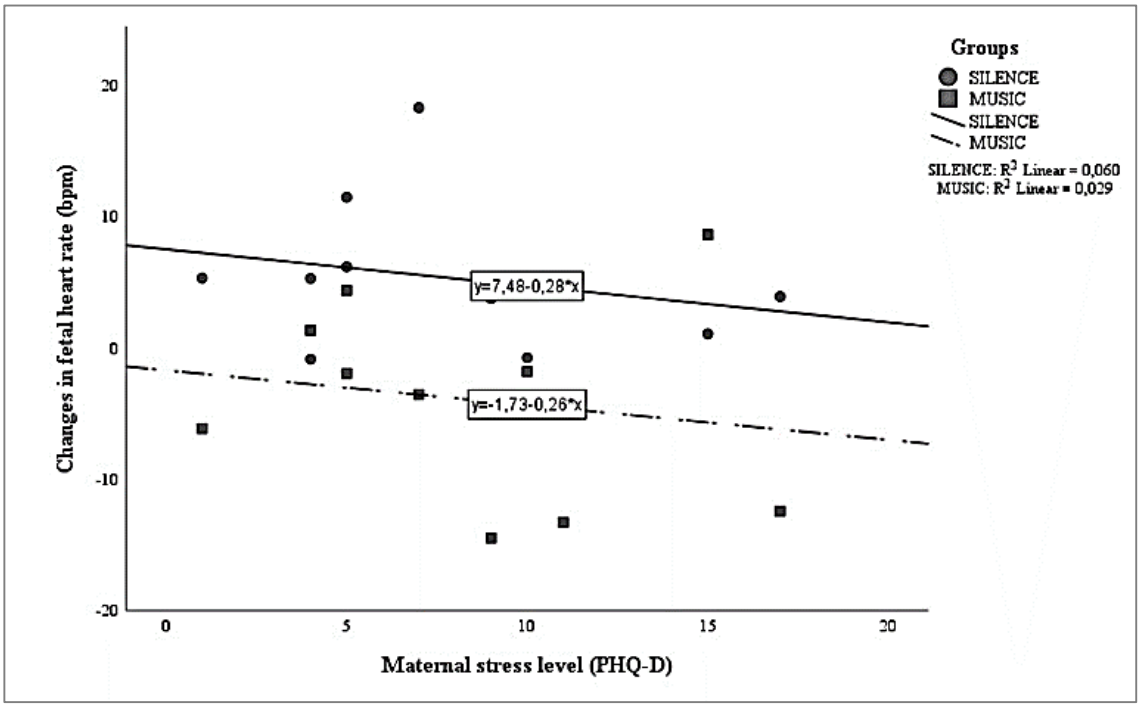


Figure 10. Study 2: Maternal stress levels (PHQ-D) and their impact on changes in fetal heart rate in MUSIC and SILENCE group.

Dashed line: MUSIC group: N=10; Solid line: SILENCE Group: N=10; bpm: beats per minute; maternal stress level is based on the PHQ-D (Patient Health Questionnaire – Subscale: Schweregrad Stress); N: sample size.

No significant effects of chronic stress (TICS SSCS) on changes in fetal RMSSD:

Multiple linear regression analysis for Music group showed no significant effect of the independent variables maternal age, GA or chronic stress (TICS SSCS) on fetal RMSSD (see Table 9).

No significant effects of stress levels (PHQ-D) on changes in fetal RMSSD:

Multiple linear regression analysis for Music group showed no significant effect of independent variables maternal age, GA or chronic stress (PHQ-D-Subscale: Schweregrad Stress) on fetal RMSSD changes (see Table 9).

Table 9. Study 2: Results of multiple regression analysis for time domain HRV measures (HR; RMSSD) with regard to maternal (chronic) stress levels in MUSIC Group and SILENCE Group.

	MUSIC Group						SILENCE Group					
	Questionnaires	Time domain HRV measures (delta)	Variables	beta	p-value	R^2	Questionnaires	Frequency domain HRV measures (delta)	Variables	beta	p-value	R^2
Maternal chronic stress	*TICS (SSCS)*	Fetal HR	GA	-.831	.171	.433 N=9	*TICS (SSCS)*	Fetal HR	GA	-.508	.238	.436 N=9
			Stress	.080	.835				Stress	.284	.521	
			MA	-.303	.592				MA	.367	.451	
		Fetal RMSSD	GA	1.132	.060	.577 N=9		Fetal RMSSD	GA	.070	.881	.034 N=10
			Stress	.089	.798				Stress	-.034	.946	
			MA	.915	.113				MA	-.176	.751	
Maternal stress level	*PHQ-D*	Fetal HR	GA	-.763	.207	.349 N=10	*PHQ-D*	Fetal HR	GA	-.257	.466	.349 N=10
			Stress	-.154	.661				Stress	-.312	.466	
			MA	-.285	.620				MA	.596	.155	
		Fetal RMSSD	GA	.986	.064	.579 N=10		Fetal RMSSD	GA	.099	.819	.018 N=11
			Stress	-.411	.178				Stress	.005	.990	
			MA	.834	.106				MA	-.146	.736	

MA: maternal age; GA: gestational age; Stress: maternal stress level based on the specific questionnaire; HR: heart rate; RMSSD: root mean square of successive differences; TICS (SSCS): Trier Inventory of Chronic Stress (Short Screening Scale of Chronic Stress); PHQ-D: Patient Health Questionnaire (Subscale: Schweregrad Stress); N: sample size (the sample size is different between parameters because any data sets that were not free from artifacts or disturbances caused by maternal movements were excluded); *on a statistical level of p<.05 significant.

Frequency domain HRV measures

We performed a multiple linear regression analysis and found no significant effects for frequency domain HRV measures, except for changes in LF/HF ratio in the Music group only (see Table 10).

Multiple linear regression analysis for Music group thus revealed a significant effect of stress levels (PHQ-D, Subscale: Schweregrad Stress) and GA on changes in fetal LF/HF ratio: maternal age (beta=.381, p=.183), stress level (beta=.456, p=.048) and on gestational age (beta=.976, p=.009). The overall model fit was R^2=.869. Maternal stress level (8.890 (5.061)) (Mean (SD)) had a significant effect on fetal changes of LF/HF ratio delta (1.743 (9.611)).

Non-significant results were found for Silence group: maternal age (beta=.019, p=.754), stress level (beta=.583, p=.081) and GA (beta=-.434, p=.211). The overall model fit was R^2=.535.

Table 10. Study 2: Results of multiple regression analysis for frequency domain HRV measures (LF/HF; HFn) with regard to maternal (chronic) stress levels in MUSIC Group and SILENCE Group.

	MUSIC Group						**SILENCE Group**					
	Questionnaires	**Frequency domain HRV measures (delta)**	**Variables**	**beta**	**p-value**	**R^2**	**Questionnaires**	**Frequency domain HRV measures (delta)**	**Variables**	**beta**	**p-value**	**R^2**
Maternal chronic stress	***TICS (SSCS)***	Fetal LF/HF	GA	1.039	**.018***	.851 N=8	***TICS (SSCS)***	Fetal LF/HF	GA	.561	.213	.396 N=9
			Stress	.407	.130				Stress	.393	.398	
			MA	.084	.776				MA	-.096	.844	
		Fetal HFn	GA	-1.025	.072	.577 N=9		Fetal HFn	GA	.222	.607	.279 N=9
			Stress	.295	.395				Stress	.380	.443	
			MA	.968	.088				MA	.126	.802	
Maternal stress level	***PHQ-D***	Fetal LF/HF	GA	.976	**.009***	.869 N=9	***PHQ-D***	Fetal LF/HF	GA	.434	.211	.535 N=10
			Stress	.456	**.048***				Stress	.583	.081	
			MA	.381	.183				MA	.019	.954	
		Fetal HFn	GA	-1.007	**.040***	.668 N=10		Fetal HFn	GA	-.190	.647	.243 N=10
			Stress	-.483	.090				Stress	-.408	.294	
			MA	-1.199	**.022***				MA	-.130	.754	

MA: maternal age; GA: gestational age; Stress: maternal stress level based on the specific questionnaire; HFn: high frequency normalized; LF/HF: ratio between low frequency and high frequency; TICS (SSCS): Trier Inventory of Chronic Stress (Short Screening Scale of Chronic Stress); PHQ-D: Patient Health Questionnaire (Subscale: Schweregrad Stress); N: sample size (the sample size is different between parameters because some data sets were not free from artifacts or disturbances caused by maternal movements and were excluded); *on a statistical level of p<.05 significant.

Hair samples and changes in fetal heart rate

The hair cortisol values ranged from 1.81 pg/mg to 13.69 pg/mg (7.673 (3.572)) (Mean (SD)) in N=11 pregnant women.

A multiple linear regression analysis revealed no significant effects for hair cortisol as a marker for maternal chronic stress and changes in FHR in Music group: maternal age (beta=-.479, p=.354), GA (beta=-.973, p=.087) and hair cortisol (beta=.480, p=.152). The same non-significant effects were observed in Silence group: maternal age (beta=.568, p=.174), GA (beta=-.221, p=.586) and hair cortisol (beta=-.267, p=.469).

Moreover, no significant correlation was found between hair cortisol and MHR changes, or between hair cortisol and maternal self-reported chronic stress (TICS SSCS).

3.7 Discussion

3.7.1 No effects of maternal subjective relaxation on fetal autonomic nervous system

In study 2, we did not detect any effects of maternal relaxation on FHR. This may have been due to the fact that we could not detect any significant physiological relaxation effect in the mother. What was striking, however, was that the changes in FHR differed significantly between the groups. A study by DiPietro and colleagues (DiPietro et al., 2008) reported a decrease in FHR and increase in FHRV after a relaxation intervention. However, post-hoc analyses showed that the decrease in FHR as well as the increase in FHRV was primarily limited to the decrease from baseline measurement to the beginning of the relaxation period (DiPietro et al., 2008). Furthermore, another study (Fink et al., 2011) reported that more fetal accelerations were present in the FHR in the control group than the intervention group. This implies that there was more fetal activity in the control group than in the intervention group (Fink et al., 2011). In this study by Fink and colleagues (2011), a combination of progressive muscle relaxation and guided imagery was used for relaxation. They reported no association between MHR or systolic/diastolic blood pressure and FHR, which is in line with our study. Our results differ from those of a study by Garcia-Gonzalez and colleagues (2017), who measured a significant increase in basal FHR in the women receiving music as a relaxation intervention. This study examined 409 pregnant women who underwent a total of 14 40-minute relaxation interventions three times a week in late pregnancy (28^{th}-36^{th} weeks of gestation) (García González et al., 2017). In our study, however, we observed this increase in FHR in the Silence group only.

3.7.2 No effects of subjective chronic stress on fetal autonomic nervous system

We found no significant effect of chronic stress, assessed via TICS (SSCS), fetal HR or fetal HRV. However, we found a significant effect of maternal stress level (PHQ-D – Subscale: Schweregrad Stress) on changes in fetal LF/HF ratio. In the literature, there is evidence that fetuses of depressed mothers showed slower FHR reactions to a

vibroacoustic stimulation procedure (Allister, Lester, Carr, & Liu, 2001). This has been linked to higher FHR baseline values (Dieter, Emory, Johnson, & Raynor, 2008). Furthermore, another study revealed greater increases in FHRV in anxious or depressed mothers during a Stroop color-word test (DiPietro et al., 2003). We cannot, of course, establish maternal chronic stress as a single factor for changes in the fetal ANS. Nevertheless, this data constitutes a sign for a connection between the maternal emotional state and fetal HRV. However, due to different concepts of depression, anxiety and stress, we cannot speak of maternal chronic stress on the basis of these studies. In future, we will certainly need to consider such inter-conceptual (depression, anxiety, stress) differences. What our results *do* show, however, is that there might be a link between maternal chronic stress and fetal HRV.

4 Study 3: Maternal stress and its impact on maternal IL-6 levels and insulin sensitivity during pregnancy

All the women included in study 3 were participants of an ongoing, multicenter study (PREG) (Fritsche et al., 2021) of the German Diabetes Center, Germany. The PREG study is a prospective follow-up study to determine whether insulin secretory dysfunction is a risk factor for the occurrence of type 2 diabetes mellitus in women with and after GDM. In addition, a subgroup-specific intervention to investigate whether lifestyle intervention in women with GDM and thus a high-risk group for type 2 diabetes mellitus. Intensive phenotyping is used to define subgroups at risk of diabetes and to improve individual diabetes prevention and therapy in the long term. Furthermore, in this cohort, it is measured how the fetal ANS is influenced in fetus of mothers with GDM compared to mothers with no GDM diagnosis (Fehlert et al., 2017). In the follow-up, additional measurements include cognitive testing e.g., language, intelligence etc., in the offspring at one, two, five and ten years of age.

4.1 Study design and research question

This study was based on the data of the PREG cohort. The PREG study (see Figure 11) is registered with clinicialtrials.gov (NCT04270578).

Our hypotheses were:

(1) We assumed that elevated stress levels during pregnancy lead to an increase in maternal IL-6 values independent of pre-pregnancy BMI.

(2) We expected elevated stress levels during pregnancy to lead to a decrease in maternal insulin sensitivity independent of pre-pregnancy BMI.

(3) We assumed that an increase in IL-6 values leads to a decrease in maternal insulin sensitivity.

For overview of study parameters, see Table 11.

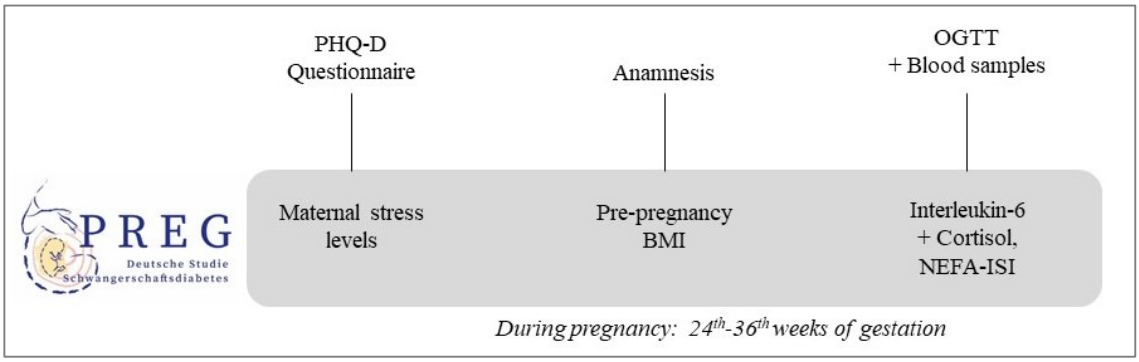


Figure 11. Study 3 design.

OGTT: Oral Glucose Tolerance Test; NEFA-ISI: non-esterified-free-fatty-acids insulin sensitivity index; PHQ-D: Patient Health Questionnaire.

Table 11. Study 3: Parameters.

Physiological parameters	Psychological parameters
• Blood samples (Cortisol, IL-6) • Pre-pregnancy BMI	• PHQ-D

PHQ-D: Patient Health Questionnaire; BMI: Body-Mass-Index; IL-6: Interleukin-6.

4.2 Psychological parameters - Maternal subjective stress

In this study, we used the PHQ-D questionnaire (for details, see study 2, section 3.2.2 (see Appendix A15)).

4.3 Neurophysiological, hormonal and metabolic parameters

4.3.1 Pre-pregnancy BMI and Maternal weight gain

Pre-gestational weight was taken from the maternal 'Mutterpass' and the pre-pregnancy BMI was calculated with weight (kg)/ height (m)². Women were assigned to three groups, depending on their pre-pregnancy BMI: women with normal weight (BMI 18.5-24.9), overweight (BMI 25.0-29.9) and obese (BMI ≥ 30.0) (Rasmussen & Yaktine, 2009).

Relative maternal weight gain and weight gain categories were calculated as in Mat Husin et al. (2020) on the basis of recommendations from the Institute of Medicine (Rasmussen & Yaktine, 2009).

For the first trimester, the recommended weight gain of 1.25 kg was assumed. In each case, maternal weight gain up to the measurement time point in pregnancy was calculated using the following formula as in Mat Husin et al. (2020):

(Maternal weight gain (MWG) up to the time of measurement in pregnancy)

$$\frac{Weight\ during\ pregnancy\ (kg) - Weight\ before\ pregnancy\ (kg) - 1.25\ kg}{Gestational\ Age\ (weeks) - 12\ weeks} + \frac{1.25\ kg}{12}$$

Weight during pregnancy was taken as the weight at the time of the visit. To calculate the weekly weight gain in the 2nd/3rd trimester until the time of measurement only, the first part of the formula was used without adding 1.25kg/12 (Breuer, 2020).

Absolute MWG was calculated by subtracting the pre-pregnancy weight from the weight at term.

4.3.2 Blood samples

A 5-point oral glucose test (OGTT) with 75 g glucose (Dextro O.G.T.; Roche Diagnostics, Mannheim, Germany) was performed at ≥27 week of gestation following an overnight fast. We placed a safety multifly needle (Sarstedt (23G)) in the antecubital vein before the measurement and OGTT. During the appointment, venous blood samples were taken at minutes 0, 30, 60, 90 and 120.

IL-6 and cortisol were assessed from the fasting blood of the pregnant women. Maternal IL-6 levels (ng/L) were analyzed with Enzyme Linked Immunosobent assay (ELISA) (R&D systems, Minneapolis, MN, USA) according to the manufactures instructions. Cortisol levels were analyzed on ADVIA Centaur XP immunoassay system (Siemens AG, Munich, Germany).

All blood analyses were carried out at the University Hospital Tuebingen, Germany.

4.3.3 Diagnosis of GDM and maternal insulin sensitivity

GDM was diagnosed in accordance with the criteria of the International Association of Diabetes and Pregnancy Study Groups (Metzger et al., 2007) with at least one out of three values exceeding the defined limits of 5.1 mmol/L of fasting glucose, 10 mmol/L 1 hour after 75g glucose ingestion and 8.5 mmol/L 2 hours after glucose ingestion.

Maternal insulin sensitivity, was calculated with the nonesterified-free-fatty-acids insulin sensitivity index (NEFA-ISI) (Wagner et al., 2016).

Formula:

$NEFA{-}ISI_{0,60,120} = 60 \times e^{3.853-0.9} \times \ln BMI - 0.205 \times \ln Insulin_0 - 0.128 \times \ln Insulin_{60} - 0.256 \times \ln Insulin_{120} - 0.138 \times NEFA_{120}$

(Insulin in pmol/l, NEFA in µmol/l, BMI in kg/m^2)

4.4 Study population and procedure

The data from this study was collected between 2012 and 2021. The Ethics Committee of the Medical Faculty of the University of Tuebingen, Germany approved the study plan (339/2010BO1). Informed consent of all participants was obtained prior to the start of the measurements.

All women included in study 3 are N=338. GDM was diagnosed in 99 women. Gestational age was between 24th and 36th weeks of gestation (mean: 27.34 (standard deviation (SD) ±2.22)), maternal age was 32.3 ±4.9 (years) and preBMI was 26.3 ±5.5 (kg/m^2).

Criteria for inclusion at study entry are women in the 24th-31st + 6th week of pregnancy. Exclusion criteria were under 18 years of age, Diabetes mellitus type 1 or type 2, Glomerular Filtration Rate < 60 ml/min/1.73$m2$, C-Reactive Protein > 1mg/dl, Transaminases elevation of twice the upper norm, cardiac history, weight loss of > 10% within the past 6 months, psychological disorders (self-report), blood glucose elevating or lowering drug therapy, such as steroids, antidiabetics and insulin.

First, the PREG cohort database was evaluated over the period from 2012 - 2021. On the basis of the inclusion criteria, all data were queried. The PHQ-D was also evaluated and all blood parameters were determined at the University Hospital of Tuebingen, Germany.

4.5 Statistical analysis

We have used the same procedure with regard to statistical program, data preparation and outlier removal as study 1 and study 2 (see 2.5 Statistical analysis or 3.5 Statistical analysis).

The testing of normality revealed that the IL-6 values were not normally distributed (KS-statistic=0.137, p=.000). The histogram showed a positive skewed distribution of the IL-6 values. For this reason, we used a Generalized Linear Model (GzLM) in SPSS with gamma as distribution and log as canonical link function. The GzLM is an extension of the general linear model to dependent variables with different distributions and less statistical assumptions (no variance homogeneity of the residuals, residuals can be correlated) instead of repeated measurement ANOVA. To determine the goodness of fit of the different statistical models and to prevent overfitting, we used the Akaike-Information-Criterium (AIC). The model with the lowest AIC was selected. A Wald-Chi-Square test with a significance level at $p<0.05$ was used to test the effects in the model. For significant model effects, pairwise comparisons for factors and interactions are computed and parameter estimates are reported. A Bonferroni-Holm correction was applied, and corrected significance levels were listed to account for multiple comparisons.

In accordance with the study of Braig et al. (2020), we divided the subjects into three groups on the basis of their stress scores (no stress (ratings 0 and 1, N=82), low stress (ratings 2 and 3, N=90) and mild stress (ratings ≥ 4, N=105). For analysis, and taking all participants with stress levels into account (N=277), the mean stress level was 3.23 ±0.17.

In addition to the categorical defined stress levels, we integrated the categorical defined preBMI (normal weight, overweight, obese), the GDM status (yes/no), the gestational age (in weeks) and the cortisol level (nmol/l) as independent variables into the model. Moreover, two-way interactions between stress and the other variables were computed.

Mediation analysis was performed to determine possible causal effects (see Figure 12). Mediation analyses were performed using the PROCESS macro by Hayes (2018), which uses ordinary least squares regression, yielding unstandardized path coefficients for total, direct, and indirect effects. Bootstrapping with 5000 samples together with heteroscedasticity consistent standard errors (Davidson & MacKinnon, 1993) was

employed to compute the confidence intervals and inferential statistics. Effects were deemed significant when the confidence interval did not include zero.

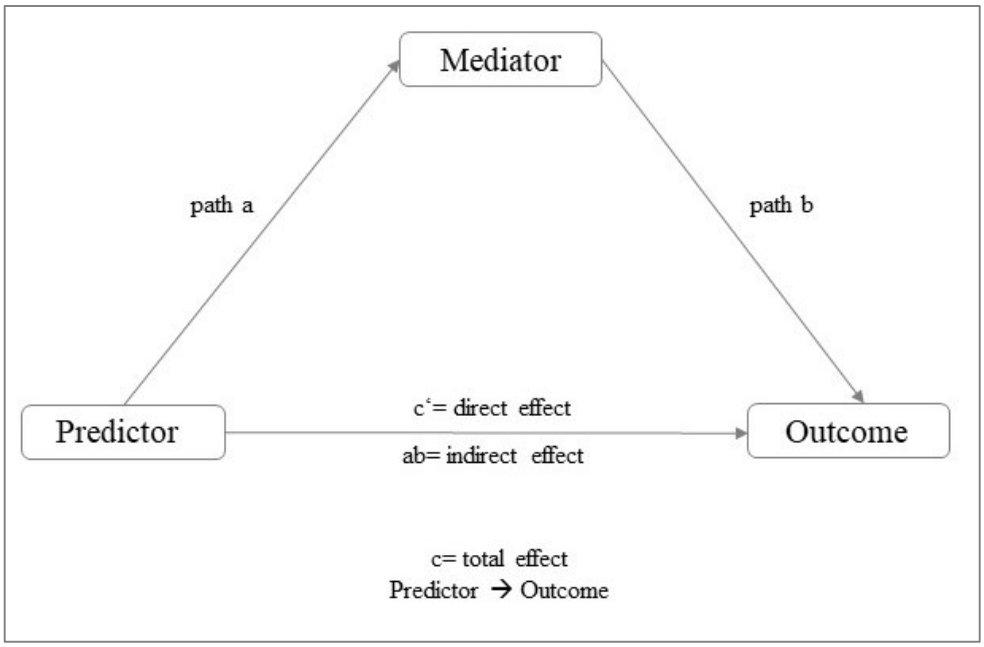


Figure 12. General Model of mediation effects.

Path c=total effect between mediator and outcome; path a=effect of predictor on mediator; path b=effect of the mediator on the outcome; path ab=multiplication between the effect of the predictor and the mediator. (from Mat Husin, 2020, *Figure 2.8*)

4.6 Results

Overall, we observed a right-skewed distribution of the stress levels, indicating that the majority of subjects were only moderately/mildly stressed (see Figure 13).

