Workplace Bullying, Depression and Cortisol

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PhD Thesis

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PREFACE

This Ph.D. thesis arises from the Modena Project, which was funded by the Danish Working Environment Research Fund in 2010. The overall aim of the project was to investigate exposure to workplace bullying and risk of mental distress with a longitudinal design. The Modena Project gave us the opportunity to develop and use independent measurements of both exposure and outcome. The Modena Project is a joint collaboration between four Danish research institutions; the Department of Occupational and Environmental Medicine at Bispebjerg University Hospital, the Department of Occupational Medicine at Aarhus University Hospital, the National Research Centre for the Working Environment, and the Institute of Psychology at the University of Copenhagen.

I have held a key position in the Modena Project throughout the whole process; planning the data collection, establishing the survey, undertaking screenings for standardized diagnostic interviews, making contact to respondents, planning and inviting respondents to interviews and clinical sessions, cleaning data, and completing the Modena database. This work has resulted in the present Ph.D thesis, and a complete, three-wave database, which can be used in future work on workplace bullying and the psychological and physiological consequences of this.

I would like to thank my supervisors, Jens Peter Bonde, Åse Marie Hansen and Annie Høgh for their support and advice throughout the process. I have enjoyed being inspired by you and I truly appreciate all your knowledge and expertise in the field of occupational health from which I have learned a lot, developed my competencies within many areas, and strengthened my integrity and wisdom.

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the group, and even though I have only worked with you for a short time, I owe you a great deal of recognition for your help with my work on cortisol.

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In my everyday life as a Ph.D candidate I have spent many hours in the office at Bispebjerg University Hospital, so to all my officemates; Ina, Marianne, Emina, Thomas, Kasper, Dorthe, Rikke, Kristian and Hanne, thank you so much for fun and inspiring lunch- and coffee breaks, as well as support, when processes have been frustrating, but most of all, thank you all for the lovely and trusting friendships that have developed throughout the years.

To my dearest mom and dad, my family and friends; all of you have been a fantastic support on this journey. I appreciate your continued support and would like to thank you all for always standing by me.

Finally, Trond and Birk, my lovely little family, you two are my greatest supporters. To you, my dearest Trond, you deserve my deepest and greatest gratitude. Your positive and never-ending help and assistance has been encouraging and appreciated, more than you will ever realize.

Maria Gullander
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LIST OF ORIGINAL PAPERS

This thesis is based on three papers:

Paper I
Manuscript: Exposure to workplace bullying and risk of depression

Gullander Maria (M.Sc), Hogh Annie (Prof.), Hansen Åse Marie (Prof.), Persson Roger (Ph.D.), Rugulies Reiner (Prof.), Kolstad Henrik Albert (Prof.), Thomsen Jane Frølund (Ph.D), Willert Morten Veis (Ph.D), Grynderup Matias (Ph.D), Mors Ole (Prof.), Bonde Jens Peter (Prof.)

Submitted in the Journal of Occupational and Environmental Medicine
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Paper II
Manuscript: Is workplace bullying related to changes in levels of cortisol in saliva?

Gullander Maria (M.Sc), Grynderup Matias (Ph.D), Hansen Åse Marie (Prof.), Hogh Annie (Prof.), Persson Roger (Ph.D.), Kolstad Henrik Albert (Prof.), Thomsen Jane Frølund (Ph.D), Mors Ole (Prof.), Andersen Johan Hviid (Ph.D), Kærlev Linda (Ph.D), Bonde Jens Peter (Prof.)

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Paper III
Manuscript: An examination of cortisol reactivity and recovery during low level exercise among workplace bullied persons

Gullander Maria (M.Sc), Harris Anette (Ph.D), Hansen Åse Marie (Prof.), Hogh Annie (Prof.), Grynderup Matias (Ph.D), Persson Roger (Ph.D.), Kolstad Henrik Albert (Prof.), Kærlev Linda (Ph.D), Bonde Jens Peter (Prof.)

Manuscript to be submitted

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LIST OF ABBREVIATIONS

LIPT	Leymann Inventory of Psychological Terror
NAQ	Negative Acts Questionnaire
BDI	Beck Depression Inventory
ZSDS	Zung Self-rating Depression Scale
MDI	Major Depression Inventory
HSCL-25	Hopkins Symptom Check List
CES-D	Center for Epidemiological Studies – Depression Scale
CMDQ	Common Mental Disorder Questionnaire
HAD Scale	Hospital Anxiety and Depression Scale
ICD	International Classification of Diseases
SCAN	Schedules for Clinical Assessment in Neuropsychiatry

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1 INTRODUCTION

Over the last decade workplace bullying has received an increased amount of attention from researchers within different disciplines, and it has been revealed as a complex and serious phenomenon. Among psychologists and physicians working with victims of workplace bullying, it is well known that people who perceive themselves as being bullied, feel humiliated and report harsh and unfair treatment at work. Employees exposed to workplace bullying often express feelings in line with symptoms of anxiety, depression, Post-Traumatic Stress Disorders and burnout; and they also often describe their emotional state as being frustrated, helpless, powerless, unable to concentrate, as well as having low self-esteem, and low self-efficiency [1]. Their stories are often characterized by descriptions of situations where colleagues or managers deliberately try to hurt and demean them. For some employees it has meant exclusion from a community, where a person's social position has been seen as uninteresting, dull and unworthy [2].

In 2009, The National Board of Industrial Injuries in Denmark acknowledged that it was necessary to obtain more knowledge on the causality of workplace bullying and development of mental distress. This is due to an increase of reports of work-related injury arising from workplace bullying and harassment. It is usually difficult to have mental disorders be acknowledged as a work related injury, as it is problematic to prove that the mental disorder has emerged from work environment, e.g. because of exposure to workplace bullying. Most often, there are so-called "competitive conditions", e.g. contributory factors at home, or having previously suffered from a depression.

The main focus of this Ph.D. thesis is exposure to workplace bullying, depression and cortisol. It contributes to the debate on the health consequences of workplace bullying, and includes a deeper understanding of the methodological issues that are associated with the investigation of workplace bullying. The background chapter gives an overview of workplace bullying, depression and cortisol, and includes a review of studies in the research field. Subsequently, the aims of the thesis will be presented, followed by an overview of methods used in *Paper I*, *II* and *III* and the ensuing results.

Lastly, methodological issues and the findings of the thesis will be discussed, followed by conclusion and future implications.

2 BACKGROUND

2.1 Workplace bullying – what does it mean?

Within bullying research, several terms and definitions have been used in the investigation of the phenomenon: Workplace Harassment [3], Mobbing [4], or Workplace Abuse [5]. However, the most used definition is from Einarsen (1996) [6], who describes workplace bullying as *harassing, offending, socially excluding someone or negatively affecting someone's work repeatedly and regularly over a longer period, e.g. six months*. The definition is developed from a definition by Olweus (1993) [7], who stated that three criteria have to be present if a situation can be defined as bullying: (1) The bullying is a negative and unwanted behavior by the target, (2) The bullying is carried out systematically and over time, and (3) The bullying occurs in an interpersonal relationship, characterized by an imbalance of power [7]. The definition by Einarsen (1996) [6] refers to the escalation of the bullying process where the target is persistently and over time, subjected to negative behavior and increasingly unable to defend him- or herself against this behavior. Because the definition of workplace bullying includes the above-mentioned criteria, it is significant for the understanding of workplace bullying to be aware of the chronicity part of the definition.

Workplace bullying not only influences the person exposed to the humiliating behavior, but also the workplace and society at large [8]. Studies have also shown that low satisfaction with the leadership, low work control [9], high role conflict [9, 10], poor job content, and a negative social environment as well as a negative social climate [6] are the consequences of bullying in a working environment.

Research has revealed that the prevalence of workplace bullying varies between countries, cultures and job categories [11], and furthermore among ways of measurements of workplace bullying [12]. For example, in a study among 1657 employees in the Royal Norwegian Navy, 2,5% reported being bullied at work [13]. This was measured with a single question, and the result did not provide information about the frequency of the exposure to bullying. In a study among the French working population (n=7694) Niedhammer and and co-workers (2006) [14] revealed that 4% males and 6% females were

exposed to bullying at work daily or almost daily. In this study, cases of bullying were defined using both the Leymann Inventory of Psychological Terror (LIPT) and self-labeling. Furthermore, in a British study among 5288 employees in different organizations, current exposure to bullying at work was reported by 11%, 2% of whom were *Frequently Bullied* [15]. This study was measured by self-report with five answer alternatives. The three studies illustrate the diversity in methods used to measure workplace bullying.

In 2012, 16,300 Danish employees responded on the national survey *Work Environment and Health* provided by the National Research Center for Work Environment. Among those, 10% reported being *Occasionally* exposed to workplace bullying, and 2% reported being exposed to workplace bullying *Frequently,* within the last 12 months [16]. Several studies have estimated the prevalence of workplace bullying among employees in Denmark. A representative population study showed an overall prevalence of workplace bullying of 8.3%, among those 6.2% reported *Occasionally Bullied* whereas 1.6% reported being *Frequently Bullied* at work [17]. Rugulies and co-workers (2012) [18] reported 10% *Occasionally Bullied* and 1.9% *Frequently Bullied* among 5629 women in the eldercare sector, whereas Hogh and co-workers(2011) [19] revealed that among newly educated health care workers 7.4% reported being *Occasionally Bullied* and 1.8% reported being *Frequently Bullied* in the first year after their graduation.

2.1.2 Assessment of workplace bullying

Assessment of workplace bullying is difficult since the concept reflects many aspects, and may be influenced by reporting bias. However, the most used methods are 1) to determine if employees feel victimized by bullying, or 2) to assess employee's perception of being exposed to a range of specific bullying behaviors.

The first assessment is referred to as the self-labeling method and is measured with a single item with response categories as either *Yes* or *No*, or with a scale that also indicates the frequency of exposure [20]. This method does not indicate what kind of bullying behavior the employee has been exposed to, but reports if an employee identifies him or herself as a victim of bullying at work. When using this method, it is recommended to present a definition of

workplace bullying to the respondent, prior to the interview [20]. The second method is referred to as the *Behavioral Experience Method*, which defines what kind of bullying behaviors employees are exposed to [20]. This method does not provide information about whether or not a person perceives the acts as workplace bullying. This assessment is predominantly measured with the *Negative Acts Questionnaire* (NAQ) [21], which includes 22 items of bullying behaviors with a five-point response category [20]. Other measurements for the behavioral experience method is the LIPT [22], or adjusted versions of the NAQ that are developed to specific occupational groups.

2.1.3 Witnesses to workplace bullying

Witnesses to workplace bullying often constitute a larger group than those being bullied in a working environment [23]. This group represents employees in a work unit who are not directly victims of bullying behavior or directly perpetrators. Assessing witnesses to workplace bullying is often done in line with the self-labeling method; with a single item and with response categories as *Yes* or *No*, or with a scale that indicates the frequency of employees witnessing workplace bullying in the working environment [20].

2.2 Depression

Depression is a mental disease characterized by episodes of depressed mood, loss of interests and decreased energy that persists for at least 14 days [24]. The diagnosis of depression covers a spectrum of disorders ranging from relatively light conditions to a severe life-threatening disease. The World Health Organization has placed depression as the fourth out of ten most common diseases in the world [25, 26]. In the European population the lifetime prevalence of depression is approximately 13% [24] and it is currently the leading burden of disease assessed by disability-adjusted life years in middle and high-income countries [25].

Depression is more prevalent among women than men, and the peak of first-onset depression episode is between 25 and 45 years [27]. Several circumstances have been related to the occurrence of depression, such as old

age, low socioeconomic status, low educational level, alcohol consumption, smoking, a family history of depression, certain personality traits, previous depression, and stressful life events [28-31]. The duration of a depression is normally 3-12 months, and 10-30% of the patients are at risk of developing chronic depression due to the risk of new depressive episodes increasing with the number of previous depressive episodes [32]. Depression has a high co-morbidity with other mental disorders, and although depressive episodes rarely last more than a year, the disorder is highly recurrent and can have an impact for life.

2.2.1 Assessment of depression

Depression rating scales are the preferred instruments to measure depression in surveys where several self-rating scales have been used in studies: the Beck Depression Inventory (BDI) [33] Zung Self-rating Depression Scale (ZUNG-SDS) [34], Major Depression Inventory (MDI) [35], and the Center for Epidemiological Studies – Depression Scale (CES-D) [36] and Common Mental Disorder Questionnaire (CMDQ) [37]. The conventional clinical procedure is to have a trained person to interview the person with suspected depression and check for the occurrence and duration of a predefined set of symptoms, which form the basis for the diagnosis according to International Classification of Diseases (ICD-10) or Diagnostic and Statistic Manual of Mental Disorders (DSM) [38]. An often used interview scale is the Hamilton Depression Scale [39], the Schedules for Clinical Assessment in Neuropsychiatry (SCAN) [40] and the Composite International Diagnostic Interview [41].

2.3 Cortisol

Cortisol is a stress hormone produced in the cortex of the adrenal glands and is involved in the psychopathology reflecting emotional arousal and regulates the metabolic system and the anti-inflammatory pathways [42]. The release of cortisol is facilitated by the Hypothalamus-Pituitary-Adrenal (HPA)-axis where the primary stimulus comes from the Corticotrophin-Releasing Hormone (CRH). CRH is locally produced in hypothalamus. A stimulus of an acute stressor increases the CRH and induces increased secretion of

Adrenocorticotropic Hormone (ATCH) from the pituitary gland. The cortex of the adrenal gland is stimulated by ATCH and triggers the secretion of cortisol into the circulation.

Cortisol exhibits a distinct diurnal variation with a peak in the morning approximately 45 minutes after awakening where cortisol increases up to 50% [43]. Cortisol declines during the day and is low during the evening and at night [43]. Several secretory episodes of short duration and high amplitudes, which depend on the activity, are seen in the variation of cortisol during a day. The short term activations are thereby necessary and adaptive for humans; however, a long term activation of the HPA-axis may be related to negative health outcomes [44]. A cortisol rise increases the oxygen and energy supply, which translates into a temporary increase in blood pressure, levels of blood glucose and free fatty acids. For example, a hypersecretion of cortisol may have implications for the physiological pathways that mediates effects of chronic stress, and can lead to dysregulation of multiple other physiological systems including the autonomic nervous system, the metabolic system, the gut, the kidneys and the immune system [45]. Among others, the changes of the diurnal rhythm have been associated with psychological and somatic complaints; e.g. major depression [46].

In a perspective of workplace bullying, Dickerson and Kemeny (2009) have suggested that even though a variety of situations can elicit negative feelings, only the threatening component of a situation elicits a particular physiological response [47]. They further propose that the motive to defend the Social Self is supported by specific biological processes that include the HPA activation. Dickerson and Kemeny (2004) uses the term *Social Self-Preservation System* for a biological skill that monitors the environment for threats to one's social esteem or social status, and coordinates psychological, physiological and behavioral responses to cope with such threats [48]. Responses to threatening situations include an increase in negative self-evaluations (negative self-related thoughts and feelings), increase in cortisol, and changes in other physiological parameters. This is defined as the *Social-Evaluative-Threat*. The magnitude of these responses depends on the intensity of the threat, its context, and the presence of vulnerability and protective factors in the individual and social environment [48].