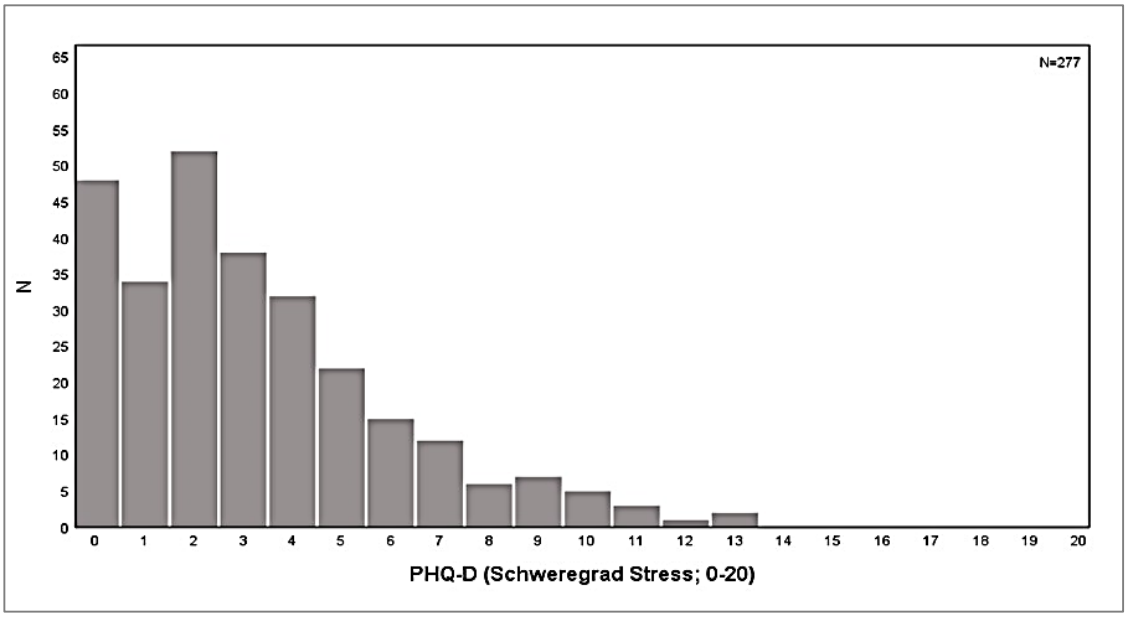


Figure 13. Study 3: Maternal stress levels (based on PHQ-D - Subscale: Schweregrad Stress).

PHQ-D: Patient Health Questionnaire; N: sample size.

Detailed characteristics of the study population are displayed in Table 12.

Table 12. Study 3: Demographic data, questionnaire values and biological data of participants.

	N	Mean	SD	Minimum	Maximum
Gestational Age (weeks)	338	27.35	2.20	24	36
Maternal Age (years)	338	32.29	4.86	19	44
Pre-pregnancy BMI (kg/m2)	295	26.27	5.46	19.81	46.60
Maternal insulin sensitivity (NEFA-ISI)	329	3.28	1.30	0.96	7.92
Interleukin-6 (ng/l)	331	1.22	0.78	0.03	4.24
Cortisol (nmol/l)	336	721.64	143.17	300	1251
PHQ-D (Schweregrad Stress; Scale 0-20)	277	3.23	2.80	0	13
GDM/NGT	GDM: N=99 / NGT: N=239				

SD: standard deviation: N: sample size; GDM: gestational diabetes mellitus; NGT: women with normal glucose tolerance.

Maternal stress is not associated with IL-6 levels during pregnancy:

The goodness-of-fit statistics showed the smallest AIC using a model without two-way interactions. The GzLM revealed no significant main effect of maternal stress on IL-6 (see Figure 14). However, a profound main effect for preBMI on IL-6 was observed ($\chi2$=36.64, p<.001). Post-hoc comparisons revealed a significant higher IL-6 value in obese than in normal weight (p<.001), in obese than in overweight (p=.042) and in overweight than in normal weight (p=.026) women (all Bonferroni-Holm corrected) (see Appendix A5). In an explorative approach, we found a significant positive association of cortisol with IL-6 ($\chi2$=6.21 p=.008, beta=0.001) (see Figure 15). Women with higher GA exhibited increased cortisol values ($\chi2$=8.93, p=.003, beta=0.055).

We also tested for interaction effects between maternal stress and preBMI, maternal stress and cortisol or maternal stress and GA on IL-6 but we found no significant interaction effects.

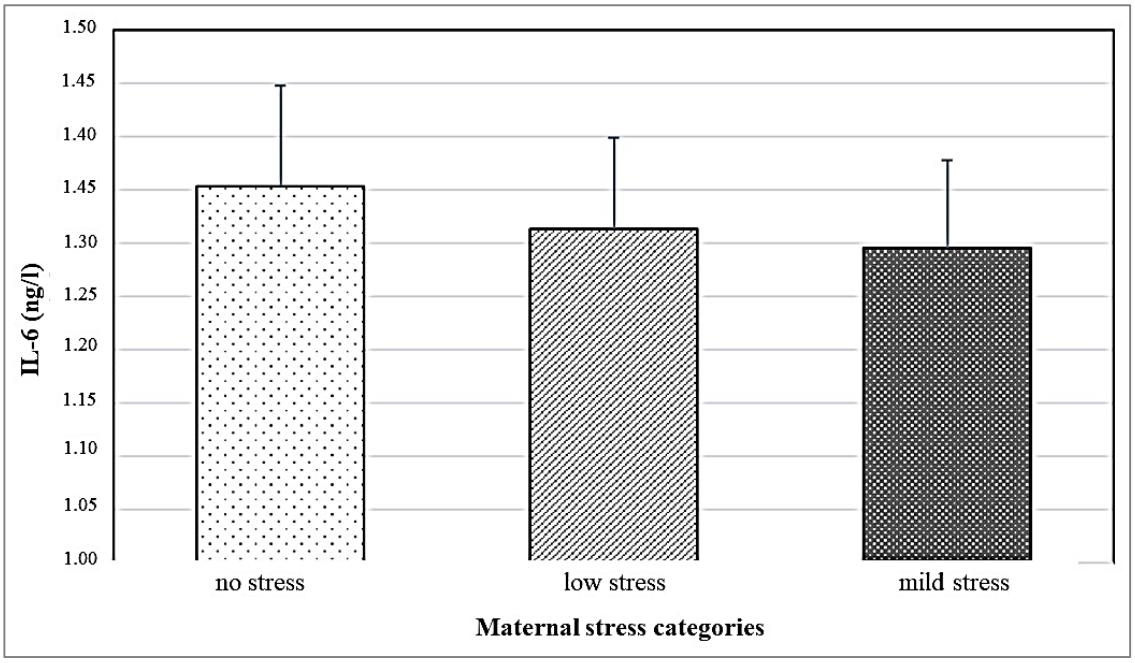


Figure 14. Study 3: Maternal stress level categories and IL-6 levels.

no stress: N=76; low stress: N=87; mild stress: N=97; IL-6: Interleukin-6. Adjusted for gestational age and cortisol levels.

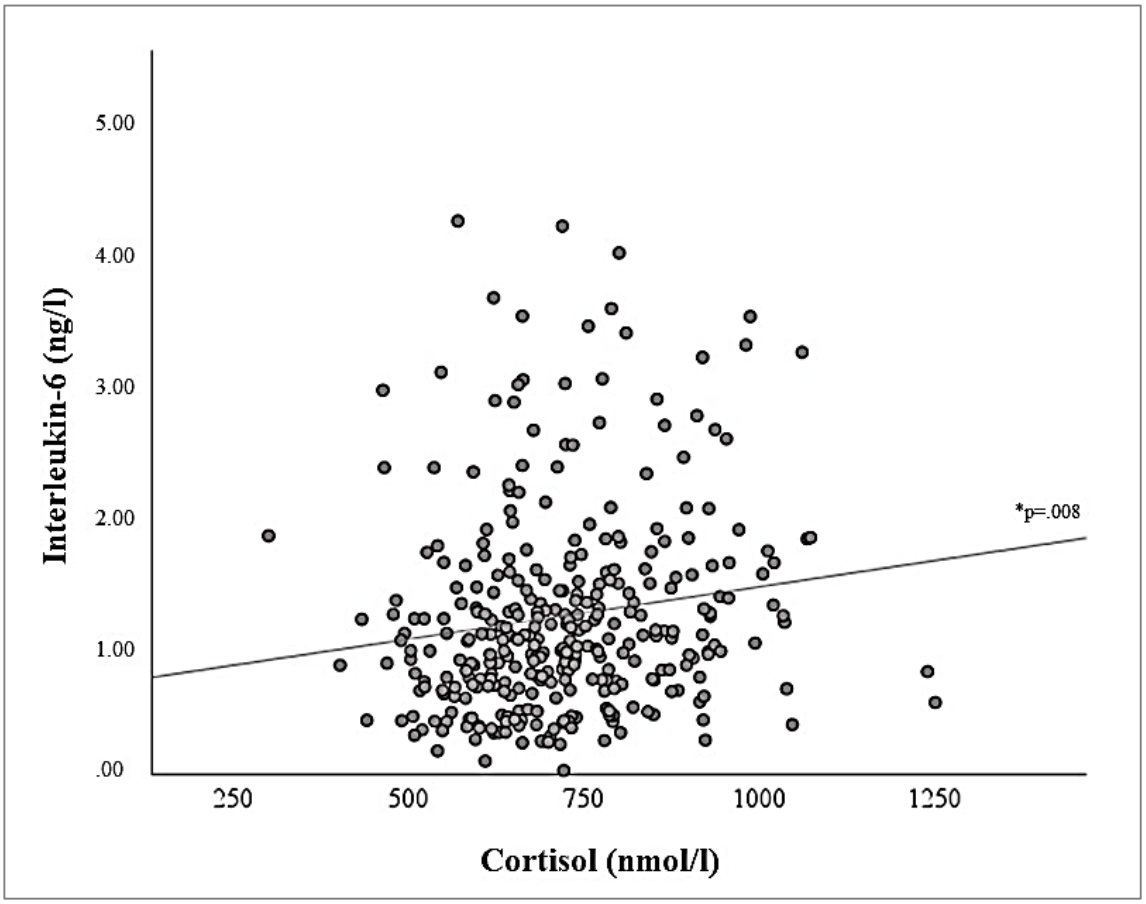


Figure 15. Study 3: Correlation between cortisol and IL-6 concentration.

Total sample size: N=242; *on a statistical level of p<.05 significant; Adjusted for gestational age.

Pre-pregnancy BMI mediates effect of stress on IL-6 levels:

We performed a mediation analysis to determine whether the direct path between maternal stress and IL-6 is mediated by the preBMI of the mother. We found that the relationship between maternal stress and IL-6 is fully mediated by the support of the preBMI with an indirect effect ab=.0177, 95%-Cl [.0024, .0364] (see Figure 16).

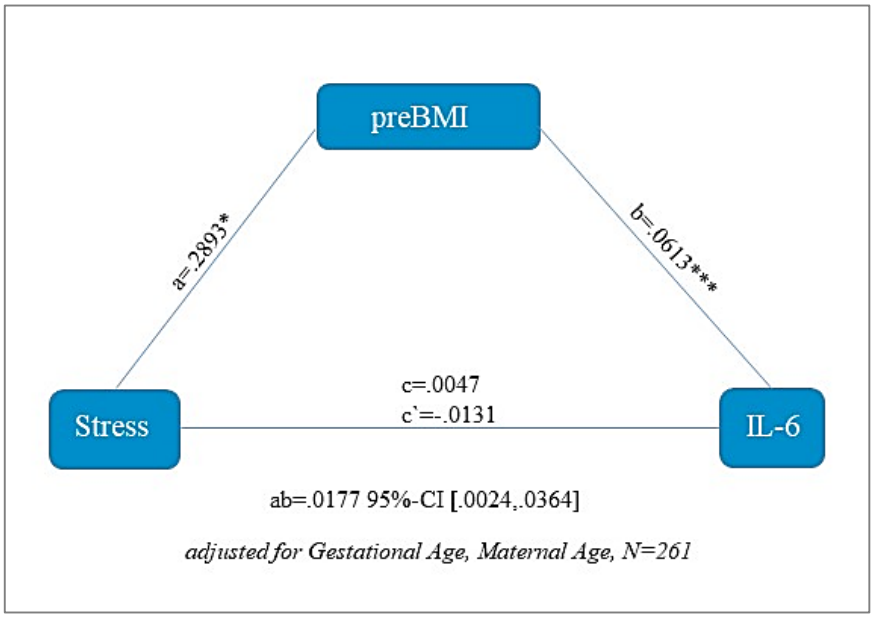


Figure 16. Mediation analysis: Maternal stress levels and IL-6.

Stress: maternal stress level based on Patient Health Questionnaire (PHQ-D - Subscale: Schweregrad Stress); IL-6: Interleukin-6; preBMI: pre-pregnancy BMI.

No significant effect of maternal stress on maternal insulin sensitivity:

The goodness-of-fit statistic showed the lowest AIC values in the models without two-way interaction terms. We therefore included only the main effects in this study. We found no significant effect of maternal stress on insulin sensitivity. However, we observed a significant main effect of preBMI on maternal insulin sensitivity ($\chi 2=97.76$, $p<.001$). Pairwise comparison revealed the lowest insulin sensitivity in obese than in overweight or lean women (both $p<.001$), whereby the difference between overweight and normal weight women was also significant ($p<.001$). This means that women with increased preBMI had a significant decrease in maternal insulin sensitivity.

PreBMI mediates effect of maternal stress on insulin sensitivity:

We performed a mediation analysis to determine whether maternal stress predicts maternal insulin sensitivity and whether the direct path is mediated by the preBMI of the mother. We ascertained that the relationship between maternal stress and insulin sensitivity is fully mediated by the support of the preBMI with an indirect effect ab=.0486, 95%-Cl [-.0858, -.0144] (see Figure 17**)**.

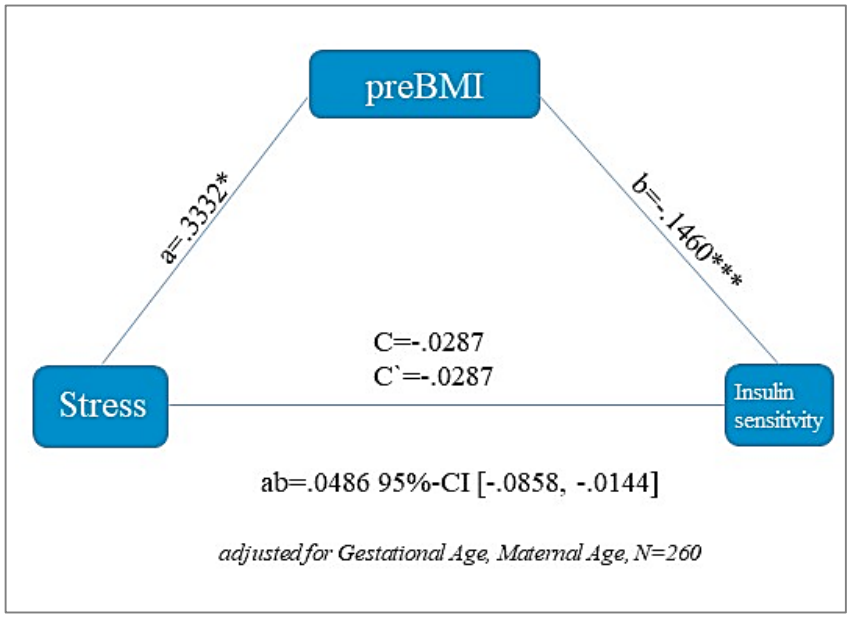


Figure 17. Study 3: Mediation analysis: Maternal stress levels and insulin sensitivity.

Stress: maternal stress levels (based on Patient Health Questionnaire (PHQ-D - Subscale: Schweregrad Stress); preBMI: pre-pregnancy BMI; Insulin sensitivity: maternal insulin sensitivity (NEFA-ISI)

Explorative analysis: No significant effect of maternal stress on maternal weight gain

On the basis of a linear regression analysis with stress, GA, preBMI and maternal age as independent variables, we found no significant effect of stress on MWG, F (4,186) = 4.768, p=.059 (see Appendix A11). However, a significant effect of preBMI on MWG was observed, F (4,186) = 4.768, p<.001.

IL-6 in GDM/NGT women:

Different hormonal and endocrine factors may influence the IL-6 levels in women with GDM differently than in women with normal glucose tolerance (NGT), and we found significantly higher IL-6 in GDM than in NGT ($\chi2$=5.25 p=.022, beta=0.181) (see Appendix A6).

We therefore excluded GDM cases for the current analysis, but still found a significant main effect of preBMI on IL-6 ($\chi2$=14.34, p=.001; Bonferroni-Holm correction: obese versus normal weight: p=.003) and GA on IL-6 ($\chi2$=7.74, p=.005). Cortisol showed only a marginally significant effect ($\chi2$=3.56, p=.059). Stress had no effect on IL-6 values. Again, the model without any interaction showed the best goodness-of-fit statistic.

Insulin sensitivity is associated with preBMI and IL-6 levels in women with normal glucose tolerance:

We found a significant main effect for IL-6 on maternal insulin sensitivity (p=.001) and a significant main effect for preBMI on insulin sensitivity ($\chi 2$=63.56, p<.001) in women with normal glucose tolerance. Post hoc comparisons confirmed the previously presented results that, of all the women in the analyses, the most adverse maternal insulin sensitivity is found in the obese group (obese versus normal weight, p<.001; obese versus overweight, p<.001; overweight versus normal weight, p<.001). IL-6 values showed a significant ($\chi 2$=11.76, p<.001) negative association (beta=-0.111) with respect to insulin sensitivity (see Appendix A7).

4.7 Discussion

4.7.1 Moderate stress as a mediator for increases in Interleukin-6 and cortisol levels

We did not find a direct effect of maternal stress on either IL-6 levels or maternal insulin sensitivity. Overall, our cohort was not highly stressed as shown by the scores not higher than 13 on a scale between 0 (no stress), and 20 (high stress).

These results are not in line with a study by Cousson-Read and colleagues (2007), who indeed showed that there was a significant association between prenatal stress and the cytokine profile in 52 pregnant women. They used the Denver Maternal Health Assessment, which quantifies the psychological stress and social support to assess stress levels. On the basis of these stress levels, they divided the women in three stress groups (low, average, and high stress) (Coussons-Read et al., 2007). For the cytokine profile, they collected blood samples in each trimester (14^{th}-16^{th}, 22^{nd}-24^{th}, and 34^{th}-36^{th} weeks of gestation). It must be noted that they did not have any exclusion criteria, and so a higher BMI, risk pregnancies or GDM, all of which can influence IL-6 levels during pregnancy, were included. Similar effects were seen in a previous study from the same authors, who reported a significant correlation between IL-6 levels and prenatal stress in 24 pregnant women (Coussons-Read et al., 2005). Here, they used the same questionnaire as described above which was administered once in each trimester. Unlike our study, they calculated a ‘Global Stress Score’ to provide a measure for all trimesters and had a

homogeneous distribution of women in the three stress scores (low stress (N=16), average stress (N=18) and high stress (N=18)) (Coussons-Read et al., 2005). IL-6 levels (SEM) ranged from 1.7 (0.9) pg/ml in first trimester, over 1.83 (.07) pg/ml in second trimester to 3.03 (.35) pg/ml in third trimester. They found that higher stress levels across pregnancy were predictive of increased production of IL-6 in the 3rd trimester (Coussons-Read et al., 2005).

In the context of stress during pregnancy, depressive symptoms and anxiety are investigated in a number of studies. One recent study (Nazzari, Molteni, Valtorta, Comai, & Frigerio, 2020) investigated both symptoms with State Scale of the State-Trait-Anxiety Inventory and Edinburgh Postnatal Depression Scale in healthy pregnant women between 34th and 36th weeks of gestation (Nazzari et al., 2020). IL-6 range (SEM) was 1.68 (1.04) in all N=97 women. They reported that tryptophan activated by kynurenine, which increases inflammation and HPA axis, moderated the association between IL-6 and depressive symptoms during the perinatal and postpartum phase, but this does not apply to anxiety (Nazzari et al., 2020). Unlike our study, inclusion criteria also took into account any women with psychiatric disorders (depression and anxiety only) which can increase the general stress level.

The fact that stress has an impact on the metabolism and homeostatic system is usually shown in studies on psychosocial stress, traumatic stress or childhood abuse in healthy non-pregnant subjects (Hartwell et al., 2013; Steptoe et al., 2007). In the literature, the effects are particularly severe when it comes to high maternal stress. Adversities during childhood and adulthood are known to be associated with increased IL-6 levels during pregnancy (Finy & Christian, 2018). We were unable to measure this in study 3, since our participants had reported the most no stress to mild stress. However, another study (Haeri, Baker, & Ruano, 2013) showed that a diagnosed depression was related to elevated IL-6 and tumor necrosis factor-alpha between 11th and 14th weeks of gestation. It is important to note that the authors also included women who had been receiving anti-depressant pharmacotherapy, who smoked or engaged in illicit substance abuse etc., all of which could influence the maternal inflammatory milieu during pregnancy (Dantzer, 2009; Elisia et al., 2020). It is also known that the cytokine profile during pregnancy differs between women who reported severe anxiety symptoms during pregnancy or severe depressive symptoms with comorbid severe anxiety symptoms (Leff Gelman et

al., 2019). The IL-6 concentrations were, nonetheless, elevated in both types of symptoms.

With regard to maternal insulin sensitivity and stress levels, Valsamakis and colleagues (2017) reported a negative correlation between prenatal anxiety symptoms and maternal insulin sensitivity in 82 pregnant women. They investigated maternal anxiety symptoms using the State Trait Anxiety Inventory and state and trait questionnaire for stress assessment. They did not find any correlation between prenatal stress and IL-6 levels (Valsamakis et al., 2017). This could be due to the fact that they had similar IL-6 levels (between 1-4 pg/ml) to our cohort (between 0-5 pg/ml) during second and third trimester which also constitutes a normal range in pregnancy (GREIG et al., 1997).

To define the role of IL-6 in this interplay, we performed a mediation analysis and detected a mediating role for stress on the direct pathway between preBMI and IL-6. This suggests that increased preBMI and increased stress is associated with increased IL-6 levels during third trimester. This result is consistent with several other studies that have already reported a strong connection between BMI and IL-6 levels (Eder, Baffy, Falus, & Fulop, 2009; Patsalos, Dalton, & Himmerich, 2020). The fact that moderate maternal stress in combination with elevated preBMI does affect IL-6 levels in third trimester contributes to our knowledge in this current field of research.

4.7.2 Pre-pregnancy BMI is the driving factor for increases in Interleukin-6 and decreases in maternal insulin sensitivity

Our results confirmed that preBMI is one of the dominant factors with regard to the IL-6 modulation during pregnancy. This result raises the question as to whether women with an elevated BMI are more stressed before pregnancy and whether they therefore gain more weight.

The overall result of our study, namely that the preBMI has an impact on maternal insulin sensitivity and IL-6 levels, is well studied in the literature. Recent studies reported preBMI as a risk factor for cellular inflammation (Bjørke-Monsen et al., 2016) and systemic inflammation based on C-reactive protein (Witteveen et al., 2020). An earlier study by Morisset and colleagues (2008) reported increased omental adipocyte glycerol release with elevated plasma IL-6 in ten female patients. Pantham and colleagues (2015) described this inflammation in pre-pregnancy obese women as a meta-inflammation

state. It is a well-known fact that obese women show higher IL-6 levels throughout pregnancy and the postpartum period than women of normal weight (Christian & Porter, 2014; Denison et al., 2010; 2010).

Obese women with GDM might therefore have a higher inflammatory profile than obese women without GDM. Another study (Bastard et al., 2000) investigated the effects of adipose cytokines in the obesity-associated insulin resistance. The results of this study show that circulating IL-6 concentrations reflect adipose tissue production (Bastard et al., 2000). Furthermore, there is a hint that this reduction after weight loss might play a role in an improved insulin sensitivity (Bastard et al., 2000).

The question is whether women who are more stressed also gain more weight during pregnancy. However, we could not conclude this from study 3. On the basis of our analysis, we found no significant relationship between stress levels and MWG during pregnancy. In the literature, one systematic review also reported that e.g., negative maternal body image, or lack of knowledge with regard to gestational weight gain, were related to excess gestational weight gain, but not to depression, anxiety or stress (Kapadia et al., 2015). A study by Vehmeijer and colleagues (2020) is in line with our findings. They used the Brief Symptom Inventory (at 20th week of gestation) to measure psychological stress in 3393 pregnant women and enquire about weight gain during pregnancy. The Brief Symptom Inventory includes nine subscales about e.g., anxiety, depression, hostility etc (Vehmeijer et al., 2020). For analysis, the Global Severity Index was used as an indicator of overall psychological distress (Vehmeijer et al., 2020). In this population, 45% of the pregnant women experienced inadequate or excessive weight gain during pregnancy according to the Institute of Medicine guidelines ("Weight Gain During Pregnancy," 2009; Gilmore & Redman, 2015; Rasmussen, Catalano, & Yaktine, 2009). Similar to our results, they did not observe consistent associations of overall psychological distress, depression, and anxiety with gestational weight gain (Vehmeijer et al., 2020). Of note, they found that women with anxiety symptoms had a lower risk of excessive weight gain during pregnancy than those without.

An earlier publication by Webb and colleagues (Webb, Siega-Riz, & Dole, 2009) showed that increased stress and low socioeconomic status tend to be associated with inadequate MWG during pregnancy (Webb et al., 2009).

On the other hand, a study by Kominiarek and colleagues (2018) reported an association between lower stress levels and adequate gestational weight gain in 744 pregnant women in third trimester. It must be noted that, in this study the questionnaire used mainly asked about the following topics: events that had occurred since becoming pregnant such as e.g., marriage, death of partner or close family member/friend, change in sleeping or eating habits (Kominiarek et al., 2018). These topics differ somewhat from the questionnaire we used, and referred to more severe life changing events.

Significant positive correlation between cortisol levels and IL-6 concentration

In study 3, we investigated a positive correlation between maternal IL-6 levels and cortisol levels. Earlier studies showed that there is an association between cortisol and IL-6. Since IL-6 acts like an acute-phase protein together with glucocorticoids from the adrenal gland, this might explain why increased IL-6 values are mainly influenced by hormonal and neuronal pathways (Zhou, Kusnecov, Shurin, DePaoli, & Rabin, 1993).

We found no correlation between cortisol levels and maternal stress. The fact that they did not correlate in our cohort may simply be because the stress level in our cohort was too low.

This finding also fits with a recent study by Bleker and colleagues (Bleker et al., 2017), who investigated maternal serum cortisol levels in pregnancy at different gestational ages and at various time points during the day and their relation to biological factors, lifestyle factors and stress factors. They used the following questionnaires: State-Trait-Anxiety Inventory for anxiety symptoms, Pregnancy-related Anxiety Questionnaire-Revised for pregnancy-related anxiety and Center for Epidemiologic Studies Depression screening test for depressive symptoms. They reported that changes in maternal cortisol levels are mainly related to biological factors (Bleker et al., 2017). The mean cortisol level was lower, 337 nmol/l (124), than our mean levels in cortisol, 721.64 nmol/l (143.175). Normal values of cortisol were reported to be between 12-50 µg/dL (331.034-1379.310 nmol/l) in third trimester (Abbassi-Ghanavati, Greer, & Cunningham, 2009). Nevertheless, our data points in the same direction. Similar results were reported by Shelton and colleagues (Shelton et al., 2015), who determine a slight positive, but non-significant correlation between prenatal stress and cortisol values. They used the Perceived Stress Scale to measure stress levels in 105 women during second trimester.

With regard to the significant correlation between IL-6 and cortisol values which we found in our cohort, there is evidence that cortisol levels and pro-inflammatory and anti-inflammatory cytokines do, in fact, correlate. Assuming that pregnancy is an 'inflammatory' condition, the same study (Shelton et al., 2015) also reported a correlation between cytokines and cortisol levels in 631 pregnant women during the second trimester of pregnancy (16^{th} to 26^{th} weeks of gestation). It should be noted that the cytokines included pro-inflammatory cytokines (Interleukin-1ß and TNF-α and Interleukin-7) and anti-inflammatory cytokines (Interleukin-4, Interleukin-5, Interleukin-10, Interleukin-13) (Shelton et al., 2015) but not IL-6.

Generally speaking, this data indicates that cortisol is mainly related to biological changes during pregnancy and not to maternal low stress during pregnancy. Our data confirms these results and reports even higher cortisol levels during third trimester.

Significant negative correlation between Interleukin-6 concentration and maternal insulin sensitivity

We investigated a significant correlation between maternal insulin sensitivity and IL-6 levels. This is in line with the literature, where the same effect was seen for women with GDM.

It is a well-known fact that IL-6 and maternal insulin sensitivity interact. There is considerable evidence in the literature that elevated IL-6 levels may be a predictor of maternal insulin disbalance and that they may play an important role in the pathogenesis of GDM (Amirian et al., 2020). One study (Morisset et al., 2011) described the changes in IL-6 levels before and after GDM. In a longitudinal hospital-based study design, they measured IL-6 levels, maternal insulin sensitivity and BMI in 47 pregnant women at 26.1±3.7 weeks of pregnancy. Women were screened for GDM via glucose tolerance test, and insulin sensitivity/resistance was determined with Matsuda index and homeostatic model assessment insulin resistance (HOMA-IR) (Morisset et al., 2011). IL-6 levels were determined in fasting blood samples. On the basis of an OGTT, the women were then assigned to either control group or GDM group (Morisset et al., 2011). During pregnancy, they reported higher IL-6 concentrations (ranging between 0.0 and 2.0 pg/ml in women with GDM) than in the control women (Morisset et al., 2011). Like in our

cohort, the maternal insulin sensitivity index was an independent and significant predictor of IL-6 concentrations at the time of GDM diagnosis.

Thus, evidence exists that IL-6 does indeed play a role in the pathogenesis of GDM, particularly especially with regard to inflammatory processes during pregnancy. Kuzmicki and colleagues (2009) confirmed these results by adding information about the relationship between resistin, IL-6 levels, and insulin sensitivity. They included 81 pregnant women with GDM and 82 pregnant women without GDM (NGT) between 24th and 31st weeks of gestation (Kuzmicki et al., 2009). The control group consisted of 25 non-pregnant healthy women. Serum IL-6 levels ranged between 0.5 and 1.5 for all groups. Serum resistin and IL-6 were significantly higher in GDM than NGT and the non-pregnant control group. But no correlation between resistin and insulin sensitivity was reported (Kuzmicki et al., 2009). This is in line with our study, where differences between NGT and GDM in IL-6 levels were also found.

5 General discussion

In sum, the main findings of these studies were that moderate stress in pregnancy has no significant effect on metabolic, hormonal or neurophysiological parameters. However, we were able to show that relaxation during pregnancy in the last trimester had a significant effect on the general well-being of the pregnant woman (see Figure 18).

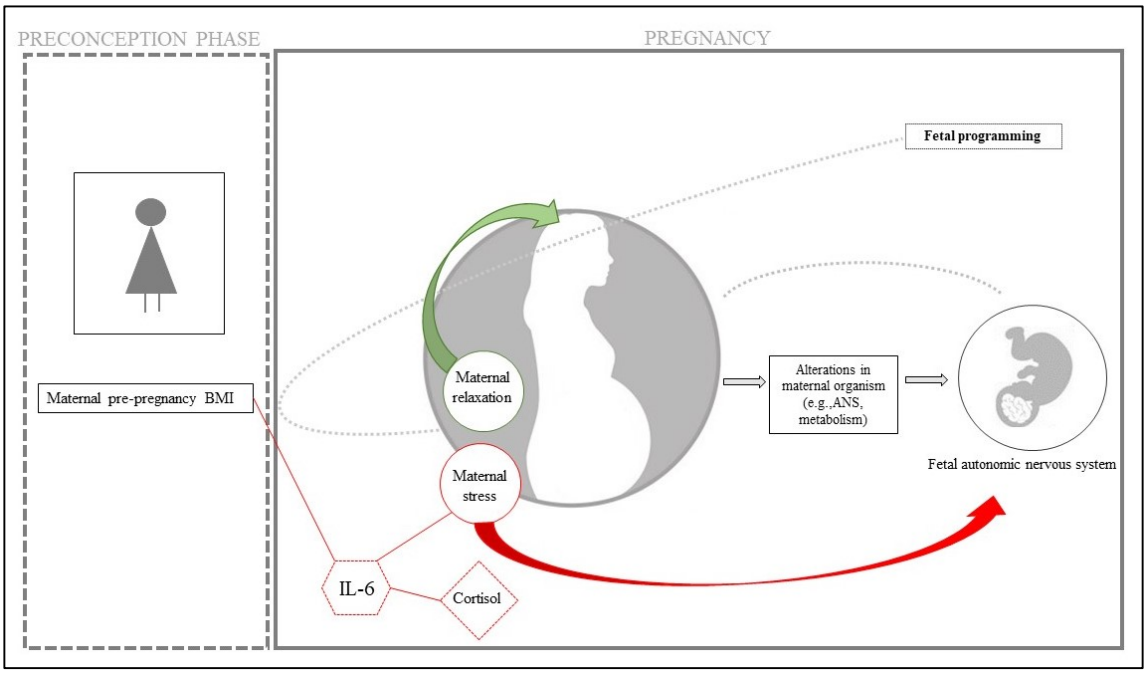


Figure 18. Overview of underlying theory with regard to reported results.

In particular, using neurophysiological measures of SCL and MHR, we ascertained that, regardless of the intervention, a relaxation effect was seen after listening to music, following a guided imagery, or resting for 20 minutes in the mother, but not to a significant extent. In addition, we saw significant acute maternal relaxation based on the subjective parameters. This relaxation effect may be related to GA of the pregnant women. However, we found no significant differences between active (guided imagery group) and passive (music group, resting group) mental-based interventions.

On the one hand, no physiological relaxation effect on the basis of SCLs or HRV of the pregnant women was found to any significant extent in study 1 or 2. On the other hand, subjective relaxation on the basis of the questionnaires was found in both studies.

Moreover, no effect on FHR during relaxation was visible, even though the changes between the groups in FHR differed significantly on the basis of study 2.

Additionally, there was no evidence to suggest that mild chronic stress affects the fetal ANS. However, we found a significant effect in changes of the LF/HF ratio in Music group only.

When metabolic factors were included, we could not detect any influence of moderate stress on IL-6 levels or maternal insulin sensitivity. Thus, no significant effect of stress on IL-6 was found, which was our main hypothesis in study 3.

On the basis of a mediation analysis, we concluded that increased stress in combination with increased preBMI could affect IL-6 levels during pregnancy. Secondary findings were that IL-6 levels in pregnancy were significantly associated with preBMI and that IL-6 levels were significantly correlated with cortisol levels in pregnant women. In this cohort, stress and cortisol showed no significant correlation.

5.1 Potential mechanisms, implications and neuroscientific outlook

Maternal stress and implications for neonatal/child development in later life

The maternal stress level and its influence on fetal development are mainly reflected by disturbances in the fetal nervous system. Many studies have therefore investigated the mechanism with regard to FHR and HRV as well as fetal brain development (Hoyer et al., 2014; Kim et al., 2018; Miranda & Sousa, 2018). The current findings show that maternal moderate stress had no significant effect on the maternal inflammatory environment. Elevated IL-6 levels during pregnancy can have far-reaching implications: Elevated IL-6 levels in pregnancy are strongly related to the fetal nervous system development and cognitive development in later life (Chiesa et al., 2015; Prins et al., 2012). In particular, extremely elevated IL-6 levels during pregnancy might indicate a potential derailment in the cytokine profile throughout pregnancy and postpartum. It influences the placental and fetal inflammatory processes that can lead to changes in the synaptic function (Buss, Entringer, Swanson, & Wadhwa, 2012).

There is evidence that IL-6 levels during pregnancy predict future working memory at two years of age (Graham et al., 2018; Rudolph et al., 2018). This links the maternal inflammation environment with cognitive development in later life and provides

evidence that changes in the maternal physiological environment during pregnancy are directly linked to changes in executive functions and therefore in goal-directed behavior. This functional architecture of brain and its network was also shown to be influenced even in the newborn amygdala (Graham et al., 2018). Regulation of emotion and subsequent behavior later in life is largely determined by the functional network of the amygdala (Graham et al., 2018). Disorders of fetal and neonatal development caused by elevated IL-6 levels are associated with greater right amygdala volume and bilateral amygdala connectivity (Graham et al., 2018). This primarily affected those brain regions involved "[…] in sensory processing and integration […], salience detection […], and learning and memory […]." (Graham et al., 2018, p. 5). These neurophysiological changes are closely linked to neuropsychiatric disorders.

Thus, severe but not mild, maternal stress is not only a negative factor for the maternal organism, but also involves a long-chain linkage of the different parameters that lead to an influence on a neurologically unremarkable development of the child and later adolescence and adulthood.

The HPA Axis – the missing link between maternal well-being and the offsprings' development

Our results showed quite clearly that maternal relaxation has an impact on maternal well-being in particular. Maternal well-being in pregnancy and resilience is partially reversely linked to stress perception in pregnancy. This inter-relationship is mainly reflected in the effects on the course of pregnancy in general and the birth experience as well as the experience of the postnatal period and increased/decreased risk for e.g., postpartum depression.

The connection between the above mentioned outcomes and maternal well-being thus lies in the reactive system itself. The main axis who reacts to maternal distress is the HPA axis which is primarily modulated through CRH (Godoy et al., 2018). Accordingly, very preterm newborns tend to have higher activation of the HPA axis (Finken et al., 2017). The secretion of CRH is subject to a circadian rhythm and relies on negative feedback from glucocorticoids (Finken et al., 2017). There is evidence that cortisol is associated with neonatal stress reactivity (Nazzari et al., 2019). This is based on the finding that the HPA axis is associated with hypercortisolemia in response to prenatal stress and reduces

inhibitory feedback (Godoy et al., 2018). Molecular pathways and mechanisms in the HPA axis underline the early life stress programming mechanism.

Earlier data suggests that inhibition of placental 11ß-hydroxysteroid dehydrogenase type 2 leads to a lower offspring birth weight and adversely affects the intracellular glucocorticoid receptor (Cottrell & Seckl, 2009). Changes in the glucocorticoid receptor are associated with exaggerated HPA responses to stress (Laryea, Schütz, & Muglia, 2013). Consequently, glucocorticoids act like key mediators of stress responses (Godoy et al., 2018; Laryea et al., 2013). This implication can be seen particularly clearly in the administration of the glucocorticoid betamethasone when there is an increased risk of premature birth in human pregnancies. Antenatal corticosteroid administration is a standard of care for women at risk of premature delivery. Neonates who were exposed to betamethasone during pregnancy tend to react with decreased cortisol values in response to a heel-stick blood draw (Davis et al., 2004). Furthermore, betamethasone as a glucocorticoid is also known to affect higher cortical functions in the fetal brain (Schneider et al., 2011), but this needs to be investigated in further studies. In sum, the role of glucocorticoids is to act as key mediators between prenatal stress and the hormonal response to stress. They primarily illuminate the mechanisms that underlie the stress response.

Maternal obesity and insulin sensitivity – mechanisms and consequences for fetal development

We showed a significant effect of preBMI on IL-6 concentration during pregnancy. Obesity as a risk factor for fetal development, as well as for the child's risk of becoming overweight later in life, is an important factor, and one that is already considered in the care of women who wish to become pregnant. Maternal obesity, which alters the inflammatory milieu, affects fetal development and maternal metabolism (Catalano, Presley, Minium, & Hauguel-de Mouzon, 2009; Catalano & Shankar, 2017; Challier et al., 2008). Maternal obesity and overweight are associated with increased FHR and decreased FHRV (Mat Husin et al., 2020) and may influence placental brain-derived-neurotrophic-factor during pregnancy (Prince, Maloyan, & Myatt, 2017).

The influence of metabolic stress, such as obesity, may increase the risk for systemic inflammation during pregnancy, which then influences fetal development via hormonal

and metabolic pathways and mechanisms (van der Burg et al., 2016). The combination of maternal stress and increased preBMI generates a dysregulation in the overall metabolism (van der Burg et al., 2016). Hence, an adverse maternal insulin sensitivity is known to be present mostly in overweight and obese women affecting the fetal development (Fehlert et al., 2017; Linder et al., 2015) and increasing the risk for diabetes type 2 and obesity in later life (Catalano et al., 2009; Godfrey et al., 2017). A derailment of maternal insulin sensitivity caused by obesity has an impact on the nutrient flux to the fetus which leads to fetal hyperinsulinemia (Shoelson, Herrero, & Naaz, 2007). In hyperglycaemia-hyperinsulinaemia, a reduced docosahexaenoic acid status, which is transported via placenta to the fetus during pregnancy occurs (Mishra, Zhao, Hattis, & Kumar, 2020).

5.2 Strengths, limitations, and suggestions for further research

One major strength of this work is the combination of different psychological and physiological methods within the studies. For physiological measurements, we measured fetal and maternal HRV and HR and SCLs. For psychological measurements, we used a wide range of validated questionnaire. Furthermore, hormonal and metabolic factors were investigated by way of hair samples and blood samples. This spectrum of methods is particularly noteworthy because it enables us to include as many factors as possible to comprehend the complex construct of stress and relaxation. It also allows us to contemplate the vast amount of psychological, neurophysiological and neuroendocrine factors that create a much more comprehensive picture of maternal stress and maternal relaxation during pregnancy.

Second, it should be emphasized that the focus of this work was on relaxation *and* stress during pregnancy. This allows the findings of the studies to be discussed in the light of both aspects. Since stress and relaxation are somehow interrelated, it is an absolute strength of our work that we had the opportunity to study both factors in a similar population, as it significantly facilitates the interpretation of the results through comparability.

Thirdly, the method of fMEG should be emphasized as it allows a unique non-invasive insight into the development of the fetal autonomic and central nervous system. The measurement of fetal ANS enables us to measure fetal HRV and HR as demonstrated in

previous studies (Mat Husin et al., 2020; Muenssinger et al., 2013; Preissl et al., 2004). Additionally, the construction of the fMEG has facilitate the use of stimulation like relaxation music via headphones and to measure maternal skin conductance levels during the measurement. Thus, it was possible to detect fetal and maternal HRV and HR in combination with different maternal physiological parameters simultaneously without disturbing the mother or the fetus.

Nevertheless, there are also some limiting factors of the results presented here. First of all, one has to mention the heterogenity in the overall sample size. A low sample size was particularly the case achieved in study 2. Secondly, the period during which the studies were carried out and the parameters measured cover only the third trimester of pregnancy. In further studies, it would be interesting to determine the extent to which the perception of stress and the ability to relax change over the entire course of pregnancy and how this affects metabolic and hormonal parameters. This would be important, since the hormonal milieu is changing during pregnancy. Thirdly, it must be mentioned that we did not detect high stress in the population. Additionally, our questionnaires often referred to mood, pregnancy-related worries, and chronic stress as well as to the general health of the women. In further studies, it would be especially helpful to query factors such as social support, adverse life events, or even strategies for coping with stress. These can have a major influence on stress perception as well as on chronic stress during pregnancy by defining individuals' resilience. Last, our population consisted mainly of women with a high socioeconomic status. Further studies should primarily seek to examine a more diverse population.

6 Conclusion

In summary, our results showed that there are neuroendocrinological and neurophysiological adaptations to maternal stress during late pregnancy. Our results confirmed the hypothesis that both maternal stress and also relaxation may have an effect on maternal well-being. Moreover, maternal stress together with high preBMI could be one of the main characteristics that unfavorably influence the maternal inflammatory environment. These findings, especially in view of the increasing incidence of obesity in the population, have important clinical consequences for both mother and fetus. The interplay of insulin sensitivity and IL-6 levels in mothers without GDM may reveal the clinical relevance even for pregnant women with normal glucose tolerance. This is particularly important since an unfavorable inflammatory milieu is known to impair the cognitive development of the child. Thus, all of these factors may play a critical role in the developmental programming of the fetal ANS.

The fact that fetal ANS measured by FHR and FHRV in our cohort did not directly depend on maternal stress may be due to the mild stress levels. However, the positive effect of relaxation on the maternal system is particularly noteworthy. Relaxation interventions in pregnancy provide a simple and non-pharmacological way to reduce stress levels and increase maternal well-being. Thus, it would be useful to develop targeted treatment and mental support for women even *before* pregnancy in combination with stress management interventions also *during* pregnancy which were seen to have a positive influence on maternal well-being and might indicate positive effects for fetal development.

References

A M. Yali and M. Lobel (1999). Coping and distress in pregnancy: An investigation of medically high risk women. *J Psychosom Obstet Gynecol*. (20), 39–52.

Abbassi-Ghanavati, M., Greer, L. G., & Cunningham, F. G. (2009). Pregnancy and laboratory studies: A reference table for clinicians. *Obstetrics and Gynecology*, *114*(6), 1326–1331. https://doi.org/10.1097/AOG.0b013e3181c2bde8

Adams, L., Winterbotham, M., Oldfield, K., McLeish, J., Large, A., & Stuart, A. (2016). Pregnancy and maternity-related discrimination and disadvantage: experiences of mothers. Retrieved from www.gov.uk/bis

Afshari, J. T., Ghomian, N., Shameli, A., Shakeri, M. T., Fahmidehkar, M. A., Mahajer, E., . . . Emadzadeh, M. (2005). Determination of Interleukin-6 and Tumor Necrosis Factor-alpha concentrations in Iranian-Khorasanian patients with preeclampsia. *BMC Pregnancy and Childbirth*, *5*(5:14), 14. https://doi.org/10.1186/1471-2393-5-14

Albani, C., Blaser, G., Geyer, M., Schmutzer, G., Brähler, E., Bailer, H., & Grulke, N. (2005). Überprüfung der Gütekriterien der deutschen Kurzform des Fragebogens "Profile of Mood States" (POMS) in einer repräsentativen Bevölkerungsstichprobe [The German short version of "Profile of Mood States" (POMS): psychometric evaluation in a representative sample]. *Psychotherapie, Psychosomatik, medizinische Psychologie*, *55*(7), 324–330. https://doi.org/10.1055/s-2004-834727

Alderdice, F., Savage-McGlynn, E., Martin, C., McAuliffe, F., Hunter, A., Unterscheider, J., . . . Malone, F. (2013). The Prenatal Distress Questionnaire: An investigation of factor structure in a high risk population. *Journal of Reproductive and Infant Psychology*, *31*(5), 456–464. https://doi.org/10.1080/02646838.2013.830210

Allister, L., Lester, B. M., Carr, S., & Liu, J. (2001). The effects of maternal depression on fetal heart rate response to vibroacoustic stimulation. *Developmental Neuropsychology*, *20*(3), 639–651. https://doi.org/10.1207/S15326942DN2003_6

Amirian, A., Mahani, M. B., & Abdi, F. (2020). Role of interleukin-6 (IL-6) in predicting gestational diabetes mellitus. *Obstetrics & Gynecology Science*, *63*(4), 407–416. https://doi.org/10.5468/ogs.20020

Barker, D. J. (1990). The fetal and infant origins of adult disease. *BMJ (Clinical Research Ed.)*, *301*(6761), 1111. https://doi.org/10.1136/bmj.301.6761.1111

Barker, D. J. (1995). Fetal origins of coronary heart disease. *BMJ (Clinical Research Ed.)*, *311*(6998), 171–174. https://doi.org/10.1136/bmj.311.6998.171

Barker, D., Osmond, C., Winter, P., Margetts, B., & Simmonds, S. (1989). WEIGHT IN INFANCY AND DEATH FROM ISCHAEMIC HEART DISEASE. *The Lancet*, *334*(8663), 577–580. https://doi.org/10.1016/s0140-6736(89)90710-1

Bastard, J. P., Jardel, C., Bruckert, E., Blondy, P., Capeau, J., Laville, M., . . . Hainque, B. (2000). Elevated levels of interleukin 6 are reduced in serum and subcutaneous adipose tissue of obese women after weight loss. *The Journal of Clinical Endocrinology and Metabolism*, *85*(9), 3338–3342. https://doi.org/10.1210/jcem.85.9.6839

Bauer, I., Hartkopf, J., Kullmann, S., Schleger, F., Hallschmid, M., Pauluschke-Fröhlich, J., . . . Preissl, H. (2020). Spotlight on the fetus: How physical activity during pregnancy influences fetal health: a narrative review. *BMJ Open Sport & Exercise Medicine*, *6*(1), e000658. https://doi.org/10.1136/bmjsem-2019-000658

Beddoe, A. E., & Lee, K. A. (2008). Mind-body interventions during pregnancy. *Journal of Obstetric, Gynecologic, and Neonatal Nursing : JOGNN*, *37*(2), 165–175. https://doi.org/10.1111/j.1552-6909.2008.00218.x

Bernd Löwe, Robert L. Spitzer, Stephan Zipfel, Wolfgang Herzog (Ed.) (2002). PHQ-D Gesundheitsfragebogen für Patienten: Manual, Komplettversion und Kurzform; Autorisierte deutsche Version des "Prime MD Patient Health Questionnaire (PHQ)" [Special issue].