2.3.1 Assessment of cortisol

Cortisol has been a tool widely used to investigate the responsiveness of the HPA axis in occupational stress studies, in both field- and experimental studies [49]. The ways of assessing cortisol vary among many options and can be measured in blood, urine, hair and saliva. Cortisol is used as a biomarker of the diurnal rhythm [50] and activation of adrenocortical activity (HPA activity) [51]. Assessing cortisol in large epidemiological studies offers different alternatives, such as cortisol awakening response, the diurnal slope, area under the curve, morning cortisol, evening cortisol, cortisol at specific time (points across the waking day), reactivity to a momentary stressor, and reactivity to a daily stressor [52].

2.4 Literature search on workplace bullying and depression

During the last 20 years, several studies have been conducted on workplace bullying and health outcomes. Within the last few years, the field of workplace bullying has received more attention and many different themes have been investigated in this relation. In order to search the literature about workplace bullying and depression, a literature review was conducted in PubMed (1960 -) on the 29th of March, 2014. To identify the relevant literature, the search was done in four combinations: *Workplace bullying* AND *Health*, *Workplace bullying* AND *Mental*, *Workplace bullying* AND *Depression,* and *Depression* AND *Bullying* AND *Work*. These search combinations produced 213 publications altogether (see Figure 1). Furthermore, the search strategy was reflected in the following inclusion criteria: 1) The study had to be cross-sectional or longitudinal, 2) The study had to include workplace bullying as exposure, (3) the study had to include depression or symptoms of depression as outcome, and 4) The study had to include a reference group. All four criteria had to be met for inclusion.

An overview of the literature search can be found in Figure 1. According to the figure, a majority of the publications were excluded as they did not meet the four criteria described above. The main explanation for this was that they used *mental distress*, *common mental disorder*, *psychological distress*, or similar as their outcome, which covers more than depression or symptoms of depression that are used as outcome in this thesis.

Furthermore, as illustrated in Figure 1, several of the included publications appeared in more than one search. Hence, after cross-checking for duplicates a total of 13 publications were included for the literature review of this thesis. Additionally, one publication by Nielsen and co-workers (2012), which was excluded after the first stage of the literature search, was re-included [53]. Nielsen and co-workers (2012) described their outcome as psychological distress, but since they used a validated scale: the HAD Scale, and defined a cut-off that reflects a depression where treatment is needed the publication was included.

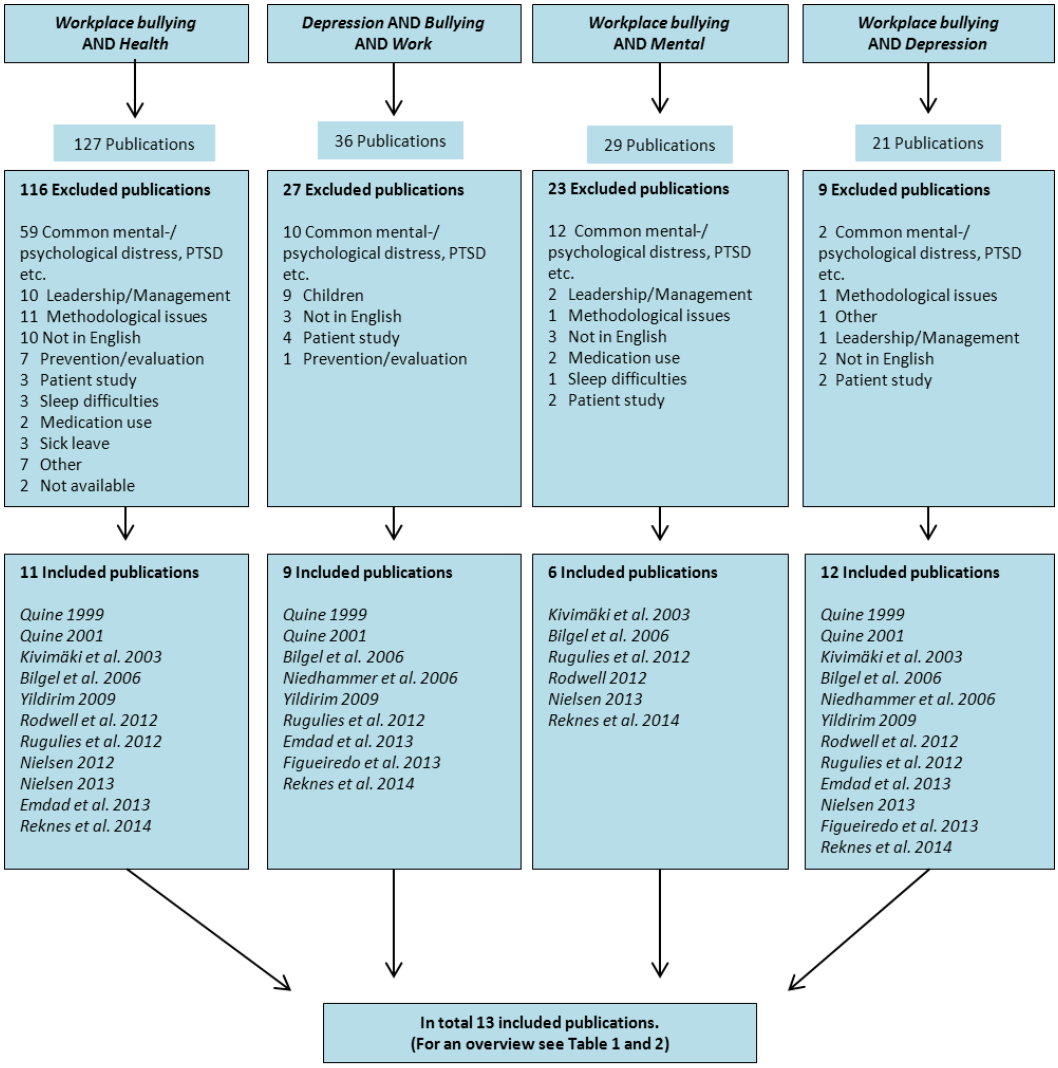

Figure 1 Diagram of literature search

2.4.1 Review of cross-sectional studies on workplace bullying and depression and symptoms of depression

Up until 2010, studies on Workplace Bullying and Depression and/or Depressive Symptoms were predominately conducted in cross-sectional designs. Quine (1999) found a significant relationship between exposure to bullying behavior and depression in a study conducted among 1100 employees in the public sector. Bullying behavior was measured with 20 items, followed by response categories of *Yes* and *No,* whereas depression was measured with the HAD Scale [54]. The study revealed that 22% of the participants had been exposed to bullying behavior during the past three months. Another study by Quine (2001) [55] was based on the same study population and used the same measurement for exposure and outcome. This study revealed that nurses were more likely to report exposure to bullying behavior than other occupational groups. Nurses who perceived one or more bullying behaviors were also more likely to report depressive symptoms. Bilgel et al. (2006) [56] revealed that employees who reported exposure to bullying behavior had an increased risk of depression. This study was conducted among 944 employees in Turkey, within the healthcare-, education- and security sector, and used the same measurements for exposure and outcome as Quine [54, 55]. The prevalence of being exposed to bullying behavior was 55% of the study sample. Another study accomplished in Turkey by Yildirim (2009) [57] was carried out among 286 nurses and revealed a significant association between being exposed to bullying behavior and depression status. This study used 33 items to determine bullying behavior and measured depression with the BDI. As in the studies above the exposure to bullying behavior was relatively high, as 21% perceived they had been directly exposed to bullying [57]. Niedhammer co-workers (2006) conducted a large cross-sectional study among 7770 employees in the general French working population. Exposure to workplace bullying was assessed by 1) The 45 items of the LIPT, and 2) By presenting a definition and asking about exposure to bullying at work by self-labeling. In this study, the prevalence of workplace bullying was 9% among men and 11% among women [14]. The prevalence in this study is lower compared to the studies above, and takes advantage of combining two ways of workplace bullying measurement. Depression was measured by the use of the CES-D and the study revealed that exposure to workplace bullying was a strong risk factor for depression.

Rodwell and Demir (2012) [58] conducted a study among 233 nurses in Australia, and in this study, the exposure to workplace bullying was measured by self-labeling prior to being presented with a definition. Depression was measured with the CES-D and the study indicated that exposure to workplace bullying had a significant effect on the association with depression.

Table 1 Cross-sectional studies on workplace bullying and depression/symptoms of depression

Author **Year** **Country**	***n*** **Response rate**	**Occupation**	**Exposure assessment** **Response categories**	**Outcome assessment**	**Prevalence of Workplace Bullying (WB) /Bullying Behavior (BB)**	**Results***
Quine 1999 England	1100 70%	Public sector	Inventory of bullying 20 items *Yes* or *No*	HAD Scale	BB: 38% (< 1 year) WB: 22% (<3 months)	Significant association between exposure to bullying behavior and depression (*p*=<0,001)
Quine 2001 England	1100 70%	Public sector	Inventory of bullying 20 items *Yes* or *No*	HAD Scale	BB: Nurses 44% Other staff 35% (<1 year)	Nurses who perceived one or more types of bullying behavior were more likely to suffer from clinical levels of depression (*p*=<0,001)
Bilgel 2006 Turkey	944 79%	Health Education Security	Inventory of bullying 20 item *Yes* or *No*	HAD Scale	BB: 55% (<1 year)	Employees reporting exposure to bullying behavior had increased risk for depression (OR 1,70 (95 % CI 1,17-2,46), *p*=0,005)
Niedhammer 2006 France	7770 40%	General working population	LIPT 45 items Definition Single item *Yes* or *No*	CES-D	WB: Men 9% Women 11% (<1 year)	Workplace bullying increased the risk for depression for both men and women OR for men: 8.0 (95% CI 6.06-10.56) OR for women: 8.44 (95% CI 6.84-10.41)
Yildirim 2009 Turkey	286 58%	Nursing	Bullying behavior 33 item 6 point scale	BDI	BB: 21% (<1 year)	Correlation between nurses' depression status and being exposed to bullying (p <0.00)
Rodwell 2012 Australia	233 29%	Nursing	Definition Single-item 6 point scale	CES-D	WB: Occasionally:16,3% Frequently: 3% (<6 months)	Bullying had a significant, main effect for depression (*F* (1,183) = 4,29, *p*=0,040 (95 % Ci -5,20 – 0,54))

*Risk estimates are shown if available

2.4.2 Review of longitudinal studies on workplace bullying and depression and symptoms of depression

During the last couple of years, several studies have been conducted with longitudinal designs in order to deal with the methodological issues that cross-sectional studies are limited by. Kiwimäki and co-workers (2003) studied hospital employees (*n*=5432) in a two year follow-up study, where exposure to workplace bullying was measured by self-labeling with response categories *Yes* and *No* at both baseline and follow-up [59]. Depression was measured with one question, where the respondents were asked if they had a physician diagnosed depression [59]. The study found an increased risk of depression after exposure to workplace bullying, and a dose-response relation was also indicated between the frequency of workplace bullying and the risk of depression. In 2012, Rugulies and co-workers (2012) [18] supported these findings in a study conducted among 5629 female, eldercare workers. In this study, workplace bullying was measured by self-labeling, and depression was measured by the MDI. Rugulies and co-workers (2012), as well as Kivimäki and co-workers (2003), indicated a similar dose-response relation between exposure to workplace bullying and the risk of depression. Furthermore, they supported Kivimäki and co-workers (2003) by stating that exposure to workplace bullying markedly increased the risk of depression two years later. Rugulies and co-workers (2012) also states that depression at baseline increases the risk of exposure to workplace bullying at follow-up. This reversed causality was confirmed by Nielsen and co-workers (2012) [53], who used the HSCL-25 among 1775 Norwegian employees to determine if depression (Psychological distress: measured as treatment needed depression) was a consequence of exposure to workplace bullying. Nielsen and colleagues (2012) used the NAQ and self-labeling to measure exposure to bullying at work, and revealed a prevalence of 4.8% self-labeled victims and 9.5% who were targets of bullying behavior at work [53]. The study confirmed earlier longitudinal studies with their findings of an increased risk of treated depression after both exposures to bullying behavior and as a self-labeled victim of bullying at work. A Spanish study among 327 employees from the public sector measured exposure to workplace bullying with 20 items based on the LIPT and the NAQ [60]. Figueiredo-Ferraz and co-workers (2013) used the ZSDS to determine depressive symptoms and found that symptoms of depression increased from baseline to follow-up in line with increased

exposure to workplace bullying. Emdad and co-workers (2013) examined the risk of symptoms of depression among bystanders to workplace bullying. Symptoms of depression were measured with the HAD Scale [61]. The study was conducted among 2563 industrial employees in Sweden and revealed that 11% of the participants reported having witnessed bullying at follow-up [61]. The study pointed at an increased risk of depression among witnesses after 18 months, when having witnessed workplace bullying. However, the study has been criticized by Nielsen and Einarsen (2013) [62] because Emdad and co-workers [61] neither controlled for depressive symptoms at baseline, nor measured the participants' own perceptions of being a victim of bullying at work. In the paper by Nielsen and Einarsen (2013) [62], a similar analysis as used in Nielsen and co-workers (2012) was conducted among the study population. Having witnessed workplace bullying was determined by a single item with 6 response categories, which was dichotomized by 0=Not witness and 1-5=Yes, witness. Depression was, as in Nielsen and co-workers (2012), measured by the HSCL-25. The study revealed that having witnessed workplace bullying does not increase the risk of depression two years later. Reknes and co-workers (2013) studied 5400 nurses and exposure to bullying behaviors, in terms of acts such as excluding someone from the work environment and/or someone receiving persistent criticism for their work effort [63]. This was associated with depressive symptoms, and it was found that exposure to bullying behavior did not increase the risk of depressive symptoms one year later.

Table 2 Longitudinal studies on workplace bullying and depression/symptoms of depression

Author Year Country	***n* Response rate**	**Occupation**	**Follow-up time**	**Exposure assessment**	**Outcome assessment**	**Prevalence of Workplace Bullying (WB)/Bullying Behavior (BB)**	**Results**
Kivimäki 2003 Finland	5432 74%	Hospital employees	Two years	Self-labeling	Physician diagnosed depression (by self-report)	WB: 7.9% in one survey 1.7% in both surveys	Increased risk of depression WB at baseline: OR 2.27 (CI 1.50-3.42) WB at baseline and follow-up: OR 4.81 (CI 2.46-9.40) Dose-response relation between frequency of exposure to bullying and risk of depression
Rugulies 2012 Denmark	5629 -	Eldercare workers	Two years	Self-labeling	MDI	WB: 10% *Occasionally* 1.9% *Frequently*	Increased risk of depression Occasionally: OR 2.48 (CI 1.09-5.65) Frequently: OR 5.61 (CI 1.29-24.36) Dose-response relation between frequency of exposure to bullying and risk of depression
Nielsen 2012 Norway	1775 -	Norwegian Central Employee Register	Two years	NAQ-R Self-labeling both 5 point scales	HSCL-25	BB: 9.5% targets of bullying behavior WB: 4.8% Self-labeled victims	Increased risk of depression where a treatment is needed BB: OR 1.68, 95% CI 1.07-2.62 WB: OR 2.47, 95% CI 1.17-5.22 Psychological distress increases the risk of bullying behavior two years later (OR 2.49, 95 % CI 1.64-3.80) Psychological distress increases the risk of exposure to workplace bullying two years later (OR 2.51, 95 % CI 1.39-5.21)
Figueiredo-Ferraz 2013 Spain	372 53%	Public sector	One year	20 items based on LIPT and the NAQ 4 point scale	ZSDS	-	Increased levels of depressive symptom from baseline to follow-up (t=-1,97, p=0.056)

Emdad 2013 Sweden	2563 60%	Paper mills Steelwork Truckers	18 months	Witnessing bullying: Single item *Yes* or *No*	HAD Scale	11% reported being a witness to bullying in the workplace at follow-up	Risk of depression increased within 18 months after being a witness of workplace bullying (OR: 1.69 (95 % CI 1.13-2.25))
Nielsen 2013 Norway	1775 57%	Norwegian Central Employee Register	Two years	Single item 6 point scale	HSCL-25	12.6% reported being a witness to bullying in the workplace at follow-up	Increased risk of anxiety/depression was not significant after controlling for symptoms at baseline and the witness own exposure to bullying (OR: 1.66 (95% CI 0.87-3.15))
Reknes 2013 Norway	5400 38%	Nurses	One year	NAQ	HAD Scale	-	Exposure to workplace bullying did not predict increased symptoms of depression (β=0,01, p=<0,47) Reciprocal relationship between symptoms of depression and exposure to workplace bullying (β=0,12, p=<0,01)

2.5 Review of literature on workplace bullying and cortisol

The literature on workplace bullying and cortisol is limited. Only five publications on workplace bullying and cortisol have been published during the last ten years (for an overview, see Table 3). In this review, four publications were included [64-67]. The fifth was excluded since it was conducted among patients [68].