Bjørke-Monsen, A.-L., Ulvik, A., Nilsen, R. M., Midttun, Ø., Roth, C., Magnus, P., . . . Ueland, P. M. (2016). Impact of Pre-Pregnancy BMI on B Vitamin and Inflammatory Status in Early Pregnancy: An Observational Cohort Study. *Nutrients*, *8*(12). https://doi.org/10.3390/nu8120776

Black, P. H. (2003). The inflammatory response is an integral part of the stress response: Implications for atherosclerosis, insulin resistance, type II diabetes and metabolic syndrome X. *Brain, Behavior, and Immunity*, *17*(5), 350–364. https://doi.org/10.1016/S0889-1591(03)00048-5

Bleker, L. S., Roseboom, T. J., Vrijkotte, T. G., Reynolds, R. M., & Rooij, S. R. de (2017). Determinants of cortisol during pregnancy - The ABCD cohort. *Psychoneuroendocrinology*, *83*, 172–181. https://doi.org/10.1016/j.psyneuen.2017.05.026

Böckelmann, I. (Ed.) (2012). *Analyse der Herzfrequenzvariabilität (HRV) — praktische Relevanz* (Vol. 62).

Braig, S., Logan, C. A., Reister, F., Rothenbacher, D., & Genuneit, J. (2020). Psychosocial stress and longitudinally measured gestational weight gain throughout pregnancy: The Ulm SPATZ Health Study. *Scientific Reports*, *10*(1), 1996. https://doi.org/10.1038/s41598-020-58808-8

Breuer, M. M. (2020). *Zusammenhang von Gestationsdiabetes, Insulinsensitivität und Depressivität auf Basis des PHQ-D in der Tübinger PREG Kohorte* (Inaugural-Dissertation). Eberhard Karls Universität Tübingen, Tübingen.

Burton, G. J., & Jauniaux, E. (2015). What is the placenta? *American Journal of Obstetrics and Gynecology*, *213*(4 Suppl), S6.e1, S6-8. https://doi.org/10.1016/j.ajog.2015.07.050

Buss, C., Davis, E. P., Shahbaba, B., Pruessner, J. C., Head, K., & Sandman, C. A. (2012). Maternal cortisol over the course of pregnancy and subsequent child amygdala and hippocampus volumes and affective problems. *Proceedings of the National Academy of Sciences of the United States of America*, *109*(20), E1312-9. https://doi.org/10.1073/pnas.1201295109

Buss, C., Entringer, S., Swanson, J. M., & Wadhwa, P. D. (2012). The Role of Stress in Brain Development: The Gestational Environment's Long-Term Effects on the Brain. *Cerebrum : the Dana Forum on Brain Science*, *2012*, 4.

Carolan, M., Barry, M., Gamble, M., Turner, K., & Mascareñas, O. (2012). The Limerick Lullaby project: An intervention to relieve prenatal stress. *Midwifery*, *28*(2), 173–180. https://doi.org/10.1016/j.midw.2010.12.006

Catalano, P. M., Presley, L., Minium, J., & Hauguel-de Mouzon, S. (2009). Fetuses of obese mothers develop insulin resistance in utero. *Diabetes Care*, *32*(6), 1076–1080. https://doi.org/10.2337/dc08-2077

Catalano, P. M., & Shankar, K. (2017). Obesity and pregnancy: Mechanisms of short term and long term adverse consequences for mother and child. *BMJ (Clinical Research Ed.)*, *356*, j1. https://doi.org/10.1136/bmj.j1

Challier, J. C., Basu, S., Bintein, T., Minium, J., Hotmire, K., Catalano, P. M., & Hauguel-de Mouzon, S. (2008). Obesity in pregnancy stimulates macrophage accumulation and inflammation in the placenta. *Placenta*, *29*(3), 274–281. https://doi.org/10.1016/j.placenta.2007.12.010

Challis, J., Sloboda, D., Matthews, S. G., Holloway, A., Alfaidy, N., Patel, F. A., . . . Newnham, J. (2001). The fetal placental hypothalamic–pituitary–adrenal (HPA) axis, parturition and post natal health. *Molecular and Cellular Endocrinology*, *185*(1-2), 135–144. https://doi.org/10.1016/s0303-7207(01)00624-4

Chang, M.-Y., Chen, C.-H., & Huang, K.-F. (2008). Effects of music therapy on psychological health of women during pregnancy. *Journal of Clinical Nursing*, *17*(19), 2580–2587. https://doi.org/10.1111/j.1365-2702.2007.02064.x

Chen, P.-Y., Ganguly, A., Rubbi, L., Orozco, L. D., Morselli, M., Ashraf, D., . . . Pellegrini, M. (2013). Intrauterine calorie restriction affects placental DNA methylation and gene expression. *Physiological Genomics*, *45*(14), 565–576. https://doi.org/10.1152/physiolgenomics.00034.2013

Chiesa, C., Pacifico, L., Natale, F., Hofer, N., Osborn, J. F., & Resch, B. (2015). Fetal and early neonatal interleukin-6 response. *Cytokine*, *76*(1), 1–12. https://doi.org/10.1016/j.cyto.2015.03.015

Christian, L. M., & Porter, K. (2014). Longitudinal changes in serum proinflammatory markers across pregnancy and postpartum: Effects of maternal body mass index. *Cytokine*, *70*(2), 134–140. https://doi.org/10.1016/j.cyto.2014.06.018

Cohen, S., Gianaros, P. J., & Manuck, S. B. (2016). A Stage Model of Stress and Disease. *Perspectives on Psychological Science : a Journal of the Association for Psychological Science*, *11*(4), 456–463. https://doi.org/10.1177/1745691616646305

Cottrell, E. C., & Seckl, J. R. (2009). Prenatal stress, glucocorticoids and the programming of adult disease. *Frontiers in Behavioral Neuroscience*, *3*, 19. https://doi.org/10.3389/neuro.08.019.2009

Coussons-Read, M. E., Okun, M. L., & Nettles, C. D. (2007). Psychosocial stress increases inflammatory markers and alters cytokine production across pregnancy. *Brain Behav Immun*, *21*(3), 343–350. https://doi.org/10.1016/j.bbi.2006.08.006

Coussons-Read, M. E., Okun, M. L., Schmitt, M. P., & Giese, S. (2005). Prenatal stress alters cytokine levels in a manner that may endanger human pregnancy. *Psychosomatic Medicine*, *67*(4), 625–631. https://doi.org/10.1097/01.psy.0000170331.74960.ad

Crist, D. A., Rickard, H. C., Prentice-Dunn, S., & Barker, H. R. (1989). The Relaxation Inventory: Self-report scales of relaxation training effects. *Journal of Personality Assessment*, *53*(4), 716–726. https://doi.org/10.1207/s15327752jpa5304_8

Dantzer, R. (2009). Cytokine, sickness behavior, and depression. *Immunology and Allergy Clinics of North America*, *29*(2), 247–264. https://doi.org/10.1016/j.iac.2009.02.002

David, M., Hirsch, M., Karin, J., Toledo, E., & Akselrod, S. (2007). An estimate of fetal autonomic state by time-frequency analysis of fetal heart rate variability. *Journal of Applied Physiology (Bethesda, Md. : 1985)*, *102*(3), 1057–1064. https://doi.org/10.1152/japplphysiol.00114.2006

Davis, E. P., & Sandman, C. A. (2010). The timing of prenatal exposure to maternal cortisol and psychosocial stress is associated with human infant cognitive development. *Child Development*, *81*(1), 131–148. https://doi.org/10.1111/j.1467-8624.2009.01385.x.

Davis, E. P., Townsend, E. L., Gunnar, M. R., Georgieff, M. K., Guiang, S. F., Ciffuentes, R. F., & Lussky, R. C. (2004). Effects of prenatal betamethasone exposure on regulation of stress physiology in healthy premature infants. *Psychoneuroendocrinology*, *29*(8), 1028–1036. https://doi.org/10.1016/j.psyneuen.2003.10.005

Denison, F. C., Roberts, K. A., Barr, S. M., & Norman, J. E. (2010). Obesity, pregnancy, inflammation, and vascular function. *Reproduction (Cambridge, England)*, *140*(3), 373–385. https://doi.org/10.1530/REP-10-0074

Dieter, J. N. I., Emory, E. K., Johnson, K. C., & Raynor, B. D. (2008). Maternal depression and anxiety effects on the human fetus: Preliminary findings and clinical

implications. *Infant Mental Health Journal*, *29*(5), 420–441. https://doi.org/10.1002/imhj.20192

DiPietro, J. A. (2012). Maternal stress in pregnancy: Considerations for fetal development. *The Journal of Adolescent Health : Official Publication of the Society for Adolescent Medicine*, *51*(2 Suppl), S3-8. https://doi.org/10.1016/j.jadohealth.2012.04.008

DiPietro, J. A., Costigan, K. A., & Gurewitsch, E. D. (2003). Fetal response to induced maternal stress. *Early Human Development*, *74*(2), 125–138. https://doi.org/10.1016/j.earlhumdev.2003.07.001

DiPietro, J. A., Costigan, K. A., Nelson, P., Gurewitsch, E. D., & Laudenslager, M. L. (2008). Fetal responses to induced maternal relaxation during pregnancy. *Biological Psychology*, *77*(1), 11–19. https://doi.org/10.1016/j.biopsycho.2007.08.008

DiPietro, J. A., Kivlighan, K. T., Costigan, K. A., & Laudenslager, M. L. (2009). Fetal motor activity and maternal cortisol. *Developmental Psychobiology*, *51*(6), 505–512. https://doi.org/10.1002/dev.20389

Dunkel Schetter, C., & Tanner, L. (2012). Anxiety, depression and stress in pregnancy: Implications for mothers, children, research, and practice. *Curr Opin Psychiatry*, *25*(2), 141–148. https://doi.org/10.1097/YCO.0b013e3283503680

Eder, K., Baffy, N., Falus, A., & Fulop, A. K. (2009). The major inflammatory mediator interleukin-6 and obesity. *Inflammation Research : Official Journal of the European Histamine Research Society … [et Al.]*, *58*(11), 727–736. https://doi.org/10.1007/s00011-009-0060-4

Elisia, I., Lam, V., Cho, B., Hay, M., Li, M. Y., Yeung, M., . . . Krystal, G. (2020). The effect of smoking on chronic inflammation, immune function and blood cell composition. *Scientific Reports*, *10*(1), 19480. https://doi.org/10.1038/s41598-020-76556-7

Entringer, S. (2013). Impact of stress and stress physiology during pregnancy on child metabolic function and obesity risk. *Current Opinion in Clinical Nutrition and Metabolic Care*, *16*(3), 320–327. https://doi.org/10.1097/MCO.0b013e32835e8d80

Entringer, S., Buss, C., Shirtcliff, E. A., Cammack, A. L., Yim, I. S., Chicz-DeMet, A., . . . Wadhwa, P. D. (2010). Attenuation of maternal psychophysiological stress responses and the maternal cortisol awakening response over the course of human

pregnancy. *Stress (Amsterdam, Netherlands)*, *13*(3), 258–268. https://doi.org/10.3109/10253890903349501

Fehlert, E., Willmann, K., Fritsche, L., Linder, K., Mat-Husin, H., Schleger, F., . . . Fritsche, A. (2017). Gestational diabetes alters the fetal heart rate variability during an oral glucose tolerance test: A fetal magnetocardiography study. *BJOG*, *124*(12), 1891–1898. https://doi.org/10.1111/1471-0528.14474

Fink, N. S., Urech, C., Cavelti, M., & Alder, J. (2012). Relaxation during pregnancy: What are the benefits for mother, fetus, and the newborn? A systematic review of the literature. *The Journal of Perinatal & Neonatal Nursing*, *26*(4), 296–306. https://doi.org/10.1097/JPN.0b013e31823f565b

Fink, N. S., Urech, C., Isabel, F., Meyer, A., Hoesli, I., Bitzer, J., & Alder, J. (2011). Fetal response to abbreviated relaxation techniques. A randomized controlled study. *Early Human Development*, *87*(2), 121–127. https://doi.org/10.1016/j.earlhumdev.2010.11.011

Finken, M. J. J., van der Voorn, B., Hollanders, J. J., Ruys, C. A., Waard, M. de, van Goudoever, J. B., & Rotteveel, J. (2017). Programming of the Hypothalamus-Pituitary-Adrenal Axis by Very Preterm Birth. *Annals of Nutrition & Metabolism*, *70*(3), 170–174. https://doi.org/10.1159/000456040

Finy, M. S., & Christian, L. M. (2018). Pathways linking childhood abuse history and current socioeconomic status to inflammation during pregnancy. *Brain Behav Immun*, *74*, 231–240. https://doi.org/10.1016/j.bbi.2018.09.012

Fritsche, L., Hummel, J., Wagner, R., Loeffler, D., Hartkopf, J., Machann, J., . . . Heni, M. (2021). The German Gestational Diabetes Study (PREG): Rationale, Methodology and Design. Advance online publication. https://doi.org/10.1101/2021.10.10.21264499

García González, J., Ventura Miranda, M. I., Manchon García, F., Pallarés Ruiz, T. I., Marin Gascón, M. L., Requena Mullor, M., . . . Parron Carreño, T. (2017). Effects of prenatal music stimulation on fetal cardiac state, newborn anthropometric measurements and vital signs of pregnant women: A randomized controlled trial. *Complementary Therapies in Clinical Practice*, *27*, 61–67. https://doi.org/10.1016/j.ctcp.2017.03.004

Gebuza, G., Zaleska, M., Kaźmierczak, M., Mieczkowska, E., & Gierszewska, M. (2018). The effect of music on the cardiac activity of a fetus in a cardiotocographic examination. *Advances in Clinical and Experimental Medicine : Official Organ Wroclaw Medical University*, *27*(5), 615–621. https://doi.org/10.17219/acem/68693

Gilmore, L. A., & Redman, L. M. (2015). Weight gain in pregnancy and application of the 2009 IOM guidelines: Toward a uniform approach. *Obesity (Silver Spring, Md.)*, *23*(3), 507–511. https://doi.org/10.1002/oby.20951

Godfrey, K. M., Reynolds, R. M., Prescott, S. L., Nyirenda, M., Jaddoe, V. W. V., Eriksson, J. G., & Broekman, B. F. P. (2017). Influence of maternal obesity on the long-term health of offspring. *The Lancet Diabetes & Endocrinology*, *5*(1), 53–64. https://doi.org/10.1016/S2213-8587(16)30107-3

Godoy, L. D., Rossignoli, M. T., Delfino-Pereira, P., Garcia-Cairasco, N., & Lima Umeoka, E. H. de (2018). A Comprehensive Overview on Stress Neurobiology: Basic Concepts and Clinical Implications. *Frontiers in Behavioral Neuroscience*, *12*, 127. https://doi.org/10.3389/fnbeh.2018.00127

Gräfe, K., Zipfel, S., Herzog, W., & Löwe, B. (2004). Screening psychischer Störungen mit dem "Gesundheitsfragebogen für Patienten (PHQ-D)". *Diagnostica*, *50*(4), 171–181. https://doi.org/10.1026/0012-1924.50.4.171

Graham, A. M., Rasmussen, J. M., Rudolph, M. D., Heim, C. M., Gilmore, J. H., Styner, M., . . . Buss, C. (2018). Maternal Systemic Interleukin-6 During Pregnancy Is Associated With Newborn Amygdala Phenotypes and Subsequent Behavior at 2 Years of Age. *Biological Psychiatry*, *83*(2), 109–119. https://doi.org/10.1016/j.biopsych.2017.05.027

GREIG, P., MURTHA, A., JIMMERSON, C., HERBERT, W., ROITMANJOHNSON, B., & ALLEN, J. (1997). Maternal Serum Interleukin-6 During Pregnancy and During Term and Preterm Labor. *Obstetrics & Gynecology*, *90*(3), 465–469. https://doi.org/10.1016/S0029-7844(97)00294-9

Grigoriadis, S., Graves, L., Peer, M., Mamisashvili, L., Tomlinson, G., Vigod, S. N., . . . Richter, M. (2018). Maternal Anxiety During Pregnancy and the Association With Adverse Perinatal Outcomes: Systematic Review and Meta-Analysis. *The Journal of Clinical Psychiatry*, *79*(5). https://doi.org/10.4088/JCP.17r12011

Guariguata, L., Linnenkamp, U., Beagley, J., Whiting, D. R., & Cho, N. H. (2014). Global estimates of the prevalence of hyperglycaemia in pregnancy. *Diabetes Research and Clinical Practice*, *103*(2), 176–185. https://doi.org/10.1016/j.diabres.2013.11.003

Haeri, S., Baker, A. M., & Ruano, R. (2013). Do pregnant women with depression have a pro-inflammatory profile? *The Journal of Obstetrics and Gynaecology Research*, *39*(5), 948–952. https://doi.org/10.1111/jog.12017

Hanson, M. A., & Gluckman, P. D. (2014). Early developmental conditioning of later health and disease: Physiology or pathophysiology? *Physiological Reviews*, *94*(4), 1027–1076. https://doi.org/10.1152/physrev.00029.2013

Hantsoo, L., Kornfield, S., Anguera, M. C., & Epperson, C. N. (2019). Inflammation: A Proposed Intermediary Between Maternal Stress and Offspring Neuropsychiatric Risk. *Biological Psychiatry*, *85*(2), 97–106. https://doi.org/10.1016/j.biopsych.2018.08.018

Hartwell, K. J., Moran-Santa Maria, M. M., Twal, W. O., Shaftman, S., DeSantis, S. M., McRae-Clark, A. L., & Brady, K. T. (2013). Association of elevated cytokines with childhood adversity in a sample of healthy adults. *Journal of Psychiatric Research*, *47*(5), 604–610. https://doi.org/10.1016/j.jpsychires.2013.01.008

Hetherington, E., McDonald, S., Williamson, T., & Tough, S. (2020). Trajectories of social support in pregnancy and early postpartum: Findings from the All Our Families cohort. *Social Psychiatry and Psychiatric Epidemiology*, *55*(2), 259–267. https://doi.org/10.1007/s00127-019-01740-8

Hoyer, D., Kowalski, E.-M., Schmidt, A., Tetschke, F., Nowack, S., Rudolph, A., . . . Schneider, U. (2014). Fetal autonomic brain age scores, segmented heart rate variability analysis, and traditional short term variability. *Frontiers in Human Neuroscience*, *8*(8/948), 948. https://doi.org/10.3389/fnhum.2014.00948

Illsley, N. P., & Baumann, M. U. (2020). Human placental glucose transport in fetoplacental growth and metabolism. *Biochimica Et Biophysica Acta. Molecular Basis of Disease*, *1866*(2), 165359. https://doi.org/10.1016/j.bbadis.2018.12.010

Janke, J. (1999). The effect of relaxation therapy on preterm labor outcomes. *Journal of Obstetric, Gynecologic, and Neonatal Nursing : JOGNN*, *28*(3), 255–263. https://doi.org/10.1111/j.1552-6909.1999.tb01990.x

JJ Braithwaite, DG Watson, R Jones, M Rowe (2013). *A Guide for Analysing Electrodermal Activity (EDA) & Skin Conductance Responses (SCRs) for Psychological Experiments: {via the BIOPAC MP36R & AcqKnowledge software}*. Birmingham.

Jones, S. A. (2005). Directing transition from innate to acquired immunity: Defining a role for IL-6. *Journal of Immunology (Baltimore, Md. : 1950)*, *175*(6), 3463–3468. https://doi.org/10.4049/jimmunol.175.6.3463

Kapadia, M. Z., Gaston, A., van Blyderveen, S., Schmidt, L., Beyene, J., McDonald, H., & McDonald, S. D. (2015). Psychological antecedents of excess gestational weight gain: A systematic review. *BMC Pregnancy and Childbirth*, *15*, 107. https://doi.org/10.1186/s12884-015-0535-y

Kapoor, A., Dunn, E., Kostaki, A., Andrews, M. H., & Matthews, S. G. (2006). Fetal programming of hypothalamo-pituitary-adrenal function: Prenatal stress and glucocorticoids. *The Journal of Physiology*, *572*(Pt 1), 31–44. https://doi.org/10.1113/jphysiol.2006.105254

Kim, H.-G., Cheon, E.-J., Bai, D.-S., Lee, Y. H., & Koo, B.-H. (2018). Stress and Heart Rate Variability: A Meta-Analysis and Review of the Literature. *Psychiatry Investig*, *15*(3), 235–245. https://doi.org/10.30773/pi.2017.08.17

Kingston, D., Tough, S., & Whitfield, H. (2012). Prenatal and postpartum maternal psychological distress and infant development: A systematic review. *Child Psychiatry and Human Development*, *43*(5), 683–714. https://doi.org/10.1007/s10578-012-0291-4

Kinsella, M. T., & Monk, C. (2009). Impact of maternal stress, depression and anxiety on fetal neurobehavioral development. *Clin Obstet Gynecol*, *52*(3), 425–440. https://doi.org/10.1097/GRF.0b013e3181b52df1.

Kocalevent, R.-D., Hinz, A., & Brähler, E. (2013). Standardization of a screening instrument (PHQ-15) for somatization syndromes in the general population. *BMC Psychiatry*, *13*(13:91), 91. https://doi.org/10.1186/1471-244X-13-91

Kominiarek, M. A., Grobman, W., Adam, E., Buss, C., Culhane, J., Entringer, S., . . . Borders, A. (2018). Stress during pregnancy and gestational weight gain. *Journal of Perinatology : Official Journal of the California Perinatal Association*, *38*(5), 462–467. https://doi.org/10.1038/s41372-018-0051-9

Kuzmicki, M., Telejko, B., Szamatowicz, J., Zonenberg, A., Nikolajuk, A., Kretowski, A., & Gorska, M. (2009). High resistin and interleukin-6 levels are associated with gestational diabetes mellitus. *Gynecological Endocrinology : the Official Journal of the International Society of Gynecological Endocrinology*, *25*(4), 258–263. https://doi.org/10.1080/09513590802653825

Laryea, G., Schütz, G., & Muglia, L. J. (2013). Disrupting hypothalamic glucocorticoid receptors causes HPA axis hyperactivity and excess adiposity. *Molecular Endocrinology (Baltimore, Md.)*, *27*(10), 1655–1665. https://doi.org/10.1210/me.2013-1187

Lazarus, R. S., & Folkman, S. (1984). *Stress, Appraisal, and Coping*. New York: Springer Publishing Company, Inc.

Leff Gelman, P., Mancilla-Herrera, I., Flores-Ramos, M., Saravia Takashima, M. F., Cruz Coronel, F. M., Cruz Fuentes, C., . . . Camacho-Arroyo, I. (2019). The cytokine profile of women with severe anxiety and depression during pregnancy. *BMC Psychiatry*, *19*(1), 104. https://doi.org/10.1186/s12888-019-2087-6

Lewis, A. J., Austin, E., & Galbally, M. (2016). Prenatal maternal mental health and fetal growth restriction: A systematic review. *Journal of Developmental Origins of Health and Disease*, *7*(4), 416–428. https://doi.org/10.1017/S2040174416000076

Linder, K., Schleger, F., Kiefer-Schmidt, I., Fritsche, L., Kümmel, S., Böcker, M., . . . Fritsche, A. (2015). Gestational Diabetes Impairs Human Fetal Postprandial Brain Activity. *The Journal of Clinical Endocrinology and Metabolism*, *100*(11), 4029–4036. https://doi.org/10.1210/jc.2015-2692

Lindsay, R. S., Lindsay, R. M., Waddell, B. J., & Seckl, J. R. (1996). Prenatal glucocorticoid exposure leads to offspring hyperglycaemia in the rat: Studies with the 11 beta-hydroxysteroid dehydrogenase inhibitor carbenoxolone. *Diabetologia*, *39*(11), 1299–1305. https://doi.org/10.1007/s001250050573

Lordier, L., Meskaldji, D.-E., Grouiller, F., Pittet, M. P., Vollenweider, A., Vasung, L., . . . Hüppi, P. S. (2019). Music in premature infants enhances high-level cognitive brain networks. *Proceedings of the National Academy of Sciences of the United States of America*, *116*(24), 12103–12108. https://doi.org/10.1073/pnas.1817536116

Malik, M. (1996). Heart Rate Variability. *Annals of Noninvasive Electrocardiology*, *1*(2), 151–181. https://doi.org/10.1111/j.1542-474X.1996.tb00275.x

Mat Husin, H. (2020). *Relationship between metabolic and anthropometric maternal parameters and the fetal autonomic nervous system* (Inaugural-Dissertation). Eberhard Karls Universität Tübingen, Tübingen.

Mat Husin, H., Schleger, F., Bauer, I., Fehlert, E., Kiefer-Schmidt, I., Weiss, M., . . . Preissl, H. (2020). Maternal Weight, Weight Gain, and Metabolism are Associated with Changes in Fetal Heart Rate and Variability. *Obesity (Silver Spring, Md.)*, *28*(1), 114–121. https://doi.org/10.1002/oby.22664

McCubbin, J., Robinson, S. E., Cropp, R., Moiseev, A., Vrba, J., Murphy, P., . . . Eswaran, H. (2006). Optimal reduction of MCG in fetal MEG recordings. *IEEE Transactions on Bio-Medical Engineering*, *53*(8), 1720–1724. https://doi.org/10.1109/TBME.2006.876619

McLeish, J., & Redshaw, M. (2017). Mothers' accounts of the impact on emotional wellbeing of organised peer support in pregnancy and early parenthood: A qualitative study. *BMC Pregnancy and Childbirth*, *17*(1), 28. https://doi.org/10.1186/s12884-017-1220-0

Metzger, B. E., Buchanan, T. A., Coustan, D. R., Leiva, A. de, Dunger, D. B., Hadden, D. R., . . . Zoupas, C. (2007). Summary and recommendations of the Fifth International Workshop-Conference on Gestational Diabetes Mellitus. *Diabetes Care*, *30 Suppl 2*, S251-60. https://doi.org/10.2337/dc07-s225

Miranda, A., & Sousa, N. (2018). Maternal hormonal milieu influence on fetal brain development. *Brain and Behavior*, *8*(2), e00920. https://doi.org/10.1002/brb3.920

Mishra, J. S., Zhao, H., Hattis, S., & Kumar, S. (2020). Elevated Glucose and Insulin Levels Decrease DHA Transfer across Human Trophoblasts via SIRT1-Dependent Mechanism. *Nutrients*, *12*(5). https://doi.org/10.3390/nu12051271

Morisset, A.-S., Dubé, M.-C., Côté, J. A., Robitaille, J., Weisnagel, S. J., & Tchernof, A. (2011). Circulating interleukin-6 concentrations during and after gestational diabetes mellitus. *ACTA Obstetricia Et Gynecologica Scandinavica*, *90*(5), 524–530. https://doi.org/10.1111/j.1600-0412.2011.01094.x

Morisset, A.-S., Huot, C., Légaré, D., & Tchernof, A. (2008). Circulating IL-6 concentrations and abdominal adipocyte isoproterenol-stimulated lipolysis in

women. *Obesity (Silver Spring, Md.)*, *16*(7), 1487–1492. https://doi.org/10.1038/oby.2008.242

Muenssinger, J., Matuz, T., Schleger, F., Kiefer-Schmidt, I., Goelz, R., Wacker-Gussmann, A., . . . Preissl, H. (2013). Auditory habituation in the fetus and neonate: An fMEG study. *Developmental Science*, *16*(2), 287–295. https://doi.org/10.1111/desc.12025

Munoz-Suano, A., Hamilton, A. B., & Betz, A. G. (2011). Gimme shelter: The immune system during pregnancy. *Immunological Reviews*, *241*(1), 20–38. https://doi.org/10.1111/j.1600-065X.2011.01002.x

Nazzari, S., Fearon, P., Rice, F., Dottori, N., Ciceri, F., Molteni, M., & Frigerio, A. (2019). Beyond the HPA-axis: Exploring maternal prenatal influences on birth outcomes and stress reactivity. *Psychoneuroendocrinology*, *101*(101), 253–262. https://doi.org/10.1016/j.psyneuen.2018.11.018

Nazzari, S., Molteni, M., Valtorta, F., Comai, S., & Frigerio, A. (2020). Prenatal IL-6 levels and activation of the tryptophan to kynurenine pathway are associated with depressive but not anxiety symptoms across the perinatal and the post-partum period in a low-risk sample. *Brain Behav Immun*, *89*, 175–183. https://doi.org/10.1016/j.bbi.2020.06.015

Nwebube, C., Glover, V., & Stewart, L. (2017). Prenatal listening to songs composed for pregnancy and symptoms of anxiety and depression: A pilot study. *BMC Complementary and Alternative Medicine*, *17*(1), 256. https://doi.org/10.1186/s12906-017-1759-3

Ohman, S. G., Grunewald, C., & Waldenström, U. (2003). Women's worries during pregnancy: Testing the Cambridge Worry Scale on 200 Swedish women. *Scandinavian Journal of Caring Sciences*, *17*(2), 148–152. https://doi.org/10.1046/j.1471-6712.2003.00095.x

Ozbay, F., Johnson, D. C., Dimoulas, E., Morgan, C. A., Charney, D., & Southwick, S. (2007). Social support and resilience to stress: From neurobiology to clinical practice. *Psychiatry (Edgmont (Pa. : Township))*, *4*(5), 35–40.

Pantham, P., Aye, I. L. M. H., & Powell, T. L. (2015). Inflammation in maternal obesity and gestational diabetes mellitus. *Placenta*, *36*(7), 709–715. https://doi.org/10.1016/j.placenta.2015.04.006

Partanen, E., Kujala, T., Tervaniemi, M., & Huotilainen, M. (2013). Prenatal music exposure induces long-term neural effects. *PloS One*, *8*(10), e78946. https://doi.org/10.1371/journal.pone.0078946

Patsalos, O., Dalton, B., & Himmerich, H. (2020). Effects of IL-6 Signaling Pathway Inhibition on Weight and BMI: A Systematic Review and Meta-Analysis. *International Journal of Molecular Sciences*, *21*(17). https://doi.org/10.3390/ijms21176290

Pawluski, J. L., Lonstein, J. S., & Fleming, A. S. (2017). The Neurobiology of Postpartum Anxiety and Depression. *Trends in Neurosciences*, *40*(2), 106–120. https://doi.org/10.1016/j.tins.2016.11.009

Persico, G., Antolini, L., Vergani, P., Costantini, W., Nardi, M. T., & Bellotti, L. (2017). Maternal singing of lullabies during pregnancy and after birth: Effects on mother-infant bonding and on newborns' behaviour. Concurrent Cohort Study. *Women and Birth : Journal of the Australian College of Midwives*, *30*(4), e214-e220. https://doi.org/10.1016/j.wombi.2017.01.007

Pinel, J. P. (1997). *Biopsychologie: Eine Einführung* (4th ed.). Heidelberg, Berlin: Spektrum Akademischer Verlag GmbH.

Pluess, M., Bolten, M., Pirke, K.-M., & Hellhammer, D. (2010). Maternal trait anxiety, emotional distress, and salivary cortisol in pregnancy. *Biological Psychology*, *83*(3), 169–175. https://doi.org/10.1016/j.biopsycho.2009.12.005

Posada-Quintero, H. F., & Chon, K. H. (2020). Innovations in Electrodermal Activity Data Collection and Signal Processing: A Systematic Review. *Sensors (Basel, Switzerland)*, *20*(2). https://doi.org/10.3390/s20020479

Preissl, H., Lowery, C. L., & Eswaran, H. (2004). Fetal magnetoencephalography: Current progress and trends. *Experimental Neurology*, *190 Suppl 1*, S28-36. https://doi.org/10.1016/j.expneurol.2004.06.016

Preissl, H., Lowery, C. L., & Eswaran, H. (2005). Fetal magnetoencephalography: Viewing the developing brain in utero. *International Review of Neurobiology*, *68*(68), 1–23. https://doi.org/10.1016/S0074-7742(05)68001-4

Prince, C. S., Maloyan, A., & Myatt, L. (2017). Maternal obesity alters brain derived neurotrophic factor (BDNF) signaling in the placenta in a sexually dimorphic manner. *Placenta*, *49*, 55–63. https://doi.org/10.1016/j.placenta.2016.11.010

Prins, J. R., Gomez-Lopez, N., & Robertson, S. A. (2012). Interleukin-6 in pregnancy and gestational disorders. *Journal of Reproductive Immunology*, *95*(1-2), 1–14. https://doi.org/10.1016/j.jri.2012.05.004

Radoš, S. N., Matijaš, M., Anđelinović, M., Čartolovni, A., & Ayers, S. (2020). The role of posttraumatic stress and depression symptoms in mother-infant bonding. *Journal of Affective Disorders*, *268*, 134–140. https://doi.org/10.1016/j.jad.2020.03.006

Rasmussen, K. M., Catalano, P. M., & Yaktine, A. L. (2009). New guidelines for weight gain during pregnancy: What obstetrician/gynecologists should know. *Current Opinion in Obstetrics & Gynecology*, *21*(6), 521–526. https://doi.org/10.1097/GCO.0b013e328332d24e

Rasmussen, K. M., & Yaktine, A. L. (2009). Weight Gain During Pregnancy: Reexamining the Guidelines. *National Academies Press.* Advance online publication. https://doi.org/10.17226/12584

Richter, P., & Hacker, W. (1998). *Belastung und Beanspruchung: Stress, Ermüdung und Burnout im Arbeitsleben*: Roland Asanger.

Rudolph, M. D., Graham, A. M., Feczko, E., Miranda-Dominguez, O., Rasmussen, J. M., Nardos, R., . . . Fair, D. A. (2018). Maternal IL-6 during pregnancy can be estimated from newborn brain connectivity and predicts future working memory in offspring. *Nature Neuroscience*, *21*(5), 765–772. https://doi.org/10.1038/s41593-018-0128-y

S. Fisher, & J. Reason (Eds.) (1988). *Handbook of Life Stress, Cognition and Health: Allostasis: A New paradigm to Explain Arousal Pathology*. Peter Sterling; Joseph Eyer: John Wiley & Sons. Retrieved from http://retina.anatomy.upenn.edu/pdfiles/5446.pdf

Sandman, C. A., Wadhwa, P. D., Chicz-DeMet, A., Porto, M., & Garite, T. J. (1999). Maternal corticotropin-releasing hormone and habituation in the human fetus. *Developmental Psychobiology*, *34*(3), 163–173. https://doi.org/10.1002/(sici)1098-2302(199904)34:3<163::aid-dev1>3.0.co;2-9

Santhanam, U., Avila, C., Romero, R., Viguet, H., Ida, N., Sakurai, S., & Sehgal, P. B. (1991). Cytokines in normal and abnormal parturition: Elevated amniotic fluid interleukin-6 levels in women with premature rupture of membranes associated with

intrauterine infection. *Cytokine*, *3*(2), 155–163. https://doi.org/10.1016/1043-4666(91)90037-E

Sardar, S., Reeby, T., Puthiyaveetil, S., Devarajan, S., Kazem, B., & Rasheed, A. (2015). Obesity is a positive modulator of Il-6R and Il-6 expression in the subcutaneous adipose tissue: significance for metabolic inflammation. *PloS one.* Advance online publication. https://doi.org/10.1371/journal.pone.0133494

Schaal, N. K., Brückner, J., Wolf, O. T., Ruckhäberle, E., Fehm, T., & Hepp, P. (2021). The effects of a music intervention during port catheter placement on anxiety and stress. *Scientific Reports*, *11*(1), 5807. https://doi.org/10.1038/s41598-021-85139-z

Scheinost, D., Sinha, R., Cross, S. N., Kwon, S. H., Sze, G., Constable, R. T., & Ment, L. R. (2017). Does prenatal stress alter the developing connectome? *Pediatric Research*, *81*(1-2), 214–226. https://doi.org/10.1038/pr.2016.197

Schneider, U., Arnscheidt, C., Schwab, M., Haueisen, J., Seewald, H. J., & Schleussner, E. (2011). Steroids that induce lung maturation acutely affect higher cortical function: A fetal magnetoencephalography study. *Reproductive Sciences (Thousand Oaks, Calif.)*, *18*(1), 99–106. https://doi.org/10.1177/1933719110381140

Schubert, C., Lambertz, M., Nelesen, R. A., Bardwell, W., Choi, J.-B., & Dimsdale, J. E. (2009). Effects of stress on heart rate complexity--a comparison between short-term and chronic stress. *Biological Psychology*, *80*(3), 325–332. https://doi.org/10.1016/j.biopsycho.2008.11.005

Schulz, P., Schlotz, W., & Becker, P. (2004). *Trierer Inventar zum chronischen Stress: Manual*. Göttingen: Hogrefe.

SELYE, H. (1950). Stress and the general adaptation syndrome. *British Medical Journal*, *1*(4667), 1383–1392. https://doi.org/10.1136/bmj.1.4667.1383

Shaffer, F., & Ginsberg, J. P. (2017). An Overview of Heart Rate Variability Metrics and Norms. *Frontiers in Public Health*, *5*, 258. https://doi.org/10.3389/fpubh.2017.00258

Shelton, M. M., Schminkey, D. L., & Groer, M. W. (2015). Relationships among prenatal depression, plasma cortisol, and inflammatory cytokines. *Biol Res Nurs*, *17*(3), 295–302. https://doi.org/10.1177/1099800414543821

Sheridan, C., Draganova, R., Ware, M., Murphy, P., Govindan, R., Siegel, E. R., . . . Preissl, H. (2010). Early development of brain responses to rapidly presented

auditory stimulation: A magnetoencephalographic study. *Brain & Development*, *32*(8), 642–657. https://doi.org/10.1016/j.braindev.2009.10.002

Shoelson, S. E., Herrero, L., & Naaz, A. (2007). Obesity, inflammation, and insulin resistance. *Gastroenterology*, *132*(6), 2169–2180. https://doi.org/10.1053/j.gastro.2007.03.059

Sippel, K., Moser, J., Schleger, F., Preissl, H., Rosenstiel, W., & Spüler, M. (2019). Fully Automated R-peak Detection Algorithm (FLORA) for fetal magnetoencephalographic data. *Computer Methods and Programs in Biomedicine*, *173*, 35–41. https://doi.org/10.1016/j.cmpb.2019.02.016

Stalder, T., Steudte, S., Alexander, N., Miller, R., Gao, W., Dettenborn, L., & Kirschbaum, C. (2012). Cortisol in hair, body mass index and stress-related measures. *Biological Psychology*, *90*(3), 218–223. https://doi.org/10.1016/j.biopsycho.2012.03.010

Steptoe, A., Hamer, M., & Chida, Y. (2007). The effects of acute psychological stress on circulating inflammatory factors in humans: A review and meta-analysis. *Brain Behav Immun*, *21*(7), 901–912. https://doi.org/10.1016/j.bbi.2007.03.011

Teixeira, J., Martin, D., Prendiville, O., & Glover, V. (2005). The effects of acute relaxation on indices of anxiety during pregnancy. *Journal of Psychosomatic Obstetrics and Gynaecology*, *26*(4), 271–276. https://doi.org/10.1080/01674820500139922

Torloni, M. R., Betrán, A. P., Horta, B. L., Nakamura, M. U., Atallah, A. N., Moron, A. F., & Valente, O. (2009). Prepregnancy BMI and the risk of gestational diabetes: A systematic review of the literature with meta-analysis. *Obesity Reviews : an Official Journal of the International Association for the Study of Obesity*, *10*(2), 194–203. https://doi.org/10.1111/j.1467-789X.2008.00541.x

Tragea, C., Chrousos, G. P., Alexopoulos, E. C., & Darviri, C. (2014). A randomized controlled trial of the effects of a stress management programme during pregnancy. *Complementary Therapies in Medicine*, *22*(2), 203–211. https://doi.org/10.1016/j.ctim.2014.01.006

Ulusar, U. D., Govindan, R. B., Wilson, J. D., Lowery, C. L., Preissl, H., & Eswaran, H. (2009). Adaptive rule based fetal QRS complex detection using Hilbert transform. *Annual International Conference of the IEEE Engineering in Medicine*

and Biology Society. IEEE Engineering in Medicine and Biology Society. Annual International Conference, *2009*, 4666–4669. https://doi.org/10.1109/iembs.2009.5334180

Urech, C., Fink, N. S., Hoesli, I., Wilhelm, F. H., Bitzer, J., & Alder, J. (2010). Effects of relaxation on psychobiological wellbeing during pregnancy: A randomized controlled trial. *Psychoneuroendocrinology*, *35*(9), 1348–1355. https://doi.org/10.1016/j.psyneuen.2010.03.008

Valsamakis, G., Papatheodorou, D. C., Chalarakis, N., Vrachnis, N., Sidiropoulou, E. J., Manolikaki, M., . . . Mastorakos, G. (2017). In pregnancy increased maternal STAI trait stress score shows decreased insulin sensitivity and increased stress hormones. *Psychoneuroendocrinology*, *84*(84), 11–16. https://doi.org/10.1016/j.psyneuen.2017.06.008

Van den Bergh, B. R. H., van den Heuvel, M. I., Lahti, M., Braeken, M., Rooij, S. R. de, Entringer, S., . . . Schwab, M. (2020). Prenatal developmental origins of behavior and mental health: The influence of maternal stress in pregnancy. *Neuroscience and Biobehavioral Reviews*, *117*, 26–64. https://doi.org/10.1016/j.neubiorev.2017.07.003

Van der Burg, J. W., Sen, S., Chomitz, V. R., Seidell, J. C., Leviton, A., & Dammann, O. (2016). The role of systemic inflammation linking maternal BMI to neurodevelopment in children. *Pediatric Research*, *79*(1-1), 3–12. https://doi.org/10.1038/pr.2015.179

Vehmeijer, F. O. L., Balkaran, S. R., Santos, S., Gaillard, R., Felix, J. F., Hillegers, M. H. J., . . . Jaddoe, V. W. V. (2020). Psychological Distress and Weight Gain in Pregnancy: A Population-Based Study. *International Journal of Behavioral Medicine*, *27*(1), 30–38. https://doi.org/10.1007/s12529-019-09832-0

Ventura, T., Gomes, M. C., & Carreira, T. (2012). Cortisol and anxiety response to a relaxing intervention on pregnant women awaiting amniocentesis. *Psychoneuroendocrinology*, *37*(1), 148–156. https://doi.org/10.1016/j.psyneuen.2011.05.016

Vrba, J., Robinson, S. E., Mccubbin, J., Lowery, C. L., Eswaran, H., Wilson, J. D., . . . Preissl, H. (2004). Fetal MEG redistribution by projection operators. *IEEE Transactions on Bio-Medical Engineering*, *51*(7), 1207–1218. https://doi.org/10.1109/TBME.2004.827265

Wagner, R., Fritsche, L., Heni, M., Fehlert, E., Stefan, N., Staiger, H., . . . Fritsche, A. (2016). A novel insulin sensitivity index particularly suitable to measure insulin sensitivity during gestation. *Acta Diabetologica*, *53*(6), 1037–1044. https://doi.org/10.1007/s00592-016-0930-5

Walter B. Cannon (1915). *Bodily cahnges in pain, hunger, fear, and rage: an account of recent researches into the function of emotional excitement*. New York, London: D. Appleton and Company.

Webb, J. B., Siega-Riz, A. M., & Dole, N. (2009). Psychosocial determinants of adequacy of gestational weight gain. *Obesity (Silver Spring, Md.)*, *17*(2), 300–309. https://doi.org/10.1038/oby.2008.490

(2009). Weight Gain During Pregnancy: Reexamining the Guidelines. Advance online publication. https://doi.org/10.17226/12584

Weinstock, M. (2005). The potential influence of maternal stress hormones on development and mental health of the offspring. *Brain Behav Immun*, *19*(4), 296–308. https://doi.org/10.1016/j.bbi.2004.09.006

Wennig, R. (2000). Potential problems with the interpretation of hair analysis results. *Forensic Science International*, *107*(1-3), 5–12. https://doi.org/10.1016/s0379-0738(99)00146-2

Witteveen, A. B., Henrichs, J., Bellers, M., van Oenen, E., Verhoeven, C. J., & Vrijkotte, T. G. M. (2020). Mediating role of C-reactive protein in associations between pre-pregnancy BMI and adverse maternal and neonatal outcomes: The ABCD-study cohort. *The Journal of Maternal-Fetal & Neonatal Medicine : the Official Journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstetricians*, 1–9. https://doi.org/10.1080/14767058.2020.1807510

Wulff, V., Hepp, P., Wolf, O. T., Balan, P., Hagenbeck, C., Fehm, T., & Schaal, N. K. (2021). The effects of a music and singing intervention during pregnancy on maternal well-being and mother-infant bonding: A randomised, controlled study. *Archives of Gynecology and Obstetrics*, *303*(1), 69–83. https://doi.org/10.1007/s00404-020-05727-8

Wurster, K. G., & Keller, E. (1985). *Endokrine Regulation und Frauenhochleistungssport*. Berlin, Heidelberg: Springer Berlin Heidelberg. Retrieved from http://dx.doi.org/10.1007/978-3-642-70743-8

Yonkers, K. A., Smith, M. V., Forray, A., Epperson, C. N., Costello, D., Lin, H., & Belanger, K. (2014). Pregnant women with posttraumatic stress disorder and risk of preterm birth. *JAMA Psychiatry*, *71*(8), 897–904. https://doi.org/10.1001/jamapsychiatry.2014.558

Zhou, D., Kusnecov, A. W., Shurin, M. R., DePaoli, M., & Rabin, B. S. (1993). Exposure to physical and psychological stressors elevates plasma interleukin 6: Relationship to the activation of hypothalamic-pituitary-adrenal axis. *Endocrinology*, *133*(6), 2523–2530. https://doi.org/10.1210/endo.133.6.8243274

Figures

Tables

APPENDIX

A 1. Study 1: Overview of maternal cardiovascular activity parameters.

Type of intervention		HR (T1)	HR (T2)	HR (T3)	HR (T4)	RMSSD (T1)	RMSSD (T2)	RMSSD (T3)	RMSSD (T4)
Music Group	Mean	-1.66	-.86	-2.33	-2.48	5.19	7.42	8.70	7.50
	Standard error	.90	.89	.88	1.63	3.96	4.58	3.90	3.45
	Standard deviation	3.12	2.96	2.90	5.63	13.71	15.87	13.53	11.44
	N	12	11	11	12	12	12	12	11
Guided Imagery Group	Mean	-3.14	-3.90	-1.87	-1.40	4.67	9.48	2.61	-.39
	Standard error	.74	1.12	.55	.91	1.21	3.13	.77	1.57
	Standard deviation	2.45	3.70	1.81	3.03	4.03	10.38	2.43	5.22
	N	11	11	11	11	11	11	10	11
Resting Group	Mean	-1.09	-2.25	-2.44	-2.58	-1.05	6.89	1.40	9.92
	Standard error	.72	1.68	.97	1.24	3.16	6.83	1.95	7.49
	Standard deviation	2.48	5.83	3.38	4.31	9.98	23.67	6.47	25.95
	N	12	12	12	12	10	12	11	12
All Groups	Mean	-1.93	-2.33	-2.22	-2.17	3.13a	7.88	4.42	5.80
	Standard error	.47	.76	.47	.74	1.79	2.91	1.64	2.93
	Standard deviation	2.77	4.43	2.72	4.39	10.27	17.20	9.42	17.10
	N	35	34	34	35	33	35	33	34

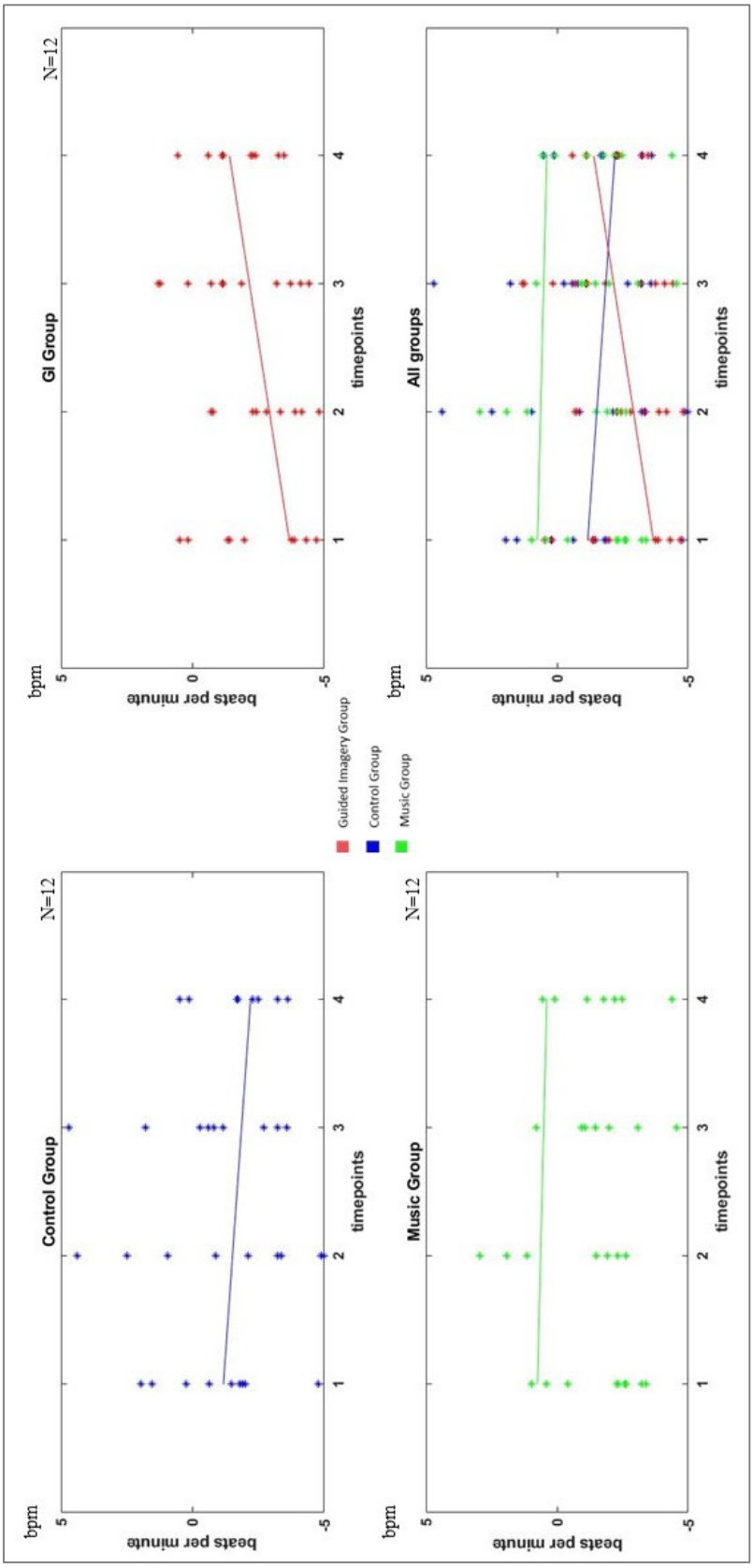
HR: heart rate; RMSSD: root mean square of successive differences; T1-T4: time points (duration: each 5 minutes); N: sample size. All values are baseline corrected.