The first publication conducted by Kudielka and Kern (2004) among sixteen persons was a pilot study, which investigated bullying and cortisol [64]. The participants were recruited over eight months and had experienced bullying according to the LIPT. Cortisol day profiles were measured with seven samples. The study showed a tendency towards an increased cortisol concentration in the morning, and a lower concentration in the evening among bullied persons [64]. However, there were no significant differences between a workday and a day off. Hansen and co-workers (2006) measured exposure to workplace bullying among Swedish employees from different occupations (*n*=437). The study used a single question to distribute respondents as *Exposed* and *Non-exposed* to bullying by a dichotomized response category. In the group of *Bullied* persons it was revealed 30% (CI 1-50%) lower cortisol concentration in the morning and 7% (CI -2-82%) lower cortisol concentration eight hours after awakening (2 AM) both compared to the *Non-bullied* employees [65]. In their second (45 minutes after awakening) and last (8 PM) sample of cortisol, no significant difference was found. Another study by Hansen and and co-workers (2011) among 1944 Danish employees measured exposure to workplace bullying by a single item and included a reference group of 1783 persons [66]. Cortisol concentrations were measured three times during a workday: At awakening, 30 minutes after awakening and at 8 PM in the evening [66]. The study revealed that *Frequently Bullied* (1% of the sample, *Weekly* or more *Frequent*) had lower cortisol values at awakening, after 30 minutes and at 8 PM (24.8% (CI 0.62-0.91)) compared to *Occasionally Bullied* persons and the reference group [66]. However, no statistically significant differences were found in the cortisol awakening response, or in the decline during the day. Hogh and co-workers (2012) investigated exposure to bullying at work and cortisol concentrations in 684 employees from public and private workplaces in Denmark [67]. The NAQ was used to define bullied persons and cortisol samples were collected in line with the

study by Hansen and co-workers (2006) [67]. The study found significantly reduced levels of cortisol in relation to two out of four factors of negative acts. A significant reduction in cortisol was found in persons who were exposed to person-related negative acts as direct harassment (12.18%) and intimidating behavior (9.62%), but not in persons subjected to work related acts [67]. The study also revealed a significantly lower cortisol concentration among persons exposed to direct harassment and intimidating behavior. However, this group of respondents represents approximately 25% of the study population and could therefore be indicative of covering more than persons labeling themselves as *Bullied*. Furthermore, it is unclear what the cortisol reduction of 12% and 10% cover in this study.

Table 3 Studies on workplace bullying and cortisol

Author Year Country	n	Occupation	Exposure assessment	Cortisol assessment	Size of exposure groups	Results
Kudielka & Kern 2004 Germany	16	-	LIPT (45 items)	Workday and day off Seven samples; Awakening, + 30 min, 8 AM, 11 AM, 3 PM, 8 PM, 10 PM	100% (included just targets)	All *p*'s were non-significant. No difference between a workday and a day off.
Hansen 2006 Sweden	437	High school Telecommunication Insurance Pharmaceutical Wood industry	Single item *Yes* or *No*	Workday Seven samples; Awakening, + 45 min, 8 h after awakening , 8 PM	Bullied *n*=22 (5%) Non bullied *n*=371 (84%)	Group of bullied persons 30% (CI 1-50%) lower cortisol in the morning and a 7 % (CI -2-82%) lower cortisol concentration 8 hours after awakening (8 PM);
Hansen 2011 Denmark	1944	Public sector Transportation Industrial Construction Finance	Definition Single item 5 point scale	Work day Three samples; Awakening, + 30 min, 8 PM	Reference group: *n*=1783 (91%) *Occasionally Bullied:* *n*=139 (7%) *Frequently Bullied:* *n*=22 (1%)	*Frequently Bullied* had lower raw cortisol values at awakening, after 30 minutes and at 8 PM (24.8%) compared to *Occasionally Bullied* and the reference group.
Hogh et al. 2012 Denmark	684	Public sector Transportation Industrial Construction Finance	NAQ 5 point scale	Workday Three samples; Awakening, + 30 min, 8 PM	-	Harassment and intimidating behavior had a lowered level of cortisol per score of negative behavior (12% (p=0.001) and 10% (p=0.030), respectively).

2.6 Workplace bullying, cortisol and depression

In both cross-sectional- and longitudinal studies, the literature review on workplace bullying, depression and depressive symptoms indicates that being exposed to bullying at work can be seen as a serious stressor, which can have negative consequences on the person being bullied. The cross-sectional studies (Table 1) show associations of depression and symptoms of depression. Only two studies [14, 56] have estimated and reports risk estimates. The four other studies [54, 55, 57, 58] only provide p-values and β-coefficients, which makes interpreting the results difficult. Among the longitudinal studies (Table 2) five out of seven studies [18, 53, 59-61] confirmed the cross-sectional studies, and pointed at an increased risk for depression and symptoms of depression after being exposed to bullying at work. Two out of seven longitudinal studies [62, 63] did not confirm that exposure to workplace bullying predicts depression or symptoms of depression. The studies on workplace bullying and cortisol [64-67] (Table 3) were able to discover associations between exposure to workplace bullying and changes in cortisol concentrations. However, the results are not entirely consistent, due to different exposure assessments.

Overall, all studies included in this literature review are based on self-report of both exposure- and outcome information. This designates a clear lack of independence between exposure- and outcome information. Additionally, some studies [53, 63] are pointing at reversed causality, which indicates that mental distress predicts exposure to workplace bullying. Furthermore, since the studies rely on self-report, mood and emotions may have affected the individual perception of being bullied and reporting of the work environment. This could indicate a major limitation of literature, which in these studies may have resulted in reporting bias [69]. In relation to studies on workplace bullying, independent measurements, such as non-self-reported measures of the bullying phenomenon, can be an opportunity to circumvent the markedly problem of reporting bias [69, 70]. To our knowledge, no studies within quantitative workplace bullying research uses other and more independent methods as exposure assessment. This thesis provided the opportunity to use independent information for both exposure and outcome, in terms of a witness approach at work-unit level, and furthermore the use of standardized interview and cortisol. To our knowledge, independent measurements of

workplace bullying and health outcome have not been used before in a longitudinal perspective.

3 AIM OF THESIS

1) To examine the risk of depression according to workplace bullying measured by
   (a) The self-labeling method.
   (b) Witnesses' reports to identify the occurrence and intensity of bullying at work-unit level.

2) To investigate changes in morning and evening cortisol concentrations
   (a) After exposure to workplace bullying.
   (b) After a discontinuance of exposure to workplace bullying.

3) To investigate reactivity and recovery of the HPA-axis measured by saliva cortisol induced by a standardized bicycle exercise challenge test following workplace *Bullied* versus *Non-bullied* persons.

4 MATERIALS AND METHODS

4.1 Design and Population

The papers are based on data from two Danish cohorts: The Workplace Bullying and Harassment Cohort (WBH) [71] and the PRISME Cohort [70]. The WBH Cohort was measured in 2006 (response rate 42%) with a follow-up in 2008 (response rate 60%). The PRISME Cohort was measured in 2007 (response rate 45%) with a follow-up in 2009 (response rate 71%). In 2010, the two cohorts were merged into the Modena Project, and in 2011 they were assessed a third time (Table 1, *Paper I* and *Paper II).* In 2011, a random sample was selected in order to complete a clinical examination and SCAN-interviews (described below in details in *Screening criteria for SCAN-interview*). Figure 2 gives an overview of the whole cohort material.

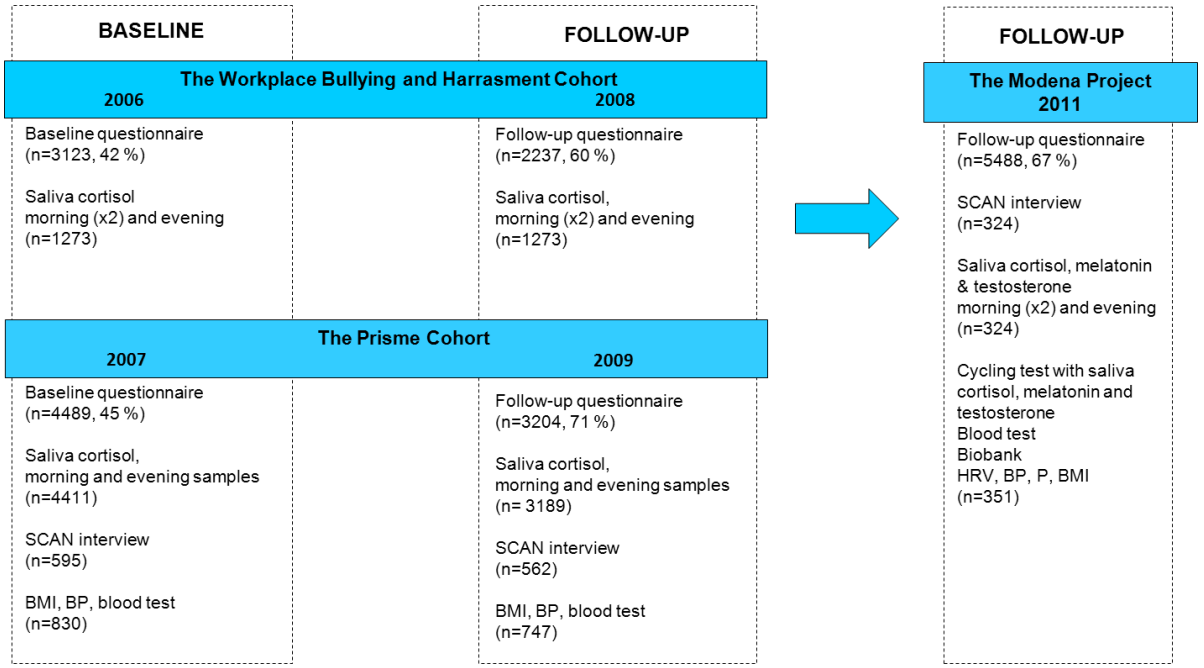

Figure 2 Cohort material

4.2 Exposure Assessment

4.2.1 Self-labeled workplace bullying

Workplace bullying was measured by the self-labeling method. Participants were presented with the definition by Einarsen (1996) followed by the question "*Have you been exposed to bullying at your current workplace within the past 6 months?*" [6]. The participants answered on a five-point scale of *Never*, *Now and again*, *Monthly*, *Weekly* and *Daily*. In line with international research we created a three-level exposure variable with the following categories: *Never Bullied*, *Occasional Bullied* (comprising the response categories *Now and again* and *Monthly*) and *Frequently Bullied* (comprising the response categories *Weekly* and *Daily*) (*Paper I, II, III*).

4.2.2 Measures of witnessed workplace bullying

Witnessing workplace bullying was measured by self-report. The participants were presented with the definition by Einarsen (1996) and asked whether or not they had witnessed a colleague being bullied at work during the past 6 months [6]. The participants answered on a five-point scale with the following categories: *Never, Now and again, Monthly, Weekly and Daily*. The responses were dichotomized between *Now and again* and *Monthly*. The proportion of witnesses per work-unit was then estimated and the value was assigned to all employees working at the work-unit (*Paper I*).

4.2.3 Work-units

The participants were affiliated with one out of a total of 455 work-units. All work-units were organized into four groups according to the proportion of employees who witnessed workplace bullying: 0% witnesses (*n*=683), 1-20% witnesses (*n*= 2,274), 21-30% witnesses (*n*=1,353), and >30% witnesses (*n*= 837) (*Paper I*).

4.2.4 Cycling test

Åstrand's Standardized Method was used as physical activity to estimate aerobic fitness during a sub-maximal workload [72]. This was done with a bicycle ergometer. The initial workload was estimated based on each participant's age and usual activity level. Heart-rates were measured using a heart-rate monitor. The goal was to achieve a heart-rate that would be 60% of maximal heart-rate reserve capacity, and at least 120 bpm. Subsequently, the workload and corresponding heart-rate were used to estimate VO2-max using the Åstrand-Rhyming Nomogram with a correction for age [72] (*Paper III*).

4.3 Outcome assessment

4.3.1 Depression

Depression was assessed by SCAN-interviews among respondents who satisfied the screening criteria (See *Screening criteria for SCAN-Interview*). The following sections of Part I were used: Section 3 (Worrying and Tension), Section 4 (Panic anxiety and Phobias), and Sections 6-8 (Depression). The interview focused on the previous 3-5 months and was computer aided and semi-structured [73, 74]. As illustrated in Figure 2, SCAN-interviews were conducted in the PRISME Cohort in 2007 and 2009, and again in 2011 in the merged Modena Project. At all occasions, the SCAN-interviews were conducted in the spring, three to six months after the main questionnaires had been completed. In line with the SCAN-interview clinical depression was assessed by MDI in the WBH Cohort in 2006 and 2008 (*Paper I*). The MDI is validated as a measure of depression in line with SCAN-interviews [75]. The MDI is a self-rated questionnaire with an algorithm that leads to ICD-10 categories of Moderate to Severe depression. The MDI consists of 10 items, where items 8 and 10 have an additional sub-item. The MDI assesses the ICD-10 symptoms of depression. Each item measures the presence of symptoms during the past two weeks on a scale ranging from 0 (the symptom has not been present at all) to 5 (the symptom is present all the time). The algorithm includes core and accompanying symptoms, and each symptom is dichotomized to indicate its presence (coded as 1) or absence (coded as

0).The questionnaire consists of 12 questions and it includes 2 algorithms, which classify participants with risk of unipolar depression (Mild, Moderate or Severe) according to the ICD-10 definition, dichotomized into depression or not by a cut-off ≥20 [75].