A 2. Study 1: Overview of maternal skin conductance level.

Overview of maternal SCLs					
Type of intervention		SCL (T1)	SCL (T2)	SCL (T3)	SCL (T4)
Music Group	Mean	1.03	.12	.01	-.04
	Standard error	.26	.07	.04	.05
	Standard deviation	.89	.21	.14	.17
	N	12	10	10	10
Guided Imagery Group	Mean	1.57	-.22	.20	-.72
	Standard error	.41	.26	.24	.46
	Standard deviation	1.28	.83	.73	1.46
	N	10	10	9	10
Resting Group	Mean	1.32	.12	-.08	-.14
	Standard error	.39	.18	.13	.17
	Standard deviation	1.36	.61	.45	.58
	N	12	12	12	12
All Groups	Mean	1.29	.01	.03	-.29
	Standard error	.20	.11	.09	.16
	Standard deviation	1.17	.61	.49	.92
	N	34	32	31	32

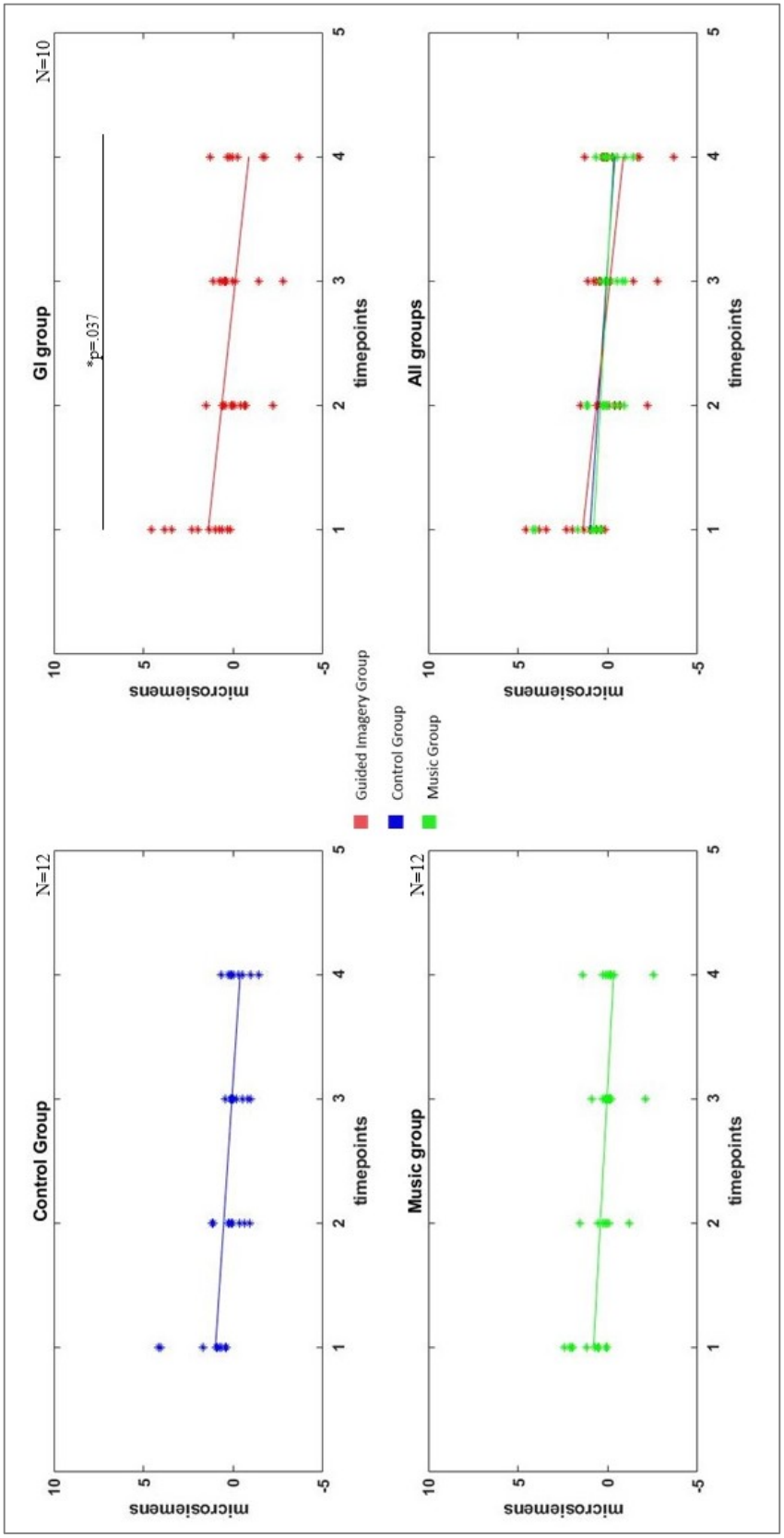
SCL: skin conductance level; T1-T4: time points (duration: each 5 minutes); N: sample size. All values are baseline corrected.

A 3. Study 1: Changes in maternal cardiovascular activity.



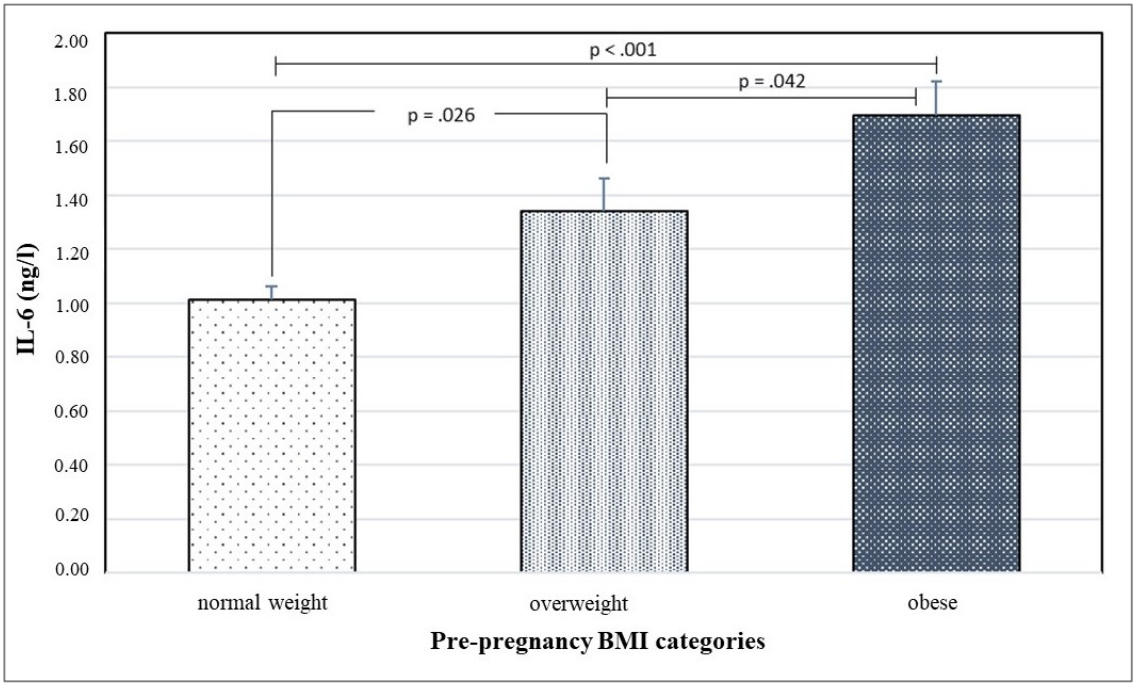
N: sample size; bpm: beats per minute; Control Group: N=12; Guided Imagery Group (GI Group): N=12; Music Group: N=12. All values are baseline corrected.

A 4. Study 1: Changes in maternal skin conductance level.



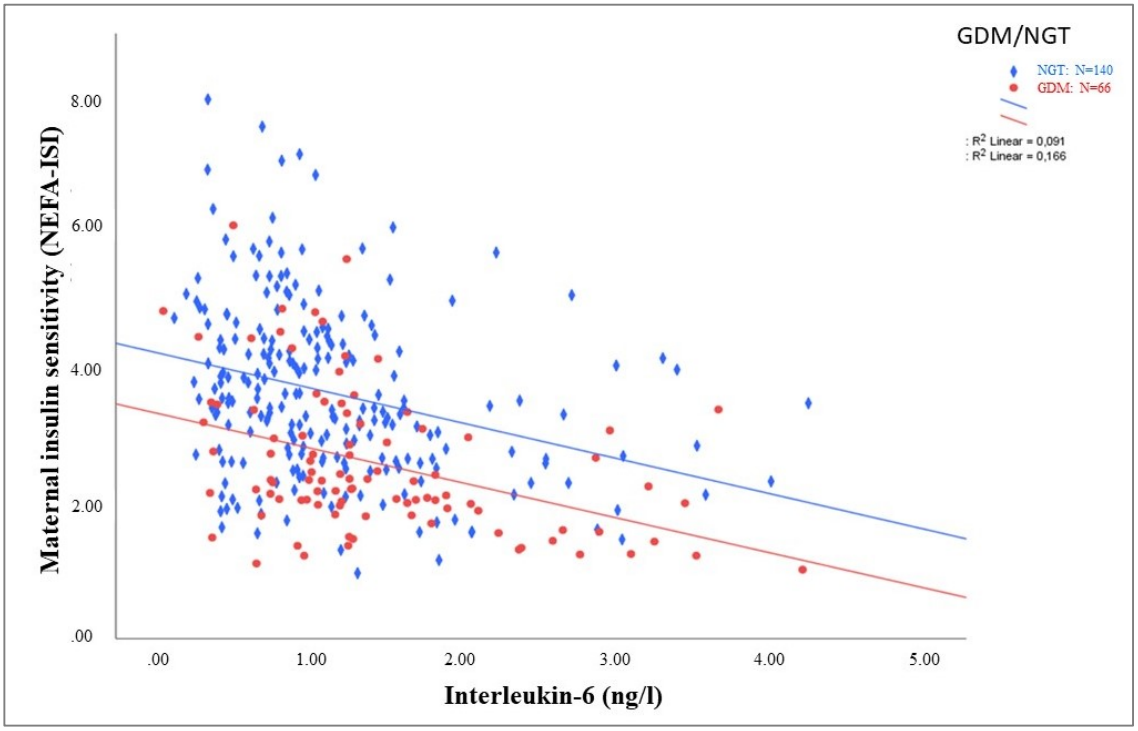
N: sample size; Control Group: N=12; Guided Imagery Group (GI Group): N=10; Music Group: N=12. All values are baseline corrected.

A5. Maternal pre-pregnancy BMI categories and IL-6 levels in all women.



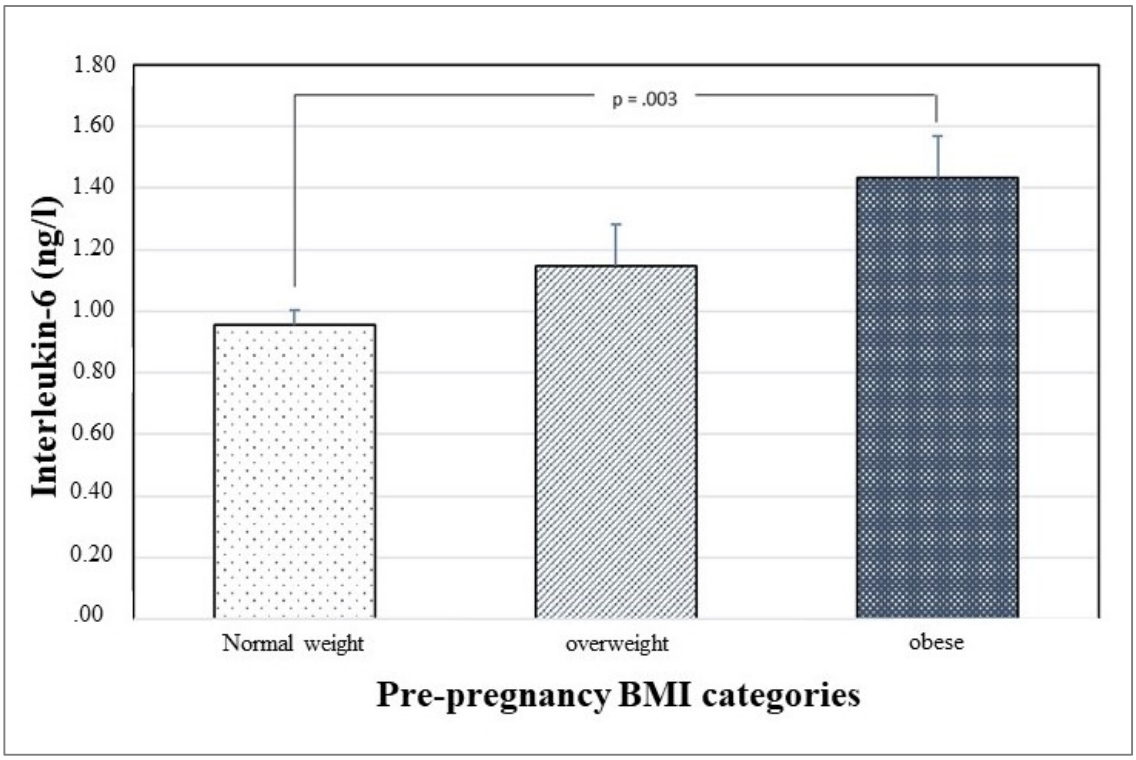
IL-6: Interleukin-6. Adjusted for gestational age and cortisol levels.

A6. Interleukin-6 and maternal insulin sensitivity in GDM and NGT.



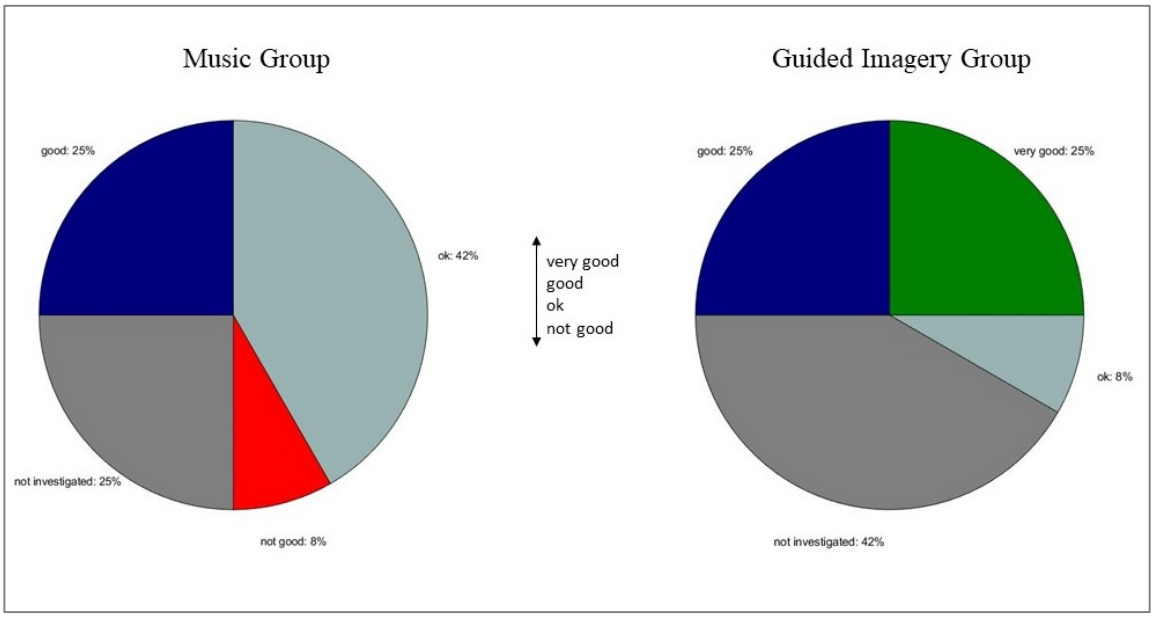
N: sample size; GDM: women with gestational diabetes mellitus; NGT: women with normal glucose tolerance.

A7. Pre-pregnancy BMI and Interleukin-6 levels for women with normal glucose tolerance.



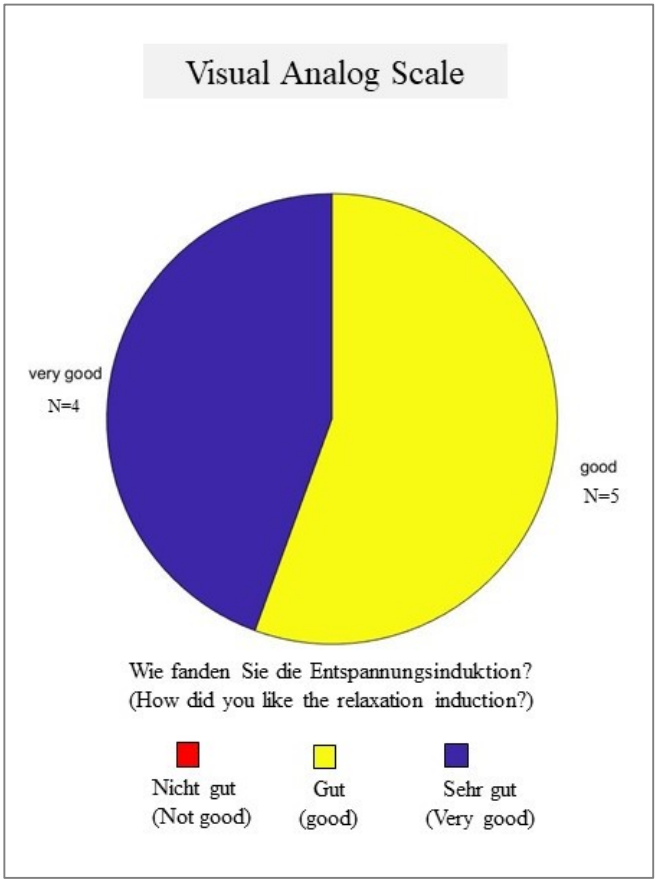
Normal weight: N=121; Overweight: N=24; Obese: N=36; Adjusted for gestational age and cortisol levels.

A8. Study 1: Maternal relaxation rating.



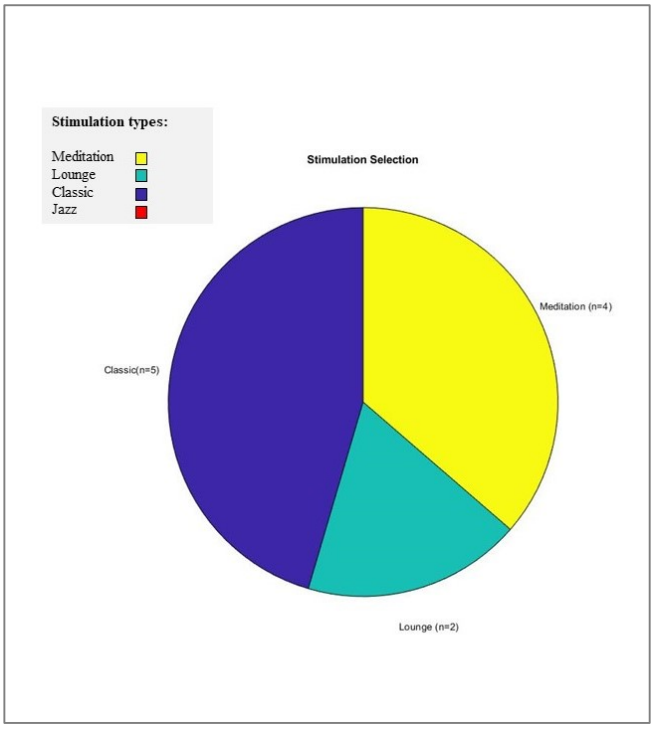
After the measurement (only in Guided Imagery and Music Group), women were asked to rate how enjoyable the relaxation stimulation was. The scale ranged from 'very good' to 'not good'. 'Not investigated': missing value. Music Group: N=9; Guided Imagery Group: N=7.

A9. Study 2: Maternal relaxation rating.



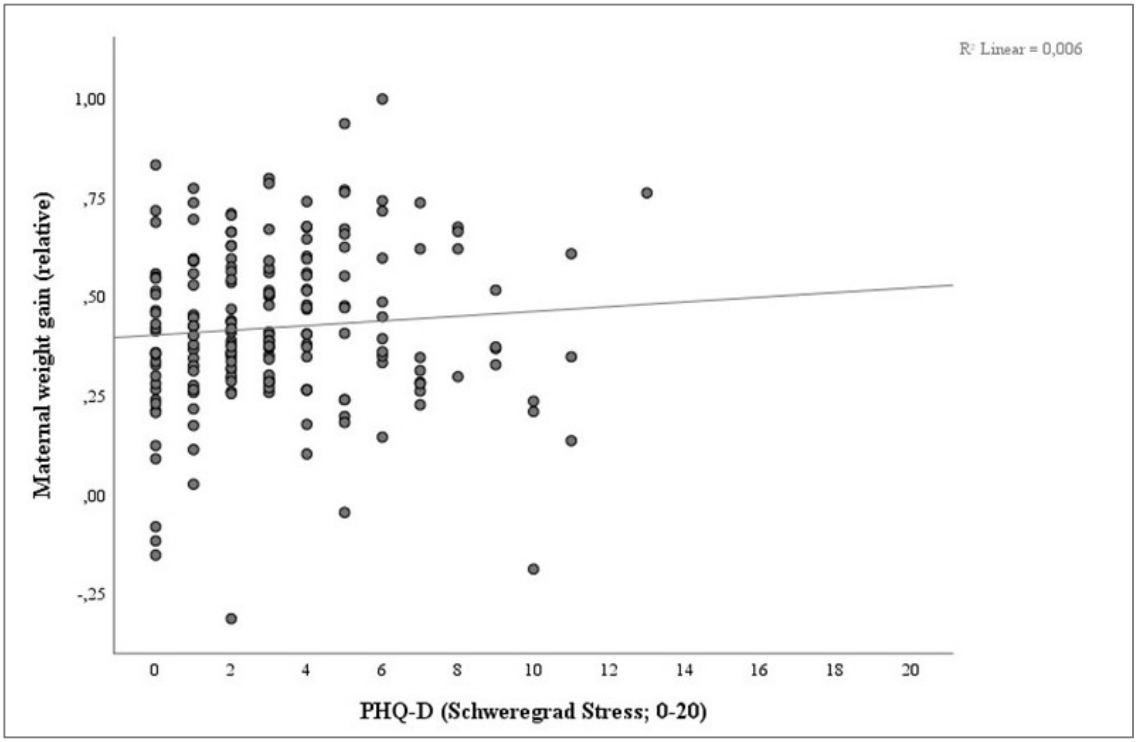
N: sample size

A10. Study 2: Stimulation Selection.



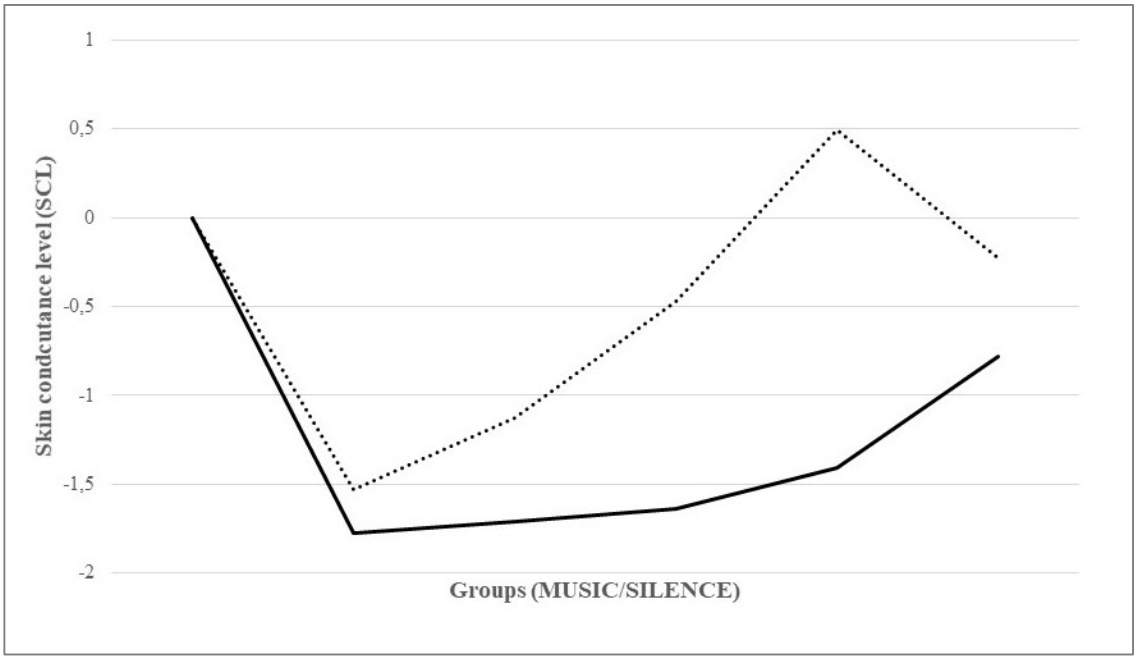
n: sample size

A11. Study 3: Correlation between relative maternal weight gain and stress levels.



PHQ-D: Patient Health Questionnaire (maternal stress level based on the subscale PHQ-D - Schweregrad Stress)

A12. Study 2: Changes in maternal skin conductance level in MUSIC/SILENCE Group.



Dashed line: Music group; Solid line: Silence group. All values are baseline corrected and presented in microsiemens.

A13. Prenatal Distress Questionnaire, PDQ (A M. Yali and M. Lobel, 1999)

Datum: _____ . _____ .

_____

Für manche Frauen sind bestimmte Erfahrungen im Zusammenhang mit der Schwangerschaft beunruhigend oder beängstigend, während andere Frauen sich über die gleichen Erfahrungen keine Sorgen machen. Bitte geben Sie bei den folgenden Aussagen jeweils an, inwieweit sie Ihren eigenen Empfindungen und Gefühlen entsprechen.

Bitte gehen Sie **der Reihe nach** vor und **lassen Sie keine Aussage aus**. Kreuzen Sie jeweils die Antwortalternative an, die Ihnen als beste Schätzung am zutreffendsten erscheint.

*Bitte wählen Sie für jede Aussage **nur eine** der folgenden Antwortalternativen:*

Durchkreuzen Sie 0 = diese Aussage trifft auf mich **gar nicht** zu

Durchkreuzen Sie 1 = diese Aussage trifft auf mich **ein wenig** zu

Durchkreuzen Sie 2 = diese Aussage trifft auf mich **mäßig stark** zu

Durchkreuzen Sie 3 = diese Aussage trifft auf mich **stark** zu

Durchkreuzen Sie 4 = diese Aussage trifft auf mich **sehr stark** zu

		trifft gar nicht zu	**trifft ein wenig zu**	**trifft mäßig stark zu**	**trifft stark zu**	**trifft sehr stark zu**
1.	Mich stören Gewichtszunahmen während der Schwangerschaft.	⓪	①	②	③	④
2.	Körperliche Symptome wie Übelkeit, Erbrechen, geschwollene Füße oder Rückenschmerzen, die im Zusammenhang mit der	⓪	①	②	③	④

	Schwangerschaft auftreten, belasten mich.					
3.	Ich bin besorgt darüber, wie ich mein Baby richtig versorge, wenn ich nach der Krankenhausentlassung wieder nach Hause komme.	⓪	①	②	③	④
4.	Mich plagen die gefühlsmäßigen Höhen und Tiefen während der Schwangerschaft.	⓪	①	②	③	④
5.	Ich mache mir Sorgen darüber, dass sich meine Beziehungen zu anderen Menschen, die mir wichtig sind, während meiner Schwangerschaft verändern.	⓪	①	②	③	④
6.	Ich mache mir Sorgen, ob mich für mein Baby gesund und ausgewogen genug nähre.	⓪	①	②	③	④

		trifft gar nicht zu	**trifft ein wenig zu**	**trifft mäßig stark zu**	**trifft stark zu**	**trifft sehr stark zu**
7.	Insgesamt belasten mich die Veränderungen meiner Figur und meines Körperumfangs.	⓪	①	②	③	④
8.	Ich mache mir Sorgen darüber, dass sich durch das Baby meine Beziehung zum Vater des Kindes ändern wird.	⓪	①	②	③	④
9.	Ich mache mir Sorgen darüber, ein krankes Baby zur Welt zu bringen.	⓪	①	②	③	④
10.	Ich habe Angst vor den Wehen und der Geburt.	⓪	①	②	③	④
11.	Ich habe Angst vor einer möglichen Frühgeburt.	⓪	①	②	③	④

12. Ich mache mir Sorgen darüber, dass ich keinen emotionalen Bezug zu meinem Baby finde.	⓪	①	②	③	④

VIELEN DANK!

Deutsche Übersetzung des „Prenatal Distress Questionnaire (PDQ)“ durch Dr. Christin Haselbeck, Universitätsklinikum Schleswig-Holstein; 2012. (Englische Originalversion: Yali & Lobel, 1999).

A14. Profile of Mood States (POMS) (Albani et al., 2005)

POMS

Anleitung: (bitte genau durchlesen)

Sie finden nachstehend eine Liste mit Wörtern, die verschiedenartige Gefühle oder Gefühlszustände beschreiben. Bitte lesen Sie sorgfältig jedes einzelne Wort und setzten Sie dann in der Spalte ein Kreuz ein, die am besten Ihre Gefühlzustände beschreibt.
Bitte lassen Sie keine Zeile aus!

		überhaupt nicht	sehr schwach	schwach	etwas	ziemlich	stark	sehr stark
1	zornig	0	1	2	3	4	5	6
2	abgeschlafft	0	1	2	3	4	5	6
3	unglücklich	0	1	2	3	4	5	6
4	lebhaft	0	1	2	3	4	5	6
5	unsicher	0	1	2	3	4	5	6
6	lustlos	0	1	2	3	4	5	6
7	traurig	0	1	2	3	4	5	6
8	aktiv	0	1	2	3	4	5	6
9	gereizt	0	1	2	3	4	5	6
10	verdrießlich	0	1	2	3	4	5	6
11	betrübt	0	1	2	3	4	5	6
12	e[illegible]sch	0	1	2	3	4	5	6
13	ä[illegible]h	0	1	2	3	4	5	6
14	h[illegible]slos	0	1	2	3	4	5	6
15	ü[illegible]	0	1	2	3	4	5	6
16	müde	0	1	2	3	4	5	6
17	verärgert	0	1	2	3	4	5	6
18	entmutigt	0	1	2	3	4	5	6
19	neidig	0	1	2	3	4	5	6
20	fröhlich	0	1	2	3	4	5	6
21	verbittert	0	1	2	3	4	5	6
22	erschöpft	0	1	2	3	4	5	6
23	schwermütig	0	1	2	3	4	5	6
24	verzweifelt	0	1	2	3	4	5	6
25	träge	0	1	2	3	4	5	6
26	hilflos	0	1	2	3	4	5	6
27	ermattet	0	1	2	3	4	5	6
28	munter	0	1	2	3	4	5	6
29	wütend	0	1	2	3	4	5	6
30	schwungvoll	0	1	2	3	4	5	6
31	schlecht gelaunt	0	1	2	3	4	5	6
32	minderwertig	0	1	2	3	4	5	6
33	erschreckt	0	1	2	3	4	5	6
34	tatkräftig	0	1	2	3	4	5	6
35	entkräftet	0	1	2	3	4	5	6

Bitte prüfen Sie, ob Sie alle Feststellungen zutreffend beantwortet haben!

A15. Patient Health Questionnaire (PHQ-D) (Bernd Löwe, Robert L. Spitzer, Stephan Zipfel, Wolfgang Herzog, 2002)

Gesundheitsfragebogen für Patienten (PHQ-D)

Gesundheitsfragebogen für Patienten (PHQ-D)

n wichtiges Hilfsmittel, um Ihnen die bestmögliche Behandlung zukommen zu lassen.
rrem Arzt helfen, Ihre Beschwerden besser zu verstehen. Bitte beantworten Sie jede Frage, so gut
n Sie Fragen bitte nur, wenn Sie dazu aufgefordert werden.

Name: _____ Alter: _____ Geschlecht: weiblich ☐ männlich ☐ Datum: _____

12 Wie stark fühlten Sie sich im Verlauf der letzten 4 Wochen durch die folgenden Beschwerden beeinträchtigt?

	Nicht beeinträchtigt	Wenig beeinträchtigt	Stark beeinträchtigt
a. Sorgen über Ihre Gesundheit	☐	☐	☐
b. Sorgen über Ihr Gewicht oder Ihr Aussehen	☐	☐	☐
c. Wenig oder kein sexuelles Verlangen oder Vergnügen beim Geschlechtsverkehr	☐	☐	☐
d. Schwierigkeiten mit dem Ehepartner, Lebensgefährten, Freundin/Freund	☐	☐	☐
e. Belastung durch die Versorgung von Kindern, Eltern oder anderen Familienangehörigen	☐	☐	☐
f. Stress bei der Arbeit oder in der Schule	☐	☐	☐
g. Finanzielle Probleme oder Sorgen	☐	☐	☐
h. Niemanden zu haben, mit dem man Probleme besprechen kann	☐	☐	☐
i. Etwas Schlimmes, das vor kurzem passiert ist	☐	☐	☐
j. Gedanken an schreckliche Ereignisse von früher oder Träume darüber – z. B. die Zerstörung des eigenen Heimes, ein schwerer Unfall, körperliche Gewalt oder eine sexuelle Handlung unter Zwang	☐	☐	☐

13 Sind Sie im letzten Jahr geschlagen, getreten oder anderweitig von jemandem körperlich verletzt worden oder hat Sie jemand zu einer ungewünschten sexuellen Handlung gezwungen? NEIN ☐ JA ☐

14 Was belastet Sie zur Zeit in Ihrem Leben am meisten? _____

15 Nehmen Sie Medikamente gegen Angst, Depressionen oder Stress? NEIN ☐ JA ☐

16 Nur für Frauen: Fragen zum Thema Monatsblutung, Schwangerschaft und Geburt

a. Wodurch wird Ihre Monatsblutung am besten beschrieben?

☐ Monatsblutung ist unverändert
☐ Keine Monatsblutung aufgrund von Schwangerschaft oder kürzlicher Geburt
☐ Monatsblutung ist unregelmäßig geworden bzw. Dauer, Abstand oder Stärke haben sich verändert
☐ Keine Monatsblutung seit mindestens einem Jahr
☐ Monatsblutung bei Hormontherapie (Einnahme von Östrogenen) oder Verhütung durch die Pille

b. Haben Sie in der Woche vor dem Beginn Ihrer Monatsblutung ausgeprägte Probleme mit Ihrer Stimmung – z. B. Depressionen, Angst, Reizbarkeit, Aggressivität oder Stimmungsschwankungen? NEIN (oder trifft nicht zu) ☐ JA ☐

c. Wenn „JA": Verschwinden diese Probleme am Ende Ihrer Monatsblutung wieder? ☐ ☐

d. Haben Sie während der letzten 6 Monate ein Kind geboren? ☐ ☐

e. Hatten Sie während der letzten 6 Monate eine Fehlgeburt? ☐ ☐

f. Haben Sie Schwierigkeiten, schwanger zu werden? ☐ ☐

1 Wie stark fühlten Sie sich im Verlauf der letzten 4 Wochen durch die folgenden Beschwerden beeinträchtigt?

	Nicht beeinträchtigt	Wenig beeinträchtigt	Stark beeinträchtigt
a. Rückenschmerzen	☐	☐	☐
b. Schmerzen in Armen, Beinen oder Gelenken (Knie, Hüften usw.)	☐	☐	☐
c. Menstruationsschmerzen oder andere Probleme mit der Menstruation	☐	☐	☐
d. Probleme beim Geschlechtsverkehr	☐	☐	☐
e. Herzklopfen oder Herzrasen	☐	☐	☐
f. Kurzatmigkeit	☐	☐	☐
g. Verstopfung, nervöser Darm oder Durchfall	☐	☐	☐
h. Übelkeit, Blähungen oder Verdauungsbeschwerden	☐	☐	☐

2 Wie oft fühlten Sie sich im Verlauf der letzten 2 Wochen durch die folgenden Beschwerden beeinträchtigt?

	Überhaupt nicht	An einzelnen Tagen	An mehr als der Hälfte der Tage	Beinahe jeden Tag
a. Wenig Interesse oder Freude an Ihren Tätigkeiten	☐	☐	☐	☐
b. Niedergeschlagenheit, Schwermut oder Hoffnungslosigkeit	☐	☐	☐	☐
c. Schwierigkeiten, ein- oder durchzuschlafen, oder vermehrter Schlaf	☐	☐	☐	☐
d. Müdigkeit, keine Energie zu haben	☐	☐	☐	☐
e. Schlechte Meinung von sich selbst; Gefühl, ein Versager zu sein oder Familie enttäuscht zu haben	☐	☐	☐	☐
f. Schwierigkeiten, sich auf etwas zu konzentrieren, z. B. beim Zeitunglesen oder Fernsehen	☐	☐	☐	☐
g. Waren Ihre Bewegungen oder Ihre Sprache so verlangsamt, dass es auch anderen auffallen würde? Oder waren Sie im Gegenteil „zappelig" oder ruhelos und hatten dadurch einen stärkeren Bewegungsdrang als sonst?	☐	☐	☐	☐
h. Waren Sie manchmal lieber tot wären oder sich Leid zufügen möchten	☐	☐	☐	☐

Deutsche Übersetzung und Validierung des „Patient Health Questionnaire (PHQ)" durch B. Löwe, S. Zipfel und W. Herzog, Medizinische Universitätsklinik Heidelberg
(Englische Originalversion: Spitzer, Kroenke & Williams, 1999).
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3 Fragen zum Thema „Angst"

	NEIN	JA
a. Hatten Sie in den letzten 4 Wochen eine Angstattacke (plötzliches Gefühl der Furcht oder Panik)?	☐	☐
Wenn „NEIN", gehen Sie bitte weiter zu Frage 5.		
b. Ist dies bereits früher einmal vorgekommen?	☐	☐
c. Treten manche dieser Anfälle völlig unerwartet auf – d. h. in Situationen, in denen Sie nicht damit rechnen, dass Sie angespannt oder beunruhigt reagieren?	☐	☐
d. Empfinden Sie diese Anfälle als stark beeinträchtigend, und/oder haben Sie Angst vor erneuten Anfällen?	☐	☐

4 Denken Sie bitte an Ihren letzten schlimmen Angstanfall.

	NEIN	JA
a. Bekamen Sie schlecht Luft?	☐	☐
b. Hatten Sie Herzrasen, Herzklopfen oder unregelmäßigen Herzschlag?	☐	☐
c. Hatten Sie Schmerzen oder ein Druckgefühl in der Brust?	☐	☐
d. Haben Sie geschwitzt?	☐	☐
e. Hatten Sie das Gefühl zu ersticken?	☐	☐
f. Hatten Sie Hitzewallungen oder Kälteschauer?	☐	☐
g. Wurde Ihnen übel, hatten Sie Magenbeschwerden oder das Gefühl, Sie würden Durchfall bekommen?	☐	☐
h. Fühlten Sie sich schwindelig, unsicher, benommen oder einer Ohnmacht nahe?	☐	☐
i. Spürten Sie ein Kribbeln oder hatten Sie ein Taubheitsgefühl in Teilen Ihres Körpers?	☐	☐
j. Zitterten oder bebten Sie?	☐	☐
k. Hatten Sie Angst, Sie würden sterben?	☐	☐

5 Wie oft fühlten Sie sich im Verlauf der letzten 4 Wochen durch die folgenden Beschwerden beeinträchtigt?

	Überhaupt nicht	An einzelnen Tagen	An mehr als der Hälfte der Tage
a. Nervosität, Ängstlichkeit, Anspannung oder übermäßige Besorgnis	☐	☐	☐
Wenn „Überhaupt nicht", gehen Sie bitte weiter zu Frage 6.			
b. Gefühle der Unruhe, sodass Stillsitzen schwer fällt	☐	☐	☐
c. Leichte Ermüdbarkeit	☐	☐	☐
d. Muskelverspannungen, Muskelschmerzen	☐	☐	☐
e. Schwierigkeiten beim Ein- oder Durchschlafen	☐	☐	☐
f. Schwierigkeiten, sich auf etwas zu konzentrieren, z. B. beim Lesen oder beim Fernsehen	☐	☐	☐
g. Leichte Reizbarkeit, Überempfindlichkeit	☐	☐	☐

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Gesundheitsfragebogen für Patienten (PHQ-D)

6 Fragen zum Thema „Essen"

	NEIN	JA
a. Haben Sie öfter das Gefühl, Sie könnten nicht kontrollieren, wie viel und was Sie essen?	☐	☐
b. Essen Sie öfter – in einem Zeitraum von 2 Stunden – Mengen, die andere Leute als ungewöhnlich groß bezeichnen würden?	☐	☐
Wenn „NEIN" bei a oder b, gehen Sie bitte zu Frage 9.		
c. Ist dies während der letzten 3 Monate im Durchschnitt mindestens zweimal in der Woche vorgekommen?	☐	☐

7 Haben Sie während der letzten 3 Monate öfter eine oder mehrere der folgenden Maßnahmen unternommen, um eine Gewichtszunahme zu vermeiden?

	NEIN	JA
a. Sich selbst zum Erbrechen gebracht?	☐	☐
b. Mehr als die doppelte empfohlene Dosis eines Abführmittels eingenommen?	☐	☐
c. Gefastet, d. h. mindestens 24 Stunden lang nichts gegessen?	☐	☐
d. Mehr als eine Stunde Sport getrieben mit dem ausschließlichen Ziel, nicht zuzunehmen, wenn Sie wie oben beschrieben (6a oder 6b) gegessen haben?	☐	☐

8 Wenn Sie bei einer oder mehrerer dieser Maßnahmen, die eine Gewichtszunahme vermeiden sollen, „JA" angekreuzt haben, kam eine davon im Durchschnitt mindestens zweimal in der Woche vor?

NEIN	JA
☐	☐

	NEIN	JA
9 Trinken Sie manchmal Alkohol (einschließlich Bier oder Wein)?	☐	☐
Wenn „NEIN", gehen Sie bitte weiter zu Frage 11.		

10 Ist bei Ihnen im Laufe der letzten 6 Monate mehr als einmal eine der folgenden Situationen eingetreten?

	NEIN	JA
a. Sie haben Alkohol getrunken, obwohl Ihnen ein Arzt angeraten hat, aus gesundheitlichen Gründen mit dem Trinken aufzuhören?	☐	☐
b. Sie haben bei der Arbeit, in der Schule, bei der Versorgung der Kinder oder bei der Wahrnehmung anderer Verpflichtungen Alkohol getrunken, waren angetrunken oder „verkatert"?	☐	☐
c. Sie sind der Arbeit, der Schule oder anderen Verpflichtungen fern geblieben oder sind zu spät gekommen, weil Sie getrunken hatten oder „verkatert" waren?	☐	☐
d. Sie hatten Schwierigkeiten, mit anderen auszukommen, weil Sie getrunken hatten?	☐	☐
e. Sie sind Auto gefahren, nachdem Sie mehrere Gläser Alkohol bzw. zu viel getrunken hatten?	☐	☐

11 Wenn eines oder mehrere der bisher in diesem Fragebogen beschriebenen Probleme bei Ihnen vorliegen, geben Sie bitte an, wie sehr diese Probleme es Ihnen erschwert haben, Ihre Arbeit zu tun, Ihren Haushalt zu regeln oder mit anderen Menschen zurecht zu kommen:

Überhaupt nicht erschwert	Etwas erschwert	Relativ stark erschwert	Sehr stark erschwert
☐	☐	☐	☐

Deutsche Übersetzung und Validierung des „Patient Health Questionnaire (PHQ)" durch B. Löwe, S. Zipfel und W. Herzog, Medizinische Universitätsklinik Heidelberg. (Englische Originalversion: Spitzer, Kroenke & Williams, 1999).

A16. Relaxation Inventory Scale (RIS) (Crist et al., 1989)

Relaxation Inventory Scale

Lesen sie sich die Aussagen durch und kreuzen sie diejenige Antwort an, mit der sie am meisten übereinstimmen.

1= Stimme ich voll zu, 2=Stimme ich eher zu, 3=Ich bin mir nicht sicher, 4=Stimme ich eher nicht zu, 5=Stimme ich gar nicht zu

A. Physiologische Spannungsskala

a. Mein Gesicht fühlt sich gerötet an.

(1) Stimme ich voll zu. (2) Stimme ich eher zu. (3) Ich bin mir nicht sicher. (4) Stimme ich eher nicht zu. (5) Stimme ich gar nicht zu.

b. Meine Stirn fühlt sich angespannt an.

(1) Stimme ich voll zu. (2) Stimme ich eher zu. (3) Ich bin mir nicht sicher. (4) Stimme ich eher nicht zu. (5) Stimme ich gar nicht zu.

c. Ich schwitze, weil ich angespannt bin.

(1) Stimme ich voll zu. (2) Stimme ich eher zu. (3) Ich bin mir nicht sicher. (4) Stimme ich eher nicht zu. (5) Stimme ich gar nicht zu.

d. Mir ist etwas heiß.

(1) Stimme ich voll zu. (2) Stimme ich eher zu. (3) Ich bin mir nicht sicher. (4) Stimme ich eher nicht zu. (5) Stimme ich gar nicht zu.

e. Mir ist heiß.

(1) Stimme ich voll zu. (2) Stimme ich eher zu. (3) Ich bin mir nicht sicher. (4) Stimme ich eher nicht zu. (5) Stimme ich gar nicht zu.

f. Ich scheine mehr als sonst zu schwitzen.

(1) Stimme ich voll zu. (2) Stimme ich eher zu. (3) Ich bin mir nicht sicher. (4) Stimme ich eher nicht zu. (5) Stimme ich gar nicht zu.

g. Meine Handflächen sind verschwitzt.

(1) Stimme ich voll zu. (2) Stimme ich eher zu. (3) Ich bin mir nicht sicher. (4) Stimme ich eher nicht zu. (5) Stimme ich gar nicht zu.

h. Meine Atmung ist schneller als normal.

(1) (2) (3) (4) (5)

i. Meine Atmung ist beschleunigt.

(1) Stimme ich voll zu. (2) Stimme ich eher zu. (3) Ich bin mir nicht sicher. (4) Stimme ich eher nicht zu. (5) Stimme ich gar nicht zu.

j. Mein Kiefer ist angespannt.

(1) Stimme ich voll zu. (2) Stimme ich eher zu. (3) Ich bin mir nicht sicher. (4) Stimme ich eher nicht zu. (5) Stimme ich gar nicht zu.

k. Einige meiner Muskeln beginnen sich zu verkrampfen.

(1) Stimme ich voll zu. (2) Stimme ich eher zu. (3) Ich bin mir nicht sicher. (4) Stimme ich eher nicht zu. (5) Stimme ich gar nicht zu.

l. Meine Herzfrequenz ist erhöht.