4.3.2 Symptoms of depression

Symptoms of depression in the PRISME Cohort were assessed by the CMDQ [37], whereas in the WBH Cohort, symptoms of depression were measured with the MDI [75]. In the merged Modena Project, depressive symptoms were measured with the CMDQ. When assessing depressive symptoms with the CMDQ, the participants were asked six questions (27, 28, 29, 30, 31 and 32) about their experiences during the last four weeks, giving their answers on a five-point scale ranging from *Not at all*, *Sometimes*, *Occasionally*, *Frequently* to *Very often*. The answers were dichotomized between *Not at all* and *Sometimes* (coded as 0), and *Occasionally*, *Frequently* and *Very often* (coded as 1). Participants with a sum score of 3 or higher on the six items were classified as having symptoms of depression. The MDI algorithm for depressive symptoms is used as described above with an MDI sum score. Participants with an MDI score of ≥ 10 were classified as having depressive symptoms (*supplementary analysis*) [75].

4.3.3 Cortisol concentrations

Saliva cortisol was provided by participants from both the WBH- and the PRISME Cohort at all occasions. The respondents were instructed to provide two saliva samples. The first sample was to be taken in the morning +30 minutes after awakening and the second sample in the evening at 8 PM (*Paper II*). Respondents who participated in the laboratory cycling test provided samples of saliva cortisol three times; at the start of the cycling test, at the end of the cycling test, and two hours after the cycling test. The sample of *Bullied* was distributed into three groups according to their bullied status within the past 12 months (baseline) and on the test day (See Figure 1, *Paper II*). Three exposure groups were distributed as follows: group A: Bullied at baseline and

answering *Never bullied* on the test day (morning sample *n*=40/afternoon sample *n*=45), group B: Bullied at baseline and answering S*ometimes*- or *Monthly bullied* on the test day (morning sample *n*=20/afternoon sample *n*=21), group C: Bullied at baseline and answering *Weekly*- or *Daily bullied* on the test day (morning sample *n*=4/afternoon sample *n*=7). The random sample (morning sample *n*=68/afternoon sample *n*=105) was used as reference group (See Figure 1, *Paper III*).

4.4 Screening criteria for SCAN interviews

In 2007, the following four screening criteria were used to select respondents for the SCAN-interviews (*n*=595): 1) symptoms of depression (point score of ≥3 on ≥3 of the six depressive symptoms items from the SCL-DEP6 [37], *n*=311), 2) A random sample of people with symptoms of burnout (with a mean score of ≥4 on the Copenhagen Burn-out Inventory, *n*=80); 3) Stress symptoms (with a mean score of ≥2.5 on the Perceived Stress Scale, *n*=79); and 4) A random sample (*n*=434). The screening criteria from 2007 are also described in Kolstad et al. 2011 [70].

In 2009, the following four criteria for SCAN-interviews (*n*=562) were: 1) Depressive, stress or burnout symptoms based on the questionnaires from 2009 (*n*=599), 2) ICD-10 depression diagnosed with the SCAN interviews in 2007 (*n*=71), 3) A random sample of people from the random sample in 2007 (*n*=201), and 4) People who reported high psycho-social load (*n*=167).

In 2011, the screening criteria for SCAN-interviews were developed from the criteria from 2007 and 2009. The used screening criteria were (*n*=655): 1) Exposure to at least one out of 11 Negative Acts of bullying weekly or more frequently, or perceived workplace bullying monthly or more often during the past 12 months (*n*=207), 2) Depressive symptoms (*n*=246), 3) Anxiety symptoms (point score of $\geq$2 of the three anxiety symptoms items, *n*=186). The final criterion was: 4) A random sample (*n*=220). The four screenings groups were overlapping.

4.5 Data structure

To utilize the cohort material, respondents were organized as courses of events. This was done in both *Paper I* and *Paper II*. This meant that those who participated at all three occasions could be included twice in our analysis. For example, a respondent who participated in 2007, 2009 and 2011 would be included with two courses; one course with baseline in 2007 and follow-up in 2009 and a second course with baseline in 2009 and follow-up in 2011. For *Paper I* and *Paper II* the included courses are different with respect to the aims of the papers. The inclusion criteria are described in the papers.

4.5.1 Confounders

In line with the different aims of the papers different confounders were included in the respective analyses. In *Paper I* we included the following confounders from the baseline questionnaire: Gender, age (≤34, 35-44, 45-54, ≥55), previous episodes of depression (*Yes* or *No*), family history of depression (*Yes* or *No*), years of education beyond primary or high school (<3/3-4/>4), weekly alcohol consumption (≤14 [for females] and ≤21 [for males]), depressive symptoms (rating of ≥3 on <2, ≥2 questions from the SCL-DEP6 [37] or MDI score of ≥10), and smoking (*Never, Ex-smoker, Smoker*). Among the respondents in the Prisme Cohort we controlled for personality traits based on the shortened version of the Eysenck Short-form Personality Questionnaire (EPQ-S) [76, 77] measuring extraversion and neuroticism. Among the respondents in the WBH Cohort we also controlled for personality traits with the scale Sense of Coherence (SOC) [78] based on Antonovsky's Concept [79]. In *Paper II* we included changes within respondents from baseline to follow-up in the following confounders: Smoking (*Never, Ex-smoker, Smoker*), weekly alcohol consumption (≤14 [for females] and ≤21 [for males]), BMI, and years of education beyond primary or high school (<3/3-4/>4). Furthermore, we included sample time + sample time^2 and time of awakening. In *Paper III* all analyses were adjusted for age, gender and sample time of the day.

4.5.2 Statistical analyses

In *Paper I* logistic regression was used to examine risk of new-onset depression by self-labeling but also among the proportion of employees who witnessed bullying in their work-units at baseline. In *Paper II* we used self-labeling of workplace bullying as exposure and investigated if cortisol change was in line with the change of the self-labeling. This was done with a multi-level mixed-effects linear regression model. In *Paper III* a paired sample t-test was used to investigate changes between cortisol levels measured in the cycling test, within the random sample. A one-way ANOVA was conducted to investigate potential differences in the relation to the response between sub-groups in the random sample, and to investigate differences in cortisol reactivity and recovery between *Bullied* and *Non-bullied* people.

5 RESULTS

In the Modena Project the prevalence of workplace bullying (Table 4) decreased during the study period from the first round, in 2006-2007, to the third round in 2011. This was the case for both *Occasionally Bullied* and *Frequently Bullied*.

Table 4 Prevalence of bullying in the Modena database

	1st round		2nd round		3rd round	
	n	**%**	***n***	**%**	***n***	**%**
Never Bullied	6802	90.7	4763	93.10	4736	94.7
Occasionally Bullied	611	8.1	317	6.2	232	4.6
Frequently Bullied	89	1.2	36	0.7	32	0.6

5.1 *Paper I:* Exposure to workplace bullying and risk of depression

The prospective association between self-labeled workplace bullying and the risk of new-onset depression showed an increased risk for both *Occasionally Bullied* (OR 2.17 [95% CI: 1.11-4.23]) and *Frequently Bullied* (OR 9.63 [95% CI: 3.42–27.10]) (Table 5, *Paper I*).

The prospective association between the proportion of employees, who witnessed workplace bullying at baseline and the risk of new-onset depression after 2 years, showed not to support that witnessing bullying at work-unit level predicts new-onset depression. The risk of depression was estimated according to the exposure groups with 1-20% witnesses (OR 0.91 [95% CI: 0.51-1.64]), 21-30% (OR 0.81 [95% CI: 0.43-1.53]) and >30% (OR 0.89 [95% CI: 0.46-1.73]) (Table 6, *Paper I*).

5.1.1 Supplementary analysis: Symptoms of Depression

The prospective association between self-labeled workplace bullying and the risk of symptoms of depression showed an increased risk of symptoms of depression among both *Occasionally Bullied* (OR 2.42 [95% CI: 1.92-3.04]) and *Frequently Bullied* (OR 2.13 [95% CI: 1.11–4.10]) (See Table 5).

Table 5 Risk of symptoms of depression by self-labeled workplace bullying

Perceived workplace bullying	*n*	New cases of with depressive symptoms	%	OR	OR adj.[a]	95 % CI
Never Bullied	8868	1546	17.4	1.0	-	-
Occasionally Bullied	551	176	31.9	2.29	2.42	1.92-3.04
Frequently Bullied	65	24	36.9	2.80	2.13	1.11-4.10

[a]Adjusted for age, gender, earlier depression, symptoms of depression, family history of depression, higher education, alcohol, and smoking.

The prospective association between the proportion of employees who had witnessed workplace bullying at baseline, and the risk of symptoms of depression after two years did not show significant increased risk of depressive symptoms: 1-20% witnesses (OR 1.09 [95% CI 0.89-1.33]), 21-30% (OR 1.12 [95% CI 0.94-1.39]) and >30% (OR 1.15 [95% CI 0.93-1.43]) (See Table 6).

Table 6 Risk of symptoms of depression by exposure groups

Percentage witnessing workplace bullying	*n*	New cases with depressive symptoms	%	OR	OR adj.[a]	95 % CI
0	1163	227	19.5	1.00	-	-
1-20%	3936	694	17.6	0.89	1.09	0.89-1.33
21-30%	2790	500	17.9	0.92	1.12	0.91-1.39
>30%	2087	404	19.4	1.00	1.15	0.93-1.43

[a]Adjusted for age, gender, earlier depression, depressive symptoms, family history of depression, higher education, alcohol, and smoking.

5.2 *Paper II:* Is workplace bullying related to changes in levels of cortisol in saliva?

Exposure to workplace bullying showed no significant change in either morning or evening cortisol levels, when respondents changed their self-labeling from *Never Bullied* to *Bullied* after approximately two years (Table 3, *Paper II*).

A discontinuance of workplace bullying showed a significant decrease in the cortisol morning sample among *Occasionally Bullied* in the PRISME Cohort. However, no significant changes were discovered in any of the samples (Table 5, *Paper II*).

5.2.1 Supplementary analysis: Cross-sectional cortisol concentrations

Table 7 presents cross-sectional analysis for morning and evening cortisol levels for participants in the PRISME- and the WBH Cohort. The adjusted values are log transformed cortisol values estimated by linear regression controlled for confounders. The adjusted estimates are differences in percent from the reference group. For the *Occasionally Bullied* no statistical significant results were found. However, a statistical significant difference was discovered among the *Frequently bullied* in the WBH Cohort for the evening values of 2006 (0.69 (95% CI 0.5-0.94) and the morning values of 2008 (0.54 (95% CI 0.37-0.80).

5.3 *Paper III:* An examination of cortisol reactivity and recovery during low level exercise – an innovative study

In the random sample the reactivity during the cycling test was 0.17 (±1.13 SD) and the mean recovery was 0.78 (±3.59 SD). The random sample was separated into the morning sample (*n*=68, 72% females) and the afternoon sample (*n*=105, 64% females). In the morning sample, there was no significant increase in cortisol reactivity (p=0.6645, 95% CI -0.346-0.222), whereas there was a significant decrease in cortisol recovery (p=0.0178, 95% CI 0.1818-1.8288). In the afternoon group, there was no significant increase in cortisol reactivity (p=0.0528, 95% CI -0.483-0.003) or in cortisol recovery (p=0.2022, 95% CI -0.341-1.5807).

In the morning, sample cortisol reactivity increased within the reference group (0.06, (Std.D 1.07)), whereas group A, B and C each had a decrease in the mean cortisol levels (group A: -0.12 (Std.D 1.20), group B: -0.07 (Std.D 1.31), and group C: -1.15 (Std.D 1.14)). There were no significant differences in reactivity between the reference group, group A and group B, whereas group C was significantly different from the reference group (p=0.029). Cortisol recovery mean values increased within all four groups (Reference group: 1.01 (Std.D 2.86), group A: 1.04 (Std.D 2.84), group B: 0.30 (Std.D 1.26), and group C: 0.72 (Std.D 2.04)) and no significant differences were found between the groups (Table 4, *Paper III*).

In the afternoon, cortisol reactivity increased within the reference group (0.24 (Std.D 1.17) and group C (0.27 (Std.D 0.55), whereas a decrease was found in group A (-0.31 (Std.D 0.71) and B (-0.15 (Std.D 0.50). There were no significant differences in reactivity between the reference group, group B and C, whereas group A was significantly different from the reference group (p=0.003). Cortisol recovery increased within all four groups (Reference group: 0.62 (Std.D 4.03), Group A: 0.82 (Std.D 2.66), Group B: 0.25 (Std.D 0.50), and Group C: 2.26 (Std.D 3.75)) and no significant differences were found between the groups (Table 5, *Paper III*).

Table 7 Cortisol concentrations for morning and evening samples in the PRISME- and the WBH Cohort in cross-sectional design

										Adjusted analyses*			
Cohort Year Sample	**Never bullied**			**Occasionally bullied**			**Frequently bullied**			**Occasionally bullied**		**Frequently bullied**	
	N	**Median**	5-95 percentile	***n***	**Median**	5-95 percentile	***n***	**Median**	5-95 percentile		(95% CI)		(95% CI)
Prisme													
2007													
Morning	3326	11.300	(3.299;25.200)	250	12.349	(2.400;31.799)	38	11.800	(3.199;34.599)	1.03	(0.94;1.12)	1.00	(0.81;1.24)
Evening	3620	1.399	(0.400;5.400)	291	1.399	(0.400;5.400)	42	1.449	(0.400;4.400)	0.97	(0.88;1.07)	1.06	(0.83;1.35)
2009													
Morning	2210	13.700	(4.199;29.700)	125	14.299	(3.499;27.100)	14	15.649	(7.500;31.500)	1.01	(0.90;1.14)	1.27	(0.89;1.81)
Evening	2459	1.399	(0.400;6.000)	148	1.499	(0.499;4.699)	18	1.299	(0.400;9.700)	0.97	(0.85;1.12)	0.94	(0.64;1.39)
2011													
Morning	171	15.035	(3.890;39.647)	32	15.554	(4.330;41.637)	12	19.515	(6.566;43.468)	1.21	(0.95;1.53)	1.47	(1.01;2.16)
Evening	173	2.816	(2.095;8.943)	35	2.869	(2.095;5.915)	11	2.658	(2.095;3.521)	0.94	(0.78;1.13)	0.78	(0.57;1.05)
WBH													
2006													
Morning	1456	11.800	(3.900;25.200)	147	12.699	(3.100;25.599)	22	8.399	(3.299;14.100)	1.06	(0.94;1.19)	0.78	(0.58;1.04)
Evening	2027	1.200	(0.4000;4.099)	204	1.100	(0.299;3.600)	29	1	(0.299;2.599)	0.97	(0.86;1.10)	**0.69**	**(0.51;0.94)**
2008													
Morning	1327	15.349	(5.800;30.100)	100	15.000	(4.950;28.499)	11	8.400	(2.599;17.399)	0.93	(0.83;1.05)	**0.54**	**(0.37;0.80)**
Evening	1514	1.499	(0.499;5.600)	114	1.700	(0.499;7.100)	13	1.700	(0.199;9.599)	0.99	(0.84;1.16)	0.73	(0.43;1.26)
2011													
Morning	106	16.883	(3.996;34.330)	21	17.077	(4.242;26.073)	4	10.096	(2.799;33.485)	0.83	(0.60;1.15)	1.79	(0.37;1.68)
Evening	104	2.737	(2.095;5.422)	23	2.693	(2.095;4.172)	3	2.711	(2.588;2.764)	0.91	(0.74;1.12)	0.90	(0.56;1.45)

*Adjusted analysis of log transformed cortisol values are showing differences in percent from reference group. Included confounders gender, age, education, BMI, smoking, alcohol, sample time+sample time^2 and time of awakening.

6 DISCUSSION

6.1 Main Results

We identified contradicting results in our study of exposure to workplace bullying and the risk of depression. Self-labeled exposure to workplace bullying was found to be a strong predictor for depression and symptoms of depression. With all due respect to the strong association between the individual perception of bullying and risk of depression and symptoms of depression, we expected to find an association between prevalence of witnesses reporting bullying at work-unit level and the risk of depression among employees working in high-risk units. However, our results based on the witness method could not support this. Furthermore, this study did not indicate that exposure to workplace bullying, or a discontinuance of workplace bullying, changed cortisol levels in a longitudinal study design. Additionally, low-level exercise did not indicate to have an impact on the HPA-axis activity among *Bullied* and *Non-bullied* employees.

6.2 Exposure Assessment

6.2.1 The self-labeling method

In line with other studies [18, 59, 62] we assessed workplace bullying by self-labeling (*Paper I, II and III*). The self-labeling method is a subjective measurement of a person's exposure to bullying. The self-labeling approach does not offer any insights into the nature of the behaviors involved and the assessment form can only reveal whether a person perceives him- or herself to be a victim of workplace bullying [20].

All previous studies on workplace bullying and depression and symptoms of depression have used self-report as exposure assessment (see Table 1 and 2). Among the cross-sectional studies one study used the self-labeling method [58], four studies assessed bullying behavior [54-57] and one study combined the two ways of assessment [14]. Among the longitudinal studies, five studies used the self-labeling method [18, 53, 59, 61, 62] and three studies assessed the bullying behavior [53, 60, 63]. The self-labeling method and assessment of

bullying behavior are different measurements and thus it can be difficult to compare the studies.

As in other studies, self-reports of exposure are connected to methodological challenges e.g. reporting bias. This is also the case in assessment of workplace bullying independently of the use of a single item, the NAQ or other scales. Mood and emotions may affect a person's perception of exposure to workplace bullying [20] and thus may result in a blunted picture of the phenomenon. Hence the causality of documented association of exposure to workplace bullying and subsequent health outcomes may be questioned.

6.2.2 The witness method

In order to develop an independent assessment that limits the risk of reporting bias we used witnesses to reflect an independent form of assessment for being an employee in a work environment where bullying exists. In our study we used the proportion of witnesses per work unit to estimate the risk of depression and symptoms of depression (*Paper I*). To our knowledge this is the first independent assessment tool to investigate workplace bullying where the problem with reporting bias is taken into account. As described in this thesis, the majority of studies have measured the workplace bullying phenomenon from the victim's or target's perspective, without obtaining information of other parties, e.g. other employees or employers. According to Agervold (2007), the closest one may come to obtain an independent assessment of workplace bullying is to use witnesses [80].

It is pertinent to ask whether the independent witnesses method encompasses the subjective self-labeling method. The witness method does not offer any information about workplace bullying on the individual level, however, the witness method reflects workplaces where bullying takes place on both a small and a larger scale and is a supplement to the traditional way of assessing workplace bullying in surveys. We included all witnesses (the cut-off was between *Never* and *Now & then*) to create four exposure groups. A relevant critique could be that a too liberal cut-off can cause uncertainty about the validity of the exposure assessment. However, workplace bullying is often hidden and without witnesses at the beginning, but tends to escalate when no

action is taken [8]. In the cases where workplace bullying escalates there will most likely be some witness. Thus we determined that in order to establish a valid estimate of an employee's perception of a working environment where bullying takes place, it is necessary to have a liberal cut-off criteria when defining exposure groups at the work-unit level. In the assessment of the witnesses' method it can be argued that the hidden bullying can be problematic in large work units. However, in the present study the risk estimates were close to equal in both large and smaller work units.

Another weakness with the witness method is that it does not necessarily reflect the number of employees exposed to workplace bullying. This can affect the results towards null. Nonetheless, the estimated risk of depression and symptoms of depression was the same in all exposure groups with no difference between workplaces with a low and high proportion of employees witnessing bullying.

6.3 Outcome Assessment

6.3.1 Depression and symptoms of depression

In our study we used SCAN-interviews to assess depression (*Paper I*). Previous studies have measured depression by use of self-administered checklists of depression [18, 53, 59, 61, 62] and symptoms of depression [60, 63].

As previously mentioned, depression was assessed differently in the PRISME- and the WBH Cohort. We used the SCAN-interview on an equal basis with the MDI. In a Danish validation study, the sensitivity and specificity of the MDI algorithms of *Moderate* to *Severe* depression according to ICD-10-DCR were analysed by using the SCAN-interview as the index of validity; the sensitivity was 0.86 and the specificity 0.86, indicating a satisfactory agreement between the two instruments [75]. Furthermore, our sensitivity analysis, when leaving out the MDI cases, revealed essentially the same results for respondents reporting *Frequently Bullied*, whereas we discovered a significantly increased risk among respondents reporting *Occasionally Bullied*. Hence, this shows that using the SCAN-interview and the MDI on an equal basis was unproblematic.

Our goal was to identify as many cases of depression as possible with clinical depression while keeping a high specificity. The selection of participants for SCAN-interviews was based on a specific screening criteria which according to earlier analyses had a sensitivity of 84% and a specificity of 96%. We invited people who: 1) Fit the screening criteria based on the questionnaire completed three to six months before the SCAN-interview period, and 2) A random sample. This can represent a limitation for the study outcome since we missed those who became cases between baseline and follow-up and were in remission at follow-up. Moreover, since the questionnaires were completed three to six months before the SCAN-interviews were completed, some cases were probably not identified because of remission before the interview was carried out. However, we excluded participants who had a SCAN diagnose or MDI depression at baseline. This ensured that the observed increased risk was based on true cases. Although a looser screening criteria could have identified more cases, incomplete ascertainment of depression is hardly related to the exposure under study and is therefore primarily expected to reduce the statistical power of the study but not to result in bias.

6.4 Confounding and reversed causality

Most longitudinal studies control for important confounders. In the study on workplace bullying and depression (*Paper I*) we included important explaining variables based on existing knowledge [28-31, 81]. Furthermore, we completed a supplementary analysis in order to control for personality traits. Still, due to the lack of independence between self-reported exposure and self-reported outcomes it can be difficult to state a total causality between exposure to workplace bullying and depression and symptoms of depression [53, 63]. Nielsen and co-workers (2012) state that psychological distress is an etiological factor for exposure to workplace bullying. Thus, the witness method is an attempt to remove the subjective influence from the traditional way of estimating workplace bullying. Because studies have revealed reversed causality it is of great importance to develop independent ways of assessing bullying at work, which can handle challenges such as reporting bias and reversed causality.

6.5 Saliva cortisol

To our knowledge, this is the first observational study that uses a longitudinal design to examine saliva cortisol response following changes in perceived bullying exposure (*Paper II*) and to examine saliva cortisol in an exercise challenge test among *Bullied* versus *Non-bullied* people (*Paper III*).

Four previous studies have indicated that cortisol secretion is lower in bullied employees (see Table 3), but these findings are not entirely consistent due to different measures of exposure (LIPT, NAQ and self-labeling). This may give different exposure groups and thus these studies are difficult to compare since the prevalence of bullying, according to the Negative Acts definition, is much more prevalent (in the range of 15% [12]) than the prevalence according to the self-labeling method (in this study 2-3%).

In our study on long term cortisol reactions (*Paper II*) we expected to find cortisol changes among employees who changed their bullied status from baseline to follow-up; either from *Not being bullied* to *Being bullied* or from *Being bullied* to *Not being bullied*. This expectation was based on the acute stress-response, where the HPA-axis plays a key role in the organism in releasing and producing stress hormones [82], and that previous studies have indicated lower cortisol among employees perceiving themselves as exposed to bullying [65, 66]. Being exposed to bullying involves central factors of the regulation of emotion and/ or physiological arousal, such as loss of control and lack of predictability [83]. A theoretical model by Dickerson and Kemeny (2004) on threats to one's Social-Self (the Social-Evaluative-Threat) shows that a social threat can be accompanied by a specific set of psychological and physiological responses, including a cortisol increase. The Social-Evaluative-Threat is an important aspect of the one's self-identity when this is judged by others in a negative way [48]. Furthermore, the context of the social evaluation is relevant in order to document a significant cortisol response. This was shown in a study by Wadiwalla and co-workers (2008), which examined the effects on several situational characteristics on the cortisol response [84]. In this study the Social-Evaluative-Threat showed to be of importance in the cortisol stress response. Therefore, when workplace bullying is used as exposure assessment, this can be seen as a serious stressor that involves humiliation and demeaning actions against another person on a very personal level. Thus, we determined that exposure to bullying was a

strong stressor in order to be able to confirm the strong findings on bullying and depression (*Paper I*) with our study on cortisol (*Paper II and Paper III*). However, our finding on cortisol in the longitudinal design (*Paper II*) and on cortisol reactivity and recovery (*Paper III*) did not confirm the hypothesis on long term stressor's implications on cortisol. Taken together, it is pertinent to ask if both exposure- and outcome assessment is an appropriate way of assessing the link between workplace bullying and cortisol. On a theoretical basis, an explanation for our findings (*Paper II* and *Paper III*) could be reflected in the exposure assessment which, according to Dickerson and Kemeny (2004) and Kemeny (2009), could be too inaccurate to be able to catch the strong and serious implications that occur in cases of workplace bullying [47, 48].

6.5.1 Strength and limitations in cortisol studies

Considering our hypothesis, we needed to deliberate if bias could explain our findings. First, our longitudinal study (*Paper II*) has few cases of *Frequently Bullied* persons: 18 persons with new-onset bullying *weekly* or *more frequently* and 19 persons who experienced a discontinuance of frequently bullying. Forty *Frequently Bullied* persons (0.4%) participated only once and were thereby excluded. Obviously, this limits the statistical power but the numbers are not much different from the numbers of *Frequently Bullied* in the few earlier cross-sectional studies. Nevertheless, we acknowledge that the study has poor power to examine possible effects of rare cases of very severe bullying. Secondly, non-differential misclassification of exposure may cause bias towards the null. We used self-labeling to measure exposure to workplace bullying, which may be distorted by the emotional- and cognitive factors. Third, 40 persons of the *Frequently Bullied* participants at baseline did not participate at follow-up. Hence, if the loss-to follow-up is differential with regard to change in cortisol concentrations, findings may be biased as well. However, this seems hypothetical since the saliva cortisol concentrations at baseline were similar among follow-up participants and non-participants (data not shown). Furthermore, our results of the exercise challenge test among *Bullied* and *Non-bullied* employees did not indicate that workplace bullying has an impact on the HPA-axis activity (*Paper III*).

Compared to previous studies using the Trier Social Stress Test, the reactivity and recovery were less pronounced in our study (*Paper III*). This can be attributed to adjustments that we can recommend for future studies. For example, the first saliva cortisol sample was measured at the start of the cycling test, about one hour after the participant arrived at the laboratory. However, all the participants had received an intensive clinical examination before the cycling test and it can be argued that the cortisol levels observed were due to this examination and not the cycling test itself. According to Balodis and co-workers (2010), cortisol levels are stable after 30 minutes in the laboratory, and we could have controlled for this bias if the participants' cortisol levels had been measured when they first entered the laboratory [85]. Furthermore, the time span between the pre- and post -exercise sample of about 10 minutes may have been too short a period. Additional samples in the post-exercise period might have revealed more pronounced changes of cortisol over time [86]. Another issue that should be considered is the physical activation in the Åstrand cycling test. A recent study indicated that a 70% physical activity load is needed to elicit a significant cortisol response [87], whereas our cycling test was designed to achieve 60% of physical activity load. Our test paradigm, however, did not activate the HPA-axis with a significant increase between *time 1* and *time 2*, either in the morning or the afternoon sample, but we found that the sample time of the day had a significant influence. From what we know about the diurnal cortisol rhythm [49, 87], it can be argued that laboratory tests on cortisol response should be conducted only in the afternoon to avoid being influenced by an increase in cortisol levels due to the cortisol awakening response [88, 89]. This relationship is supported by the non-significant increase in our samples; here the diurnal cortisol response that occurs in the morning may have played a role.

Studies on the cortisol responses in humans contribute to a better understanding of the relationship between psycho-social environment and health. The investigation of biomarkers within the bullying research is relatively new and only a couple of studies have investigated people exposed to bullying in relation to a biological response [64-67]. Most researchers know that cortisol mechanisms, which are studied in laboratory settings, vary across study designs. They can be influenced by a large range of variables, such as

meals, smoking, drinking, pharmacological stimulation, physical activity, etc. Many confounding factors and routines are needed when using saliva cortisol as a biomarker [90, 91], and the present study (*Paper II* and *III*) took variables into consideration such as age, gender and time of day. Other variables, such as information about menstrual cycles and genetic factors, were not controlled for but are not confounding results within person variation across short time spans [87]. Using the baseline information, participants were grouped by the established screening criteria, followed by a follow-up status that formed the classification for exposure groups. As such, we may assume that participants, who indicated that they were bullied at baseline and again on the test day, can be classified as people who have been exposed to bullying.

6.6 Selection bias and loss-to follow-up

The participation rate for the initial survey was low at baseline (in 2006 in the WBH Cohort 42% and in 2007 in the PRISME Cohort 45%) and could cause selection bias. In the PRISME Cohort the respondents differed from the non-respondents both by gender, age and social class [92]. However, the association between psycho-social work environment and mental health outcomes were not systematically biased [92]. In the WBH Cohort the respondents differed by age and gender [71]. Younger respondents and men were more likely to leave the cohort, and men employed in the private sector in particular. Thus, there is a risk for selection bias by loss-to follow-up. However, the data material of the present study included participants who responded at two occasions, and the difference between the included respondents versus respondents who were excluded have no influence on the result. As presented in the Result section, bullied respondents were likely to leave the cohort. However, the difference of occurrence in depressive cases was not differential between participants and loss-to follow-up and should therefore not have an impact on differences between included and excluded participants.

7 CONCLUSION

This study supports previous studies on workplace bullying and depression and symptoms of depression. By using the self-labeling method, we confirm an increased risk of depression and symptoms of depression, when one perceives him- or herself as exposed to bullying at work.

The study shows that there are weaknesses and challenges associated with the exposure assessment of workplace bullying by self-report. This study tries to deal with the weakness of the self-labeling method but is not able to confirm earlier associations between exposure to workplace bullying and depressions and symptoms of depression by classifying workplaces based on the number of witnesses.

The study could not confirm studies on exposure to workplace bullying and changes of cortisol. With the longitudinal study we could not support findings of changes in the HPA axis. Our study could not verify that exposure to workplace bullying and a discontinuance of workplace bullying changes cortisol. Additionally, low level exercise did not indicate to have an impact on the HPA-axis activity among *Bullied* and *Non-bullied* employees.

7.1 Future implications

Research on workplace bullying is important to work in the future. Studies have shown that exposure to workplace bullying has an impact on health, although, these studies tend to have methodological issues one needs to be aware of. In future studies it is important to use data other than just surveys to examine whether bullying provides health consequences. Although we can confirm that self-labeled bullying at work increases the risk of depression and symptoms of depression, it is crucial to establish independent ways of assessing workplace bullying in order to exclude competing factors and reversed causality.

Studies on bullying and health that include biomarkers are few and far between, and there are still lots of pathways to investigate. There are many biological factors to be included, such as further investigations of hormones in the HPA-axis or other markers for long term stress. Longitudinal studies using

both surveys and biological material in a large epidemiological scale is important for establishing new hypotheses and links between the psycho-social work environment and health. Furthermore, a combination of observational studies, studies in a laboratory context, and biological material would be very interesting with the aim of establishing deeper knowledge within psycho-biologics and to establish more sophisticated measurements of long term exposure to person-related stress. Additionally, there is a need for studies on the prognosis of the bullied according to development of depression and their following quality of life. A study like this could be obtained among *Bullied* versus *Non-bullied* and should look further into important life factors such as family, health and work.

8 REFERENCES

1 Tehrani N. *Workplace Bullying - symptoms and solutions*. New York: Routledge 2012.

2 Murray J. Moral distress: The face of workplace bullying. *The John Hopkins University Press* 2013;**3** (2):112-4.

3 Brodsky C. *The harassed worker*. Toronto: Lexington Books, DC Health and Company 1976.

4 Leymann H. Mobbing and Psychological Terror at Workplaces. *violence and victims* 1990;**5** (2):119-26.

5 Keashly L. Emotional abuse in the workplace: Conceptual and empirical issues. *Journal of Emotional Abuse* 1998;**1**:85-117.

6 Einarsen S. *Bullying and Harassment at work: Epidemiological and psychosocial aspects*. Bergen: Department of Psychosocial Science, Faculty of Psychology, University of Bergen 1996.

7 Olweus D. *Bullying in school: what we know and what we can do*. Oxford: Blackwell 1993.

8 Einarsen S, Hoel H, Zapf D*, et al.* The concept of bullying and harassment at work: The European Tradition. In: Einarsen S, Hoel H, Zapf D*, et al.*, eds. *Bullying and Harassment in the Workplace*. Boca Raton: CRC Press 2011:3-39.

9 Einarsen S, Raknes IB, Matthiesen SB. Bullying and harassment at work and their relationship to work environment quality: an explorative study. *Journal of work and organizational psychology* 1994;**4**:381-401.

10 Hauge LJ, Skogstad A, Einarsen S. Relationships between stressful work environments and bullying: Results of a large representative study. *Work Stress* 2007;**21** (3):220-42.

11 Niedhammer I, Sultan-Taieb H, Chastang JF*, et al.* Exposure to psychosocial work factors in 31 European countries. *Occup Med (Lond)* 2012;**62** (3):196-202.

12 Nielsen MB, Matthiesen SB, Einarsen S. The impact of methodological moderators on prevalence rates of workplace bullying. A meta-analysis. *Journal of Occupational and Organizational Psychology* 2010;**83** (4):955-79.

13 Magerøy N. Health among navy personnel: A cross-sectional study in the royal norwegian navy. University of Bergen, Norway 2009:1-64.

14 Niedhammer I, David S, Degioanni S. Association between workplace bullying and depressive symptoms in the French working population. *J Psychosom Res* 2006;**61** (2):251-9.

15 Cooper CL, Hoel H, Faragher B. Bullying is detrimental to health, but not all bullying behaviours are not necessarily equally damaging. *Birtish Journal of Guidance and Counselling* 2004;**32** (3):367-87.

16 Hansen ÅM. Skænderier eller konflikter, mobning og seksuel chikane på arbejdspladsen. The National Research Centre for the Working Environment 2012.

17 Ortega A, Hogh A, Pejtersen JH*, et al.* Prevalence of workplace bullying and risk groups: a representative population study. *Int Arch Occup Environ Health* 2009;**82** (3):417-26.

18 Rugulies R, Madsen IE, Hjarsbech PU*, et al.* Bullying at work and onset of a major depressive episode among Danish female eldercare workers. *Scand J Work Environ Health* 2012;**38** (3):218-27.

19 Hogh A, Hoel H, Carneiro IG. Bullying and employee turnover among healthcare workers: a three-wave prospective study. *J Nurs Manag* 2011;**19** (6):742-51.

20 Nielsen MB, Notelaers G, Einarsen S. Measuring Exposure to Workplace Bullying. In: Einarsen S, Hoel H, Zapf D*, et al.*, eds. *Bullying and Harassment in the Workplace*. Boca Raton: CRC Press, Taylor & Francis Group 2011:149-74.

21 Einarsen S, Raknes BI. Harassment in the workplace and the victimization of men. *Violence Vict* 1997;**12** (3):247-63.

22 Leymann H. *Handbok för användning av LIPT-formuläret för kartläggning av risker för psykologisk vald [manual of the LIPT questionaire for assessing the risk of psychological violence at work]*. Stockholm: Violen 1990.

23 Vartia MA. Consequences of workplace bullying with respect to the well-being of its targets and the observers of bullying. *Scand J Work Environ Health* 2001;**27** (1):63-9.

24 Alonso J, Angermeyer MC, Bernert S*, et al.* Prevalence of mental disorders in Europe: results from the European Study of the Epidemiology of Mental Disorders (ESEMeD) project. *Acta Psychiatr Scand Suppl* 2004 (420):21-7.

25 The World Health Organization. The Global Burden of Disease. WHO 2008.

26 Kessing LV, Videbech P. [Depression--burden of disease, diagnosis and treatment]. *Ugeskr Laeger* 2003;**165** (12):1252.

27 Kessler RC, Berglund P, Demler O*, et al.* The epidemiology of major depressive disorder: results from the National Comorbidity

Survey Replication (NCS-R). *J Am Med Assoc* 2003;**289** (23):3095-105.

28 Kessler RC. The Effects of Stressful Life Events on Depression. *Annu Rev Psychol* 1997;**48** (1):191-214.

29 Burcusa SL, Iacono WG. Risk for recurrence in depression. *Clinical Psychology review* 2007;**27** (8):959-85.

30 Boden JM, Fergusson DM, Horwood LJ. Cigarette smoking and depression: tests of causal linkages using a longitudinal birth cohort. *The British Journal of Psychiatry* 2010;**196** (6):440-6.

31 Andersen I, Thielen K, Nygaard E*, et al.* Social inequality in the prevalence of depressive disorders. *Journal of Epidemiology and Community Health* 2009;**63** (7):575-81.

32 Kessing LV, Hansen MG, Andersen PK*, et al.* The predictive effect of episodes on the risk of recurrence in depressive and bipolar disorders - a life-long perspective. *Acta Psychiatr Scand* 2004;**109** (5):339-44.

33 Beck AT, Steer RA, Ball R*, et al.* Comparison of Beck Depression Inventories -IA and -II in psychiatric outpatients. *J Pers Assess* 1996;**67** (3):588-97.

34 ZUNG WK. A self-rating depression scale. *Arch Gen Psychiatry* 1965;**12** (1):63-70.

35 Olsen LR, Jensen DV, Noerholm V*, et al.* The internal and external validity of the Major Depression Inventory in measuring severity of depressive states. *Psychol Med* 2003;**33** (2):351-6.

36 Radloff LS. The CES-D Scale: A self-report depression scale for research in generel population. *Applied Psychological Measurement* 1977;**1** (3):385-401.

37 Christensen KS, Fink P, Toft T*, et al.* A brief case-finding questionnaire for common mental disorders: the CMDQ. *Family Practice* 2005;**22**:448-57.

38 Jablensky A. The nature of psychiatric classification: issues beyond ICD-10 and DSM-IV. *Aust N Z J Psychiatry* 1999;**33** (2):137-44.

39 Hamilton M. A Rating Scale for Depression. *J Neurol Neurosurg Psychiatry* 1960;**23** (1):56-62.

40 Wing JK, Babor T, Brugha T*, et al.* SCAN: Schedules for Clinical Assessment in Neuropsychiatry. *Arch Gen Psychiatry* 1990;**47** (6):589-93.

41 Clinical Research Unit for Anxiety and Depression StVH. *Composite International Diagnostic Interview*. Sydney: Clinical Research Unit for Anxiety and Depression 2005.

42 McEwen BS. Central effects of stress hormones in health and disease: Understanding the protective and damaging effects of stress and stress mediators. *Eur J Pharmacol* 2008;**583** (2-3):174-85.

43 Kudielka BM, Gierens A, Hellhammer DH*, et al.* Salivary cortisol in ambulatory assessment--some dos, some don'ts, and some open questions. *Psychosom Med* 2012;**74** (4):418-31.

44 McEwen BS. Protective and damaging effects of stress mediators. *New Eng J Med* 1998;**338** (3):171-9.

45 Marques AH, Silverman MN, Sternberg EM. Evaluation of stress systems by applying noninvasive methodologies: measurements of neuroimmune biomarkers in the sweat, heart rate variability and salivary cortisol. *Neuroimmunomodulation* 2010;**17** (3):205-8.

46 Grynderup MB, Mors O, Hansen AM*, et al.* Work-unit measures of organisational justice and risk of depression--a 2-year cohort study. *Occup Environ Med* 2013;**70** (6):380-5.

47 Kemeny ME. Psychobiological responses to social threat: Evolution of a psychological model in psychoneuroimmunology. *Brain, Behavior, and Immunity* 2009;**23** (1):1-9.

48 Dickerson SS, Kemeny ME. Acute stressors and cortisol responses: a theoretical integration and synthesis of laboratory research. *Psychol Bull* 2004;**130** (3):355-91.

49 Hansen ÅM, Garde AH, Persson R. Sources of biological and methodological variation in salivary cortisol and their impact on measurement among healthy adults: a review. *Scand J Clin Lab Invest* 2008;**68** (6):448-58.

50 Born J, Fehm HL. The neuroendocrine recovery function of sleep. *Noise Health* 2000;**2** (7):25-38.

51 Clow A, Thorn L, Evans P*, et al.* The awakening cortisol response: methodological issues and significance. *Stress* 2004;**7** (1):29-37.

52 Adam EK, Kumari M. Assessing salivary cortisol in large-scale, epidemiological research. *Psychoneuroendocrinology* 2009;**34** (10):1423-36.

53 Nielsen MB, Hetland J, Matthiesen SB*, et al.* Longitudinal relationships between workplace bullying and psychological distress. *Scand J Work Environ Health* 2012;**38** (1):38-46.

54 Quine L. Workplace bullying in NHS community trust: staff questionnaire survey. *BMJ* 1999;**318** (7178):228-32.

55 Quine L. Workplace bullying in nurses. *J Health Psychol* 2001;**6** (1):73-84.

56 Bilgel N, Aytac S, Bayram N. Bullying in Turkish white-collar workers. *Occup Med (Lond)* 2006;**56** (4):226-31.

57 Yildirim D. Bullying among nurses and its effects. *Int Nurs Rev* 2009;**56** (4):504-11.

58 Rodwell J, Demir D. Psychological consequences of bullying for hospital and aged care nurses. *Int Nurs Rev* 2012;**59** (4):539-46.

59 Kivimäki M, Virtanen M, Vartia M*, et al.* Workplace bullying and the risk of cardiovascular disease and depression. *Occup Environ Med* 2003;**60** (10):779-83.

60 Figueiredo-Ferraz H, Gil-Monte PR, Olivares-Faundez VE. Influence of mobbing (workplace bullying) on depressive symptoms: a longitudinal study among employees working with people with intellectual disabilities. *J Intellect Disabil Res* 2013.

61 Emdad R, Alipour A, Hagberg J*, et al.* The impact of bystanding to workplace bullying on symptoms of depression among women and men in industry in Sweden: an empirical and theoretical longitudinal study. *Int Arch Occup Environ Health* 2013;**86** (6):709-16.

62 Nielsen MB, Einarsen S. Can observations of workplace bullying really make you depressed? A response to Emdad et al. *Int Arch Occup Environ Health* 2013;**86** (6):717-21.

63 Reknes I, Pallesen S, Mageroy N*, et al.* Exposure to bullying behaviors as a predictor of mental health problems among Norwegian nurses: results from the prospective SUSSH-survey. *Int J Nurs Stud* 2014;**51** (3):479-87.

64 Kudielka BM, Kern S. Cortisol day profiles in victims of mobbing (bullying at the work place): preliminary results of a first psychobiological field study. *J Psychosom Res* 2004;**56** (1):149-50.

65 Hansen AM, Hogh A, Persson R*, et al.* Bullying at work, health outcomes, and physiological stress response. *J Psychosom Res* 2006;**60** (1):63-72.

66 Hansen AM, Hogh A, Persson R. Frequency of bullying at work, physiological response, and mental health. *J Psychosom Res* 2011;**70** (1):19-27.

67 Hogh A, Hansen AM, Mikkelsen EG*, et al.* Exposure to negative acts at work, psychological stress reactions and physiological stress response. *J Psychosom Res* 2012;**73** (1):47-52.

68 Lac G, Dutheil F, Brousse G*, et al.* Saliva DHEAS changes in patients suffering from psychopathological disorders arising from bullying at work. *Brain Cogn* 2012;**80** (2):277-81.

69 Kasl SV. Measuring job stressors and studying the health impact of the work environment: an epidemiologic commentary. *J Occup Health Psychol* 1998;**3** (4):390-401.

70 Kolstad HA, Hansen AM, Kaergaard A*, et al.* Job strain and the risk of depression: is reporting biased? *Am J Epidemiol* 2011;**173** (1):94-102.

71 Hogh A, Hansen AM, Bloch C*, et al.* Mobning og negativ adfærd på arbejdspladsen [Bullying and negative behaviour in the workplace]. Copenhagen: The National Research Centre for the Working Enviroment 2009.

72 Åstrand P-O, Rodahl K. *Textbook of work physiology. Physiological basis of exercise*. Singapore: McGraw-Hill Book Company 1986:523-82.

73 Wing J.K, Sartorius N, Ustun T.B. Diagnosis and clinical measurement in psychiatry. A reference manual for SCAN. *WHO* 1998.

74 Celik C. *Computer assisted personal interview application for the Schedules for Clinical Assessment in Neuropsychiatry, version 2.1, and diagnostic algorithms for WHO ICD 10 chapter V DCR and for Diagnostic and Statistical Manual for Mental disorders, version IV*. Geneva: WHO 2005.

75 Bech P, Rasmussen NA, Olsen LR*, et al.* The sensitivity and specificity of the Major Depression Inventory, using the Present State Examination as the index of diagnostic validity. *J Affect Disord* 2001;**66** (2-3):159-64.

76 Eysenck HJ. A Short Questionnaire for the Measurement of two Dimensions of Personality. *J Appl Psychol* 1958;**42** (1):14-7.

77 Francis L.J, Lewis C.A, Ziebertz H. The short-form revised Eysenck personality Questionnaire (EPQ-S): A german edition. *Social Behavior and Personality* 2006;**34** (2):197-204.

78 Setterlind S, Larsson G. The stress profile: A psychosocial approach to measuring stress. *Stress Med* 1995;**11**:85-92.

79 Antonovsky A. The structure and properties of the sense of coherence scale. *Soc Sci Med* 1993;**36** (6):725-33.

80 Agervold M. Bullying at work: a discussion of definitions and prevalence, based on an empirical study. *Scand J Psychol* 2007;**48** (2):161-72.

81 Hasin DS GRSFGB. Epidemiology of major depressive disorder: Results from the national epidemiologic survey on alcoholism and related conditions. *Arch Gen Psychiatry* 2005;**62** (10):1097-106.

82 Hellhammer DH, Wüst S, Kudielka BM. Salivary cortisol as a biomarker in stress research. *Psychoneuroendocrinology* 2009;**34**:163-71.

83 Zapf D, Einarsen S. Individual Antecedents of Bullying: Victims and Perpetrators. In: Einarsen S, Hoel H, Zapf D*, et al.*, eds. *Bullying and Harassment in the Workplace*. Boca Raton: CRC Press 2011:177-200.

84 Wadiwalla M, Andrews J, Lai B*, et al.* Effects of manipulating the amount of social-evaluative threat on the cortisol stress response in young healthy women. *Stress* 2010;**13** (3):214-20.

85 Balodis IM, Wynne-Edwards KE, Olmstead MC. The other side of the curve: examining the relationship between pre-stressor physiological responses and stress reactivity. *Psychoneuroendocrinology* 2010;**35** (9):1363-73.

86 Eriksen HR, Olff M, Murison R*, et al.* The time dimension in stress response: relevance for survival and health. *Psychiatry Res* 1999;**85**:39-50.

87 Kudielka BM, Hellhammer DH, Wüst S. Why do we respond so differently? Reviewing determinants of human salivary cortisol responses to challenge. *Psychoneuroendocrinology* 2009;**34** (1):2-18.

88 Kristenson M, Garvin P, Lundberg U. The Role of Saliva Cortisol Measurement in Health and Disease Introduction - Why This Book? In: Kristenson M, Garvin P, Lundberg U, eds. *The Role of Saliva Cortisol Measurement in Health and Disease* 2011:3-16.

89 Wilhelm I, Born J, Kudielka BM*, et al.* Is the cortisol awakening rise a response to awakening? *Psychoneuroendocrinology* 2007;**32** (4):358-66.

90 Bellingrath S, Weigl T, Kudielka BM. Chronic work stress and exhaustion is associated with higher allostastic load in female school teachers. *Stress* 2009;**12** (1):37-48.

91 Maina G, Bovenzi M, Palmas A*, et al.* Psychosocial environment and health: Methodological variability of the salivary cortisol measurements. *Toxicol Lett* (0).

92 Kaerlev L, Kolstad HA, Hansen AM*, et al.* Are risk estimates biased in follow-up studies of psychosocial factors with low base-line participation? *BMC Public Health* 2011;**11**:539.

93 Mikkelsen EG, Einarsen S. Bullying in Danish work-life: Prevalence and health correlates. *Eur J Work Org Psychol* 2001;**10** (4):393-413.

94 Ortega A, Hogh A, Borg V. Bullying, absence and presenteeism in Danish elderly care sector: A one-year follow-up study. June 4-6, Montreal, Canada: Sixt International Conference on Workplace Bullying 2008:84-6.

95 Einarsen S, Gemzøe ME. Individual effects of exposure to bullying at work. In: Einarsen S, Hoel H, Zapf D*, et al.*, eds. *Bullying and*

Emotional Abuse in Workplace: International Perspectives in research and practice. London: Taylor & Francis 2003:127-44.

96 Hogh A, Mikkelsen EG, Hansen AM. Individual consequences of workplace bullying / mobbing. In: Einarsen S, Hoel H, Zapf D*, et al.*, eds. *Workplace Bullying: Development in Theory, Research and Practice. CRC Press, Taylor & Francis* 2011.

97 Moayed FA, Daraiseh N, Shell R*, et al.* Workplace Bullying: a systematic review of risk factors and outcomes. *Theoretical Issues in Ergonomics Science* 2006;**7** (3):311-27.

98 Mikkelsen EG, Iversen GF. Bullying at work: Perceived effects on health, well-being and present job situation. In: Liefooghe APD, Hoel H, eds. London: Birkbeck, University of London 2002:31.

99 O'Morre M, Seigne E, McGuire L*, et al.* Bullyign at work. Victims of bullying at work in Ireland. *Journa of Occupational Health and Safety* 1998;**16** (6):569-74.

100 Zapf D, Dormann C, Frese M. Longitudinal studies in organizational stress research: a review of the literature with reference to methodological issues. *J Occup Health Psychol* 1996;**1** (2):145-69.

101 World Health Organization. The ICD-10 Classification of Mental and Behavioural Disorders. Diagnostic criteria for research. Geneva, Switzerland 1993.

102 Twisk JW. *Applied Multilevel Analysis: A practical Guide for Medical Researchers*. New York: Cambridge University Press 2006.

103 Dai J, Li Z, Rocke DM. Hierarchical logistic regression modelling with SAS GLIMMIX. 2006.

104 Setterlind S, Larsson G. The stress profile: A psychosocial approach to measuring stress. *Stress Med* 1995;**11** (1):85-92.

105 Vie TL, Glaso L, Einarsen S. How does it feel? Workplace bullying, emotions and musculoskeletal complaints. *Scand J Psychol* 2012;**53** (2):165-73.

106 Hauge LJ, Skogstad A, Einarsen S. The relative impact of workplace bullying as a social stressor at work. *Scand J Psychol* 2010.

107 Dickerson SS, Kemeny ME. Acute Stressors and Cortisol Responses: A Theoretical Integration and Synthesis of Laboratory Research. *Psychol Bull* 2004;**130** (3):355-91.

108 Karlson B, Lindfors P, Roberto R*, et al.* Psychosocial Work Stressors and saliva Cortisol. In: Kristenson M, Garvin P, Lundberg U, eds. *The Role of Saliva Cortisol Measurement in Health and Disease*: Bentham Science Publisher 2011:43-66.

109 Dickerson SS, Gruenewald TL, Kemeny ME. When the social self is threatened: shame, physiology, and health. *J Pers* 2004;**72** (6):1191-216.

110 Hansen AM, Hogh A, Garde AH*, et al.* Workplace bullying and sleep difficulties: a 2-year follow-up study. *Int Arch Occup Environ Health* 2013.

111 Grynderup MB, Kolstad HA, Mikkelsen S*, et al.* A two-year follow-up study of salivary cortisol concentration and the risk of depression. *Psychoneuroendocrinology* 2013;**38** (10):2042-50.

112 Hansen AM, Garde AH, Christensen JM*, et al.* Evaluation of a radioimmunoassay and establishment of a reference interval for salivary cortisol in healthy subjects in Denmark. *Scand J Clin Lab Invest* 2003;**63** (4):303-10.

113 Westgard JO, Barry PL, Hunt MR*, et al.* A multi-rule Shewhart chart for quality control in clinical chemistry. *Clin Chem* 1981;**27** (3):493-501.

114 Garde AH, Hansen ÅM, Nikolajsen TB. An inter-laboratory comparison for determination of cortisol in saliva. *Accred Qual Assur* 2003;**8** (1):16-20.

115 Jensen MA, Hansen AM, Abrahamsson P*, et al.* Development and evaluation of a liquid chromatography tandem mass spectrometry method for simultaneous determination of salivary melatonin, cortisol and testosterone. *J Chromatogr B Analyt Technol Biomed Life Sci* 2011;**879**:2527.

116 Garde AH, Persson R, Hansen AM*, et al.* Effects of lifestyle factors on concentrations of salivary cortisol in healthy individuals. *Scand J Clin Lab Invest* 2009;**69** (2):242-50.

117 Niedhammer I, David S, Degioanni S*, et al.* Workplace bullying and psychotropic drug use: the mediating role of physical and mental health status. *Ann Occup Hyg* 2011;**55** (2):152-63.

118 Lahelma E, Lallukka T, Laaksonen M*, et al.* Workplace bullying and common mental disorders: a follow-up study. *Journal of Epidemiology and Community Health* 2011.

119 Lambert E, Lambert G. Stress and Its Role in Sympathetic Nervous System Activation in Hypertension and the Metabolic Syndrome. *Current Hypertension Reports* 2011:1-5.

120 Chrousos GP, Gold PW. A healthy body in a healthy mind--and vice versa--the damaging power of "uncontrollable" stress. *J Clin Endocrinol Metab* 1998;**83** (6):1842-5.

121 Kirschbaum C, Hellhammer DH, Pirke K-M. The 'Trier Social Stress Test'--a tool for investigating psychobiological stress responses in a laboratory setting. *Neuropsychobiology* 1993;**28**:76-81.

122 Burke HM, Davis MC, Otte C*, et al.* Depression and cortisol responses to psychological stress: A meta-analysis. *Psychoneuroendocrinology* 2005;**30** (9):846-56.

123 Chopra KK, Ravindran A, Kennedy SH*, et al.* Sex differences in hormonal responses to a social stressor in chronic major depression. *Psychoneuroendocrinology* 2009;**34** (8):1235-41.

124 Hamilton LD, Newman ML, Delville CL*, et al.* Physiological stress response of young adults exposed to bullying during adolescence. *Physiol Behav* 2008;**95** (5):617-24.

125 Garde AH, Persson R, Hansen AM*, et al.* Effects of lifestyle factors on concentrations of salivary cortisol in healthy indviduals. *Scand J Clin Lab Invest* 2008.

SUMMARY

Background: During the past year, approximately 10% of the Danish workforce has been exposed to bullying at work. Furthermore, 1-2% has reported that bullying occurs weekly or more frequently at their workplace. People exposed to workplace bullying report more health problems than people who are not exposed to workplace bullying, and knowledge about the nature of bullying and the victims' social and psychological characteristics is well established. A methodological issue of previous studies has been the complete reliance on self-report of both exposure- and outcome measurements. Thus, it remains uncertain whether or not bullying can cause persistent mental illness and disturbances of the hypothalamic-pituitary-adrenal (HPA) axis.

Aims: The aim of this thesis was to examine if exposure to workplace bullying is associated with an increased risk of depression and changes of the stress hormone, cortisol, as a biomarker of the HPA-axis in a longitudinal design. Furthermore, the aim was to investigate reactivity and recovery of the HPA-axis measured by saliva cortisol, induced by a standardized bicycle exercise challenge test among persons who are bullied at work, versus those who are not.

Methods: The thesis is based on the Workplace Bullying and Harassment Cohort (WBH) (n=3123 at baseline in 2006) and the PRISME Cohort (n=4351 at baseline in 2007). Both cohorts were measured at two occasions (follow-ups: 2008 and 2009, respectively) and were merged into a joint cohort in 2011 (The Modena Project). At all occasions, the participants received an extensive survey about perceptions of health, work-life and bullying at work. In the follow-ups, new cases of depression were diagnosed with the aid of Schedules for Clinical Assessment in Neuropsychiatry interviews and the Major Depression Inventory questionnaire. Furthermore, the participants provided two samples of saliva cortisol (30 minutes after awakening and at 8 PM at all three occasions. Based on four predefined screening criteria, a group of 718 respondents were invited to a test session with an interview, a clinical examination, and a cycling test (participation rate 48%).

Results: We identified 147 new cases of depression and found an increased risk for new-onset depression among participants who reported workplace bullying *Occasionally* and *Frequently*. There was no association between exposure to workplace bullying and depression when we assessed workplace bullying by co-workers' joint evaluation of the occurrence of bullying in a working environment. Likewise, we found no significant change in cortisol, neither for participants who reported *Not Bullied* at baseline, but *Bullied* at follow-up, nor for participants who reported a discontinuance of bullying after two years. The analysis of cortisol among *Bullied* and *Non-bullied* persons indicated an increase in saliva cortisol between time 1 and time 2 at low exercise level among the most frequently bullied persons in the morning sample. However, this result was not supported by the analysis of the afternoon-samples, and may be a chance finding.

Conclusion: Self-labeled workplace bullying predicts depression and supports earlier studies on this issue, but in our investigations of a work environment with a high proportion of employees who witness bullying, we could not confirm previous findings. Furthermore, we were not able to support previous associations on workplace bullying and changes in cortisol levels. We did not discover any impact of long-term workplace bullying on cortisol, not in a longitudinal design, nor as a measurement of cortisol in an exercise challenge test. Due to methodological issues, future studies should develop independent measurements of exposure assessment, in order to discover the causality of the association between workplace bullying and depression.

RESUMÉ

Baggrund: Gennem det seneste år har omkring 10 % af den danske arbejdsstyrke været udsat for mobning på deres arbejdsplads, og 1-2 % oplyser, at mobning på arbejdspladsen foregår ugentligt eller hyppigere. Lønmodtagere som oplyser at have været udsat for mobning, rapporterer også, at de har flere helbredsproblemer end personer der ikke har været udsat for mobning. Man ved en del om mobningens natur og om mobbeofrenes sociale og psykologiske karakteristika, men studier som tidligere har vist sammenhænge mellem mobning og depression er baseret på selv-rapporterede data for både eksponering og udfaldsmål. Det er derfor fortsat usikkert, om mobning i arbejdslivet kan medføre vedvarende psykisk lidelse og resultere i forandringer i hypothalamus-hypofyse-binyrebark (HPA)-aksen.

Formål: Formålet med projektet var at undersøge sammenhængen mellem udsættelse for mobning i arbejdslivet, risiko for depression, og forandringer i niveauet af stresshormonet kortisol i et longitudinelt design. Derudover var formålet at undersøge kortisols reaktivitet og normalisering, målt i spytkortisol ved en standardiseret cykeltest mellem personer, som oplyste at været udsat for mobning på deres arbejdsplads, og personer som ikke var mobbede.

Metode: Projektet bygger på Mobningskohorten (n=3132 ved baseline i 2006) og PRISME kohorten (n=4351 ved baseline i 2007). Begge kohorter blev fulgt op med en opfølgningsundersøgelse to år senere (henholdsvis i 2008 og 2009). I 2011 blev kohorterne flettet sammen til et fælles projekt (Modena-projektet). Ved alle målinger modtog deltagerne et omfattende spørgeskema om helbred, arbejdsliv og mobning på arbejdspladsen og leverede yderligere to spytkortisolprøver (30 minutter efter opvågning og kl. 20:00). Ved begge opfølgningsundersøgelser blev nye tilfælde med depression diagnosticeret med Schedules for Clinical Assessment in Neuropsychiatry og spørgeskemaet Major Depression Inventory. Ud fra fire foruddefinerede udvælgelseskriterier, blev spørgeskemabesvarelserne brugt til at udtrække en gruppe på 718 respondenter, som blev inviteret til en undersøgelse indeholdende interview, klinisk undersøgelse og cykeltest (48 % deltagelse).

Resultat: Vi identificerede 147 nye tilfælde af depression og fandt en øget risiko for depression og depressive symptomer blandt deltagere som rapporterede at have været udsat for mobning *af & til* og *ofte*. Vi fandt ingen sammenhæng mellem udsættelse for mobning og depression eller depressive symptomer, i den del af analysen hvor vi målte mobning på arbejdspladsniveau. Ligeledes fandt vi ingen forandringer i kortisol, hverken for deltagere som rapporterede ingen mobning ved baseline men mobning ved opfølgning, eller blandt deltagere som rapporterede at mobning var ophørt fra baseline til opfølgning to år senere. Analyser af kortisol blandt mobbede og ikke mobbede personer indikerede en stigning i kortisol mellem første og anden måling ved lav fysisk aktivitet blandt de ofte mobbede i morgengruppen. Imidlertid kunne vi ikke bekræfte samme stigning i eftermiddagsgruppen.

Konklusion: rojektet bekræfter tidligere undersøgelser og viser at selv-rapporteret mobning i arbejdslivet giver en øget risiko for depression. Imidlertid kunne vi ikke i vores analyser på arbejdspladsniveau ikke understøtte samme fund. Derudover var vi ikke i stand til at understøtte sammenhængen mellem udsættelse for mobning og forandringer i kortisol. Vi fandt hverken en sammenhæng mellem langvarig mobning og forandringer i kortisol, eller forskelle i kortisol ved lav fysisk aktivitet blandt mobbede og ikke mobbede. På grund af de metodiske udfordringer som tidligere undersøgelser har været begrænset af, anbefales det at fremtidige studier udvikler nye uafhængige metoder til at måle mobningseksponering, for at kunne undersøge kausaliteten af mobning og depression.

PAPER I

Exposure to workplace bullying and risk of depression

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ABSTRACT

Objective: We examined the prospective association between self-labelled and witness-reported bullying and the risk of newly-onset of depression.

Methods: Employees were recruited from two cohorts of 3196 and 2002 employees, respectively. Participants received a questionnaire at baseline in 2006-07 with follow-up in 2008-09 and 2011. New cases of depression were diagnosed in the follow-up using Schedules for Clinical Assessment in Neuropsychiatry interviews and the Major Depression Inventory questionnaire.

Results: We identified 147 new cases of depression. The odds ratio for newly-onset depression among participants reporting bullying occasionally was 2.17 [95% CI: 1.11-4.23] and among frequently bullied 9.63 [95% CI: 3.42-27.1]. There was no association between percentage witnessing bulling and newly-onset depression.

Conclusion: Frequent self-labelled bullying predicts development of depression but a work environment with high proportion of employees witnessing bullying does not.

INTRODUCTION

Workplace bullying has been defined as *harassing, offending, socially excluding someone or negatively affecting someone's work* repeatedly and regularly over a longer period, e.g. six months (1). The concept has gained considerable interest in the past 10 years and other terms used interchangeably are mobbing and harassment. It has been argued that exposure to workplace bullying can be a severe social stressor that can have stronger adverse effects on workers' health than the effect of all other work-related stressors combined (2). The prevalence of self-reported bullying varies across studies (1). For example in a study among hospital- and manufacturing employees 3 % reported workplace bullying (3), whereas in a study among employees in the elderly care sector 12 % reported workplace bullying (4).

Cross-sectional studies have found correlations between reports of bullying and several psychological and physical symptoms (for reviews see e.g., (5-7)). Other studies have found that bullying was associated with reduced self-confidence, low self-worth, shyness, an increased sense of vulnerability as well as feelings of guilt and self-contempt among victims (8, 9)**.** So far only two follow-up studies on the prospective association between workplace bullying and depression have been published (10, 11). A two-year follow-up study among hospital employees found that self-reported bullying at work predicted increased risk of depression (10). Another prospective study found an exposure-response relation between workplace bullying and risk of onset of a major depressive episode among eldercare workers (11).

A serious limitation in previous research including the few prospective follow-up studies on workplace bullying and health has been the reliance on self-report for the assessment of both the exposure and the endpoint, rendering results vulnerable to common method variance (12, 13). The high relative risk estimates in the range of 4-9 observed even in prospective studies cast doubt as to whether bullying as an objective characteristic of human behaviour violating norms for interpersonal relations is causally related to development of major depression. This fundamental problem in earlier research aimed to establish cause-effect relations is approached in our study by use of an independent measure of bullying, namely the co-workers' joint evaluation of the occurrence of bullying in a work unit. This study was designed to examine the risk of depression according to workplace bullying measured by the self-labelling method (individual perception of being bullied) and by witnesses reports to identify the occurrence and intensity of bullying at work-unit level.

Furthermore, we used SCAN-interviews for ascertainment of the outcome in most cases to obtain reliable data on the occurrence of newly-onset depression in a prospective design.

METHOD AND MATERIAL

Study design and population

We recruited participants for the present study from two Danish cohorts, the Workplace Bullying and Harassment Cohort (WBH) (14) and the Prisme Cohort (15), see Table 1. The study participants were investigated three times with approximately two years between the examinations. At all occasions the participants received an extensive questionnaire measuring health status, working conditions, social and family life, education level, history of depression and mental distress.

Inclusion criteria

The current study population comprised employees who responded to at least two out of three surveys during the three-wave study period. Because the aim of this study is to investigate depression two years after exposure to bullying respondents with four years follow-up time (n=1162) and respondents who only participated at one occasion (n=1778) were excluded. A total of 5,198 respondents participated at two or three occasions giving a total of 9,120 periods to follow-up (see Table 2).

The Workplace bullying and Harassment cohort and the Prisme cohort

The WBH Cohort enrolled employees (n=2,002) from workplaces throughout Denmark. Participants received a questionnaire at baseline in 2006, and again in 2008. *The Prisme Cohort* comprised hospital and civil-service employees (n=3,196) from different workplaces in the Central Denmark Region. Participants received a questionnaire at baseline in 2007 and again in 2009. The WBH- and the Prisme-cohort have a comparable demographic profile and for that reason the two cohorts were combined in a joint study in 2011. E.g., both cohorts are dominated by female respondents and the workplaces are mainly situated in the public sector. Furthermore, in both cohorts younger people and men were more likely to leave the cohort after one round (14, 16). There was however a difference between the two cohorts in that the WBH-cohort had a larger loss to follow-up compared to the Prisme-cohort (data not shown).

Work unit

The 5,198 participants were affiliated to one of in total 455 work units that were defined by the lowest managerial level (Table 3). The number of employees in a work unit ranged from 1 to 161 workers. All work

units were organised into four groups according to the proportion of employees who witnessed workplace bullying: 0% witnesses (n=683), 1-20% witnesses (n= 2274), 21-30% witnesses (n=1353) and >30% witnesses (n= 837) (see Table 3). This grouping was defined before analyses were carried out as an appropriate trade-off between exposure contrast and number of participants in each group. Table 3 also illustrates the relation between witnesses of bullying and perception of bullying in the four exposure groups.

Measures of self-labelled workplace bullying

To calibrate the participants' understanding of bullying, we first presented a definition similar to the one by Einarsen et al. (1); " Bullying occurs when one or more persons repeatedly over a longer period, are exposed to unpleasant or negative actions or behaviours at work, that it is difficult to defend one-self against". Then we asked "Have you been exposed to bullying at your current workplace within the last 6 months?" Response categories were "never", "now and again", "monthly", "weekly" and "daily". We created a three-level exposure variable with the following categories: "no", "occasional bullying" (comprising the response categories "now and again" and "monthly") and "frequent bullying" (comprising the response categories "weekly" and "daily") (Table 3).

Measures of witnessing workplace bullying

To assess witnesses reporting bullying, we first presented the same bullying definition described above. Next, we asked the participants to state whether or not they had witnessed a colleague being bullied at work during the last 6 months. The five-point scale was used with the following categories: "never", "now and again", "monthly", "weekly" and "daily". We dichotomized the responses between "now and again" and "monthly". The proportion of witnesses per work-unit was then estimated and the value was assigned to all employees working at the work-unit.

Measures of diagnosis of depression

Diagnosis of depression was measured differently in the two cohorts. In the *WBH Cohort,* depression was identified with the Major Depression Inventory (MDI) questionnaire (17). In the *Prisme Cohort,* depression was measured using the SCAN interview. In the joint study in 2011, SCAN interviews were again used to diagnose depression according to ICD-10 DCR-criteria. The SCAN interviews were conducted during the spring, three to six months after the respondents had filled out the screening questionnaire.

MDI in the Workplace Bullying and Harassment cohort

MDI is a self-rated questionnaire with an algorithm that leads to ICD-10 categories of mild to severe depression (17). MDI consists of 10 symptoms, where items 8 and 10 have a sub-item; thus, there were 12 questions in total. Each item measures the presence of symptoms during the past two weeks on a scale ranging from 0 (the symptom has not been present at all) to 5 (the symptom is present all the time). The algorithm includes core and accompanying symptoms, and each symptom is dichotomised to indicate whether it is clinically significant present (=1) or absent (=0).

SCAN interview in the Prisme cohort and the joint study in 2011

We diagnosed depression according to the International Classification of Diseases, Tenth Revision, Diagnostic Criteria for Research (ICD-10-DCR) (18) by applying the Schedules for Clinical Assessment in Neuropsychiatry (SCAN) interview, version 2.1, part I (19). The following sections of part I were used: section 3 (worrying and tension), section 4 (panic anxiety and phobias), sections 6-8 (depression). The interview focused on the previous 3 till 5 months and was computer aided and semi-structured (19, 20). A single interview took about one hour, and each was conducted by psychology and medical students trained to manage these interviews.

Screening criteria for SCAN interviews

In 2007, the following four screening criteria were used to select respondents for the SCAN interviews (n=595): 1) depressive symptoms (point score of ≥3 on ≥3 of the six depressive symptoms items from the SCL-DEP6 (21), n=311); 2) a random sample of people with symptoms of burnout (mean score of ≥4 on Copenhagen Burn-out Inventory, n=80); 3) stress symptoms (mean score of ≥2.5 on Perceived Stress Scale, n=79); and 4) a random sample (n=434). The screening criteria from 2007 are also described in Kolstad et al. 2011 (15).

In 2009, the following four criteria for SCAN interviews (n=562) were: 1) depressive, stress or burnout symptoms, based on the questionnaires from 2009 (n=599); 2) ICD-10 depression, diagnosed with the SCAN interviews in 2007 (n=71); 3) a random sample with people from the random sample in 2007 (n=201); and 4) people who reported high psycho-social load (n=167).

In 2011 the screening criteria for SCAN interview were developed from the criteria from 2007 and 2009. The used screening criteria were (n=655): 1) exposure to at least one out of 11 Negative Acts of bullying weekly or

more frequently, or perceived workplace bullying monthly or more often during the past 12 months (n=207); 2) depressive symptoms (n=246); 3) anxiety symptoms (point score of $\geq$2 of the three anxiety symptoms items, n=186). The final criterion was 4) a random sample (n=220). The four screenings groups were overlapping.

Statistics

We used logistic regression to examine risk of newly-onset depression by self-labelling of bullying at baseline and by the proportion of employees who witnessed bullying in their work units at baseline. The follow-up period from baseline to ascertainment of outcome was approximately 2 years and each participant could contribute up to 2 follow-up periods. Among the 5,198 respondents, we identified 9,120 2-year periods of follow-up (Table 2). Cases with depression at baseline, measured with SCAN interview or MDI, were excluded from the analysis. Two different methods were used to estimate the risk of depression in relation to workplace bullying. The first method used the self-labelling method of bullying as an indicator of exposure. The second method used the proportion of colleagues within a work-unit that had witnessed bullying as an indicator of exposure. Lacking independence of the up to two follow-up periods in the same individual was accounted for by the proc genmod repeated measures procedure with SAS software, version 9.2 (SAS Institute, Inc. Cary, N.C., USA). Differences in characteristics across groups categorised by percentage of employees witnessing bullying (Table 4) were evaluated by the use of Pearson's chi square tests.

The hierarchical structure of the study population characterised by nesting employees within work units may violate the assumption of independent observations potentially causing too narrow confidence limits. To address this issue we repeated the main analysis by hierarchical logistic regression modelling using SAS 9.1 PROC GLIMMIX. In these analyses the effect of work units are accounted for by a random intercept variable (22, 23)

We included a fixed set of covariates selected a-priori based on current knowledge (24-28). From the baseline questionnaire the following confounders were measured: gender, age ($\leq$34, 35-44, 45-54, $\geq$55), previous episodes of depression (yes/no), family history of depression (yes/no), years of education beyond primary or high school (<3/3-4/>4), weekly alcohol consumption ($\leq$14 [for females] and $\leq$21 [for males]), depressive symptoms (rating of $\geq$3 on <2, $\geq$2 questions from the SCL-DEP6 (21) or MDI score of $\geq$20), smoking (never, ex-smoker, smoker). Interactive effects on risk of depression by cohort (Prisme and WBH, respectively) and

self-labelled bullying was examined by inclusion of an interaction term (cohort*bullying) in the regression models.

Among respondents in the Prisme cohort we controlled for personality traits based on the shortened version of Eysenck short-form Personality Questionnaire (EPQ-S) (29, 30) measuring extraversion and neuroticism. Among respondents in the WBH cohort we also controlled for personality traits with the scale Sense of Coherence (SOC) (31) based on Antonovskys concept (32).

RESULTS

In total 5,198 employees took part in at least one follow-up (see Table 1 and Table 2). The distribution of participants according to percentage of employees witnessing bullying past 6 months is presented in Table 3. Table 3 also presents the distribution of employees that perceive that they have been bullied by percentage of employees witnessing bullying in their work. Among those, the mean age was 48 years and the gender distribution was 75 % females and 25 % males. Table 4 presents characteristics of respondents across groups categorised by percentage of employees witnessing bullying past six months. Since associations between bullying and depression were not modified by cohort in any of the analyses we present findings for the cohorts combined.

Prospective analyses of newly-onset depression as predicted by the self-labelling method

Table 5 presents the prospective association between self-labelled workplace bullying and the risk of newly-onset depression. In the respondent groups of the "occasionally" and "frequently" bullied, the adjusted odds ratios were 2.17 [95% CI: 1.11 - 4.23] and 9.63 [95% CI: 3.42 – 27.10], respectively. The hierarchical logistic regression analysis using SAS GLIMMIX which account for possible clustering of the outcome within work units showed essentially the same results (adjusted odds ratio 1.97 [95% CI: 1.12-3.47] and 7.90 [95% CI: 3.04-20.50], respectively).

When adjusting for the participants who changed job at follow-up, the OR for occasionally bullying was 2.43 [95% CI: 1.38 – 4.26] and 10.94 [95% CI: 4.24 - 28. 20] for frequently bullying. Separating the analyses for men and women did not change the results significantly. For men the OR for occasional bullying was 1.44 [95% CI: 0.33 – 6.24] and 27.98 [95% CI: 4.96 – 157.71] for frequent bullying. For women the OR for occasional bullying was 2.38 [95% CI: 1.33 – 4.25] and for frequent bullying was 5.97 [95% CI: 1.96 -18.14].

To account for the different measures of depression we performed a sensitivity analysis based upon the more reliable SCAN interview only and found an adjusted OR of 2.66 [95 % CI: 1.48 – 4.77] for occasional bullying and for frequent bullying an OR of 5.31 [95 % CI: 1.48 – 19.04], which is close to the results of the overall analysis also including MDI cases. Both results followed the pattern of the main analysis.

The cohort specific ORs based upon SCAN verified depression were in the Prisme cohort 3.01 [95% CI: 1.58 – 5.73] for occasional bullying and 6.22 [95 % CI: 1.66 - 23.24] for frequent bullying. In the WBH cohort the corresponding ORs were 0.76 [95% CI: 0.22 – 2.64] and 11.44 [95% CI: 3.80 – 34.46] for occasional and frequent bullying, respectively.

In the Prisme cohort we adjusted for extraversion and neuroticism at baseline. The OR for occasional bullying was 2.73 [95% CI: 1.33 – 5.67] and for the group reporting frequent bullying the OR was 5.00 [95% CI: 1.36-18.46]. Among respondents in the WBH cohort we controlled for personality traits with the SOC scale**.** This did not change the result for the WBH cohort. For the group reporting occasionally bullying the OR was 0.64 [95% CI: 0.18 – 2.26] and for the group reporting frequently bullying the OR was 9.93 [95% CI: 1.49 – 66.16].

Prospective analyses of newly-onset depression as predicted by the proportion of witnesses within a work-unit

Table