(1)	(2)	(3)	(4)	(5)
Stimme ich voll zu.	Stimme ich eher zu.	Ich bin mir nicht sicher.	Stimme ich eher nicht zu.	Stimme ich gar nicht zu.

m. Die Muskeln in meinem Rücken sind angespannt.

(1)	(2)	(3)	(4)	(5)
Stimme ich voll zu.	Stimme ich eher zu.	Ich bin mir nicht sicher.	Stimme ich eher nicht zu.	Stimme ich gar nicht zu.

n. Ich spüre, dass ich Kopfschmerzen bekomme.

(1)	(2)	(3)	(4)	(5)
Stimme ich voll zu.	Stimme ich eher zu.	Ich bin mir nicht sicher.	Stimme ich eher nicht zu.	Stimme ich gar nicht zu.

o. Mein Herz schlägt schneller als sonst.

(1)	(2)	(3)	(4)	(5)
Stimme ich voll zu.	Stimme ich eher zu.	Ich bin mir nicht sicher.	Stimme ich eher nicht zu.	Stimme ich gar nicht zu.

B. Skala der körperlichen Beurteilung

a. Meine Muskeln sind locker.

(1)	(2)	(3)	(4)	(5)
Stimme ich voll zu.	Stimme ich eher zu.	Ich bin mir nicht sicher.	Stimme ich eher nicht zu.	Stimme ich gar nicht zu.

b. Mein ganzer Körper ist erholt.

(1)	(2)	(3)	(4)	(5)
Stimme ich voll zu.	Stimme ich eher zu.	Ich bin mir nicht sicher.	Stimme ich eher nicht zu.	Stimme ich gar nicht zu.

c. Ich bin zufrieden.

(1)	(2)	(3)	(4)	(5)
Stimme ich voll zu.	Stimme ich eher zu.	Ich bin mir nicht sicher.	Stimme ich eher nicht zu.	Stimme ich gar nicht zu.

d. Ich fühle mich sehr friedlich.

(1)	(2)	(3)	(4)	(5)
Stimme ich voll zu.	Stimme ich eher zu.	Ich bin mir nicht sicher.	Stimme ich eher nicht zu.	Stimme ich gar nicht zu.

e. Mein ganzer Körper fühlt sich locker an.

(2)				
Stimme ich voll zu.	Stimme ich eher zu.	Ich bin mir nicht sicher.	Stimme ich eher nicht zu.	Stimme ich gar nicht zu.

f. Ich fühle eine Art von Frieden.

(1)	(2)	(3)	(4)	(5)
Stimme ich voll zu.	Stimme ich eher zu.	Ich bin mir nicht sicher.	Stimme ich eher nicht zu.	Stimme ich gar nicht zu.

g. Meine Muskeln fühlen sich entspannt an.

(1)	(2)	(3)	(4)	(5)
Stimme ich voll zu.	Stimme ich eher zu.	Ich bin mir nicht sicher.	Stimme ich eher nicht zu.	Stimme ich gar nicht zu.

h. Ich fühle mich gerade wirklich unbeschwert.

(1)	(2)	(3)	(4)	(5)
Stimme ich voll zu.	Stimme ich eher zu.	Ich bin mir nicht sicher.	Stimme ich eher nicht zu.	Stimme ich gar nicht zu.

i. Ich bin sehr ruhig.

(1) Stimme ich voll zu. (2) Stimme ich eher zu. (3) Ich bin mir nicht sicher. (4) Stimme ich eher nicht zu. (5) Stimme ich gar nicht zu.

j. Ich fühle ein Gefühl von Ruhe in meinem Körper.

(1) Stimme ich voll zu. (2) Stimme ich eher zu. (3) Ich bin mir nicht sicher. (4) Stimme ich eher nicht zu. (5) Stimme ich gar nicht zu.

k. Ich fühle mich sehr entspannt.

(1) Stimme ich voll zu. (2) Stimme ich eher zu. (3) Ich bin mir nicht sicher. (4) Stimme ich eher nicht zu. (5) Stimme ich gar nicht zu.

l. Ich fühle mich gelassen.

(1) (2) (3) (4) (5)

m. Ich fühle mich wirklich locker.

(1) Stimme ich voll zu. (2) Stimme ich eher zu. (3) Ich bin mir nicht sicher. (4) Stimme ich eher nicht zu. (5) Stimme ich gar nicht zu.

n. Ich fühle mich wirklich extrem wohl.

(1) Stimme ich voll zu. (2) Stimme ich eher zu. (3) Ich bin mir nicht sicher. (4) Stimme ich eher nicht zu. (5) Stimme ich gar nicht zu.

o. Ich fühle mich geschmeidig.

(1) Stimme ich voll zu. (2) Stimme ich eher zu. (3) Ich bin mir nicht sicher. (4) Stimme ich eher nicht zu. (5) Stimme ich gar nicht zu.

p. Mein Kopf ist frei.

(1)	(2)	(3)	(4)	(5)
Stimme ich voll zu.	Stimme ich eher zu.	Ich bin mir nicht sicher.	Stimme ich eher nicht zu.	Stimme ich gar nicht zu.

q. Meine Muskeln sind im Ruhezustand.

(1)	(2)	(3)	(4)	(5)
Stimme ich voll zu.	Stimme ich eher zu.	Ich bin mir nicht sicher.	Stimme ich eher nicht zu.	Stimme ich gar nicht zu.

r. Sehr wenige Dinge könnten mich jetzt stören oder beunruhigen.

(1)	(2)	(3)	(4)	(5)
Stimme ich voll zu.	Stimme ich eher zu.	Ich bin mir nicht sicher.	Stimme ich eher nicht zu.	Stimme ich gar nicht zu.

s. Ich spüre im Moment überhaupt keine Anspannung in meinen Muskeln.

(1)	(2)	(3)	(4)	(5)
Stimme ich voll zu.	Stimme ich eher zu.	Ich bin mir nicht sicher.	Stimme ich eher nicht zu.	Stimme ich gar nicht zu.

t. Ich fühle mich erfrischt.

(1)	(2)	(3)	(4)	(5)
Stimme ich voll zu.	Stimme ich eher zu.	Ich bin mir nicht sicher.	Stimme ich eher nicht zu.	Stimme ich gar nicht zu.

C. Kognitive Spannungsskala

a. Versagensängste machen sich in meinem Kopf breit.

(1)	(2)	(3)	(4)	(5)
Stimme ich voll zu.	Stimme ich eher zu.	Ich bin mir nicht sicher.	Stimme ich eher nicht zu.	Stimme ich gar nicht zu.

b. Ich denke über meine Probleme nach.

(1)	(2)	(3)	(4)	(5)
Stimme ich voll zu.	Stimme ich eher zu.	Ich bin mir nicht sicher.	Stimme ich eher nicht zu.	Stimme ich gar nicht zu.

c. Ich bin wirklich gerade sehr besorgt über alle meine Probleme.

(1)	(2)	(3)	(4)	(5)
Stimme ich voll zu.	Stimme ich eher zu.	Ich bin mir nicht sicher.	Stimme ich eher nicht zu.	Stimme ich gar nicht zu.

d. Ich fühle mich ein wenig ängstlich.

(1)	(2)	(3)	(4)	(5)
Stimme ich voll zu.	Stimme ich eher zu.	Ich bin mir nicht sicher.	Stimme ich eher nicht zu.	Stimme ich gar nicht zu.

e. Ich denke über die Zukunft nach.

(1)	(2)	(3)	(4)	(5)
Stimme ich voll zu.	Stimme ich eher zu.	Ich bin mir nicht sicher.	Stimme ich eher nicht zu.	Stimme ich gar nicht zu.

f. Ich habe das Gefühl, ich bin in einem Zustand mentaler Belastung.

(1)	(2)	(3)	(4)	(5)
Stimme ich voll zu.	Stimme ich eher zu.	Ich bin mir nicht sicher.	Stimme ich eher nicht zu.	Stimme ich gar nicht zu.

g. Ich denke über meine Karriere nach.

(1)	(2)	(3)	(4)	(5)
Stimme ich voll zu.	Stimme ich eher zu.	Ich bin mir nicht sicher.	Stimme ich eher nicht zu.	Stimme ich gar nicht zu.

h. Ich mache mir Sorgen darüber, wieviel Geld ich habe.

(1)	(2)	(3)	(4)	(5)
Stimme ich voll zu.	Stimme ich eher zu.	Ich bin mir nicht sicher.	Stimme ich eher nicht zu.	Stimme ich gar nicht zu.

i. Ich möchte nicht, dass andere wissen was ich fühle.

(1)	(2)	(3)	(4)	(5)
Stimme ich voll zu.	Stimme ich eher zu.	Ich bin mir nicht sicher.	Stimme ich eher nicht zu.	Stimme ich gar nicht zu.

j. Ich scheine mich um andere zu sorgen.

(1)	(2)	(3)	(4)	(5)
Stimme ich voll zu.	Stimme ich eher zu.	Ich bin mir nicht sicher.	Stimme ich eher nicht zu.	Stimme ich gar nicht zu.

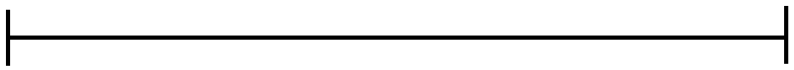
Original Version in English: The Relaxation Inventory: Self-Report Scales of Relaxation Training Effects by Dwayne A. Christ, henry C. Rickard, Steven Prentice-Dunn, Harry R. Barker. University of Alabama. 1989

A17. Visual Analog Scale (VAS_{Stress})

Visuelle Analog Skala zur Studie

”Entspannung in der Schwangerschaft”

Wie gestresst fühlen sie Sich jetzt gerade? (Bitte kreuzen sie an.)



0 10

Gar nicht gestresst *Sehr gestresst*

A18. Questionnaire for rating the relaxation stimulation (Q_{STIM})

”Entspannung in der Schwangerschaft”

Wie fanden sie die Entspannungsinduktion? (Bitte kreuzen sie an.)

☐ ☐ ☐

Nicht gut *gut* *Sehr gut*

A19. Trier Inventory of Chronic Stress (TICS) (Schulz et al., 2004)

TICS **Fragebogen**

Name: _____ Alter: _____ Testdatum: _____

Auf den folgenden Seiten finden Sie einige Fragen, die Sie danach beurteilen sollen, wie häufig Sie die darin angesprochene Erfahrung gemacht bzw. Situation erlebt haben. Ihre Aufgabe ist es, anzugeben, ob Sie die darin angesprochenen Erfahrungen bzw. Situationen **nie, selten, manchmal, häufig** oder **sehr häufig** gemacht bzw. erlebt haben. Denken Sie bei der Beantwortung bitte an die, vom heutigen Tag aus gesehen, **vergangenen drei Monate** und versuchen Sie sich daran zu erinnern, wie oft Sie in diesem Zeitraum die jeweilige Erfahrung gemacht haben.

Dabei bedeuten:

⓪ = nie (das habe ich nie erlebt)
① = selten (das habe ich selten erlebt)
② = manchmal (das habe ich manchmal erlebt)
③ = häufig (das habe ich häufig erlebt)
④ = sehr häufig (das habe ich sehr häufig erlebt)

Zum Beispiel kann eine Frage so lauten:

Erfahrung	In den letzten drei Monaten wie oft erlebt?				
	nie	selten	manch-mal	häufig	sehr häufig
Zu viele Kontakte mit anderen Menschen, denen ich lieber ausweichen würde	⓪	①	②	③	④

Haben Sie diese Erfahrung in den letzten 3 Monaten **nie** gemacht, durchkreuzen Sie bitte: ⓪
Haben Sie diese Erfahrung in den letzten 3 Monaten **selten** gemacht, durchkreuzen Sie bitte: ①
Haben Sie diese Erfahrung in den letzten 3 Monaten **manchmal** gemacht, durchkreuzen Sie bitte: ②
Haben Sie diese Erfahrung in den letzten 3 Monaten **häufig** gemacht, durchkreuzen Sie bitte: ③
Haben Sie diese Erfahrung in den letzten 3 Monaten **sehr häufig** gemacht, durchkreuzen Sie bitte: ④

Bitte beantworten Sie **alle** Fragen der Reihe nach, ohne eine auszulassen. Einige Aussagen klingen ähnlich oder haben einen ähnlichen Sinn. Bitte beantworten Sie sie trotzdem. Es kommt bei der Beantwortung nicht auf Schnelligkeit an; nehmen Sie sich Zeit, über die Beantwortung nachzudenken.

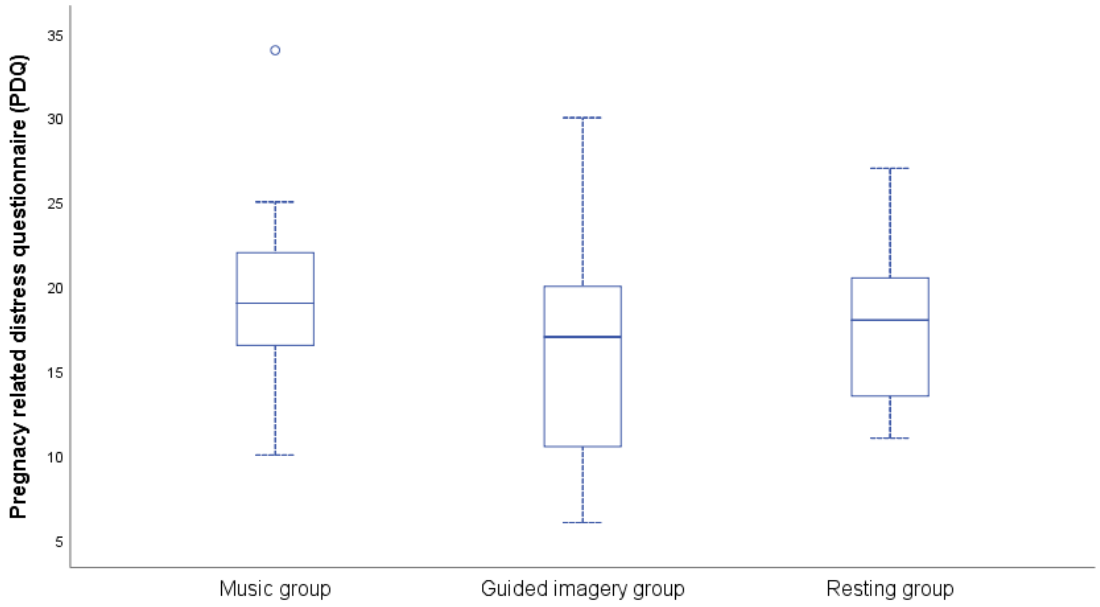
Bitte beginnen Sie jetzt.

	Erfahrung	In den letzten drei Monaten wie oft erlebt?				
		nie	selten	manch-mal	häufig	sehr häufig
01	Zeiten, in denen ich dringend benötigte Erholung aufschieben muss	⓪	①	②	③	④
02	Ich bekomme zu wenig Anerkennung für das, was ich leiste	⓪	①	②	③	④
03	Ich mache zu viele Fehler, weil ich mit dem, was ich zu tun habe, überfordert bin	⓪	①	②	③	④
04	Ich habe zu wenig Zeit, um meine täglichen Aufgaben zu erfüllen	⓪	①	②	③	④
05	Ich muss Arbeiten erledigen, die mir unsinnig erscheinen	⓪	①	②	③	④
06	Es gibt Meinungsverschiedenheiten zwischen mir und anderen, die zu Spannungen führen	⓪	①	②	③	④
07	Ich habe Arbeiten zu erledigen, bei denen ich sehr viel Verantwortung für andere Menschen trage	⓪	①	②	③	④
08	Situationen, in denen ich mich anstrengen muss, das Vertrauen anderer zu gewinnen	⓪	①	②	③	④
09	Befürchtung, dass irgendetwas Unangenehmes passiert	⓪	①	②	③	④
10	Mir fehlen interessante Aufgaben, die meinen Tag ausfüllen	⓪	①	②	③	④
11	Zeiten, in denen ich zu viel allein bin	⓪	①	②	③	④
12	Situationen, in denen ich mich um eine gute Beziehung zu anderen bemühen muss	⓪	①	②	③	④
13	Ich muss Aufgaben erledigen, die ich nicht gern mache	⓪	①	②	③	④
14	Ich habe Aufgaben zu erledigen, bei denen ich unter kritischer Beobachtung stehe	⓪	①	②	③	④
15	Ich habe Streit mit anderen, weil diese etwas anderes wollen als ich	⓪	①	②	③	④
16	Zeiten, in denen ich sorgenvolle Gedanken **nicht** unterdrücken kann	⓪	①	②	③	④
17	Zeiten, in denen sich die Termine so häufen, dass sie kaum zu bewältigen sind	⓪	①	②	③	④
18	Ich bemühe mich vergeblich, mit guten Leistungen Anerkennung zu erhalten	⓪	①	②	③	④
19	Zeiten, in denen ich mich zu viel um die Probleme anderer kümmern muss	⓪	①	②	③	④

	Erfahrung	In den letzten drei Monaten wie oft erlebt?				
		nie	selten	manch-mal	häufig	sehr häufig
20	Ich kann meine Aufgaben nur unzureichend erfüllen, obwohl ich mein Bestes gebe	⓪	①	②	③	④
21	Zeiten, in denen mir Aufgaben fehlen, die mir sinnvoll erscheinen	⓪	①	②	③	④
22	Ich habe Arbeiten zu erledigen, bei denen ich andere nicht enttäuschen darf	⓪	①	②	③	④
23	Kontakte mit anderen Personen, bei denen ich einen guten Eindruck hinterlassen muss	⓪	①	②	③	④
24	Ich werde den Anforderungen bei meiner Arbeit nicht mehr gerecht	⓪	①	②	③	④
25	Zeiten, in denen mir die Sorgen über den Kopf wachsen	⓪	①	②	③	④
26	Ich habe Streit mit anderen, weil ich mich nicht so verhalte, wie andere es von mir erwarten	⓪	①	②	③	④
27	Zeiten, in denen ich unter Termindruck/Zeitnot arbeiten muss	⓪	①	②	③	④
28	Ich [illegible] mich zu viel mit Problemen and[illegible] beschäftigen	⓪	①	[illegible]	③	[illegible]
29	Zeiten, in denen ich keine Möglichkeiten habe, mich [illegible] anderen auszusprechen	[illegible]	①	[illegible]	[illegible]	④
30	Situationen, in denen es ganz allein von mir abhängt, ob ein Konflikt zu einem anderen Menschen zufriedenstellend verläuft	⓪	[illegible]	[illegible]	③	[illegible]
31	Obwohl ich mein Bestes gebe, wird meine Arbeit nicht gewürdigt	⓪	①	②	③	④
32	Ich habe Aufgaben zu erfüllen, bei denen ich mich bewähren muss	⓪	①	②	③	④
33	Ich habe Konflikte mit anderen, weil sie sich zu viel in meine Angelegenheiten einmischen	⓪	①	②	③	④
34	Zeiten, in denen ich von anderen Menschen isoliert bin	⓪	①	②	③	④
35	Zeiten, in denen ich nicht die Leistung bringe, die von mir erwartet wird	⓪	①	②	③	④
36	Zeiten, in denen ich mir viele Sorgen mache und nicht damit aufhören kann	⓪	①	②	③	④
37	Ich muss Verpflichtungen erfüllen, die ich innerlich ablehne	⓪	①	②	③	④

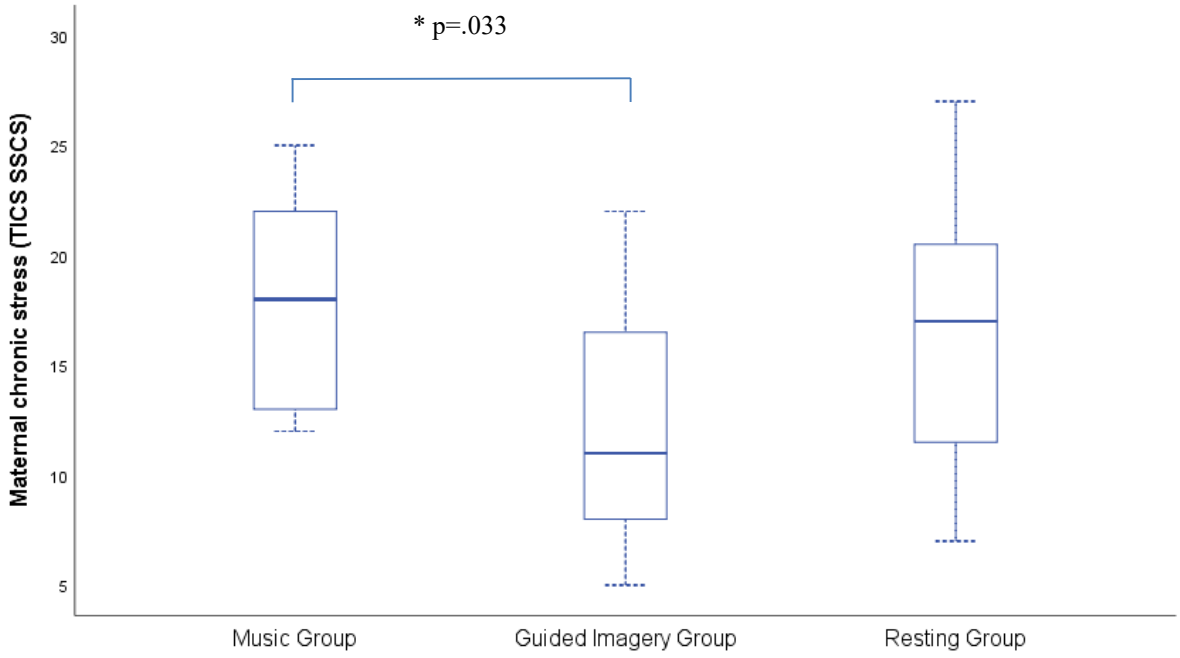
	Erfahrung	In den letzten drei Monaten wie oft erlebt?				
		nie	selten	manch-mal	häufig	sehr häufig
38	Zeiten, in denen ich zu viele Verpflichtungen zu erfüllen habe	⓪	①	②	③	④
39	Ich muss ständig aufs Neue für das Wohl anderer Menschen sorgen	⓪	①	②	③	④
40	Situationen, in denen ich mich anstrengen muss, anderen zu gefallen	⓪	①	②	③	④
41	Zeiten, in denen ich nichts Sinnvolles zu tun habe	⓪	①	②	③	④
42	Zeiten, in denen ich zu wenig Kontakte zu anderen Personen habe	⓪	①	②	③	④
43	Ich muss Aufgaben erfüllen, die mit hohen Erwartungen verbunden sind	⓪	①	②	③	④
44	Zeiten, in denen mir die Arbeit über den Kopf wächst	⓪	①	②	③	④
45	Ich habe Auseinandersetzungen mit anderen Menschen, die zu länger andauernden Konflikten führen	⓪	①	②	③	④
	r mei nsatz w de ich cht a mes lohnt		①	②	③	
4	efürchte meine Au gaben rfüllen zu nnen		①	②	③	④
4	n muss eiten ma en, bei denen ne Fähig n um zur satz kommen		①	②	③	
49	Situationen, in denen das Wohlergehen anderer davon abhängt, wie zuverlässig ich meine Arbeit mache	⓪	①	②	③	④
50	Ich habe zu viele Aufgaben zu erledigen	⓪	①	②	③	④
51	Zeiten, in denen mir Kontakte zu anderen Menschen fehlen	⓪	①	②	③	④
52	Ich habe unnötigen Streit mit anderen Personen	⓪	①	②	③	④
53	Zeiten, in denen mir Aufgaben fehlen, die mir Freude bereiten	⓪	①	②	③	④
54	Erfahrung, dass alles zu viel ist, was ich zu tun habe	⓪	①	②	③	④
55	Obwohl ich mich bemühe, erfülle ich meine Aufgaben nicht so, wie es sein sollte	⓪	①	②	③	④
56	Zeiten, in denen mir Freunde fehlen, mit denen ich etwas unternehmen kann	⓪	①	②	③	④
57	Zeiten, in denen mir die Verantwortung für andere zur Last wird	⓪	①	②	③	④

A20. Study 1: Pregnancy related distress Questionnaire (PDQ) values between groups.



Scale of pregnancy-related distress: Overview of mean values in all groups: music, guided imagery, resting. (from Bauer et al., 2021, *Figure 3*)

A21. Study 1: Trier Inventory of Chronic Stress (TICS) values between groups.



Short Screening Scale of Chronic Stress (SSCS): Overview of mean values in all groups: music, guided imagery, resting. (from Bauer et al., 2021, *Figure 2*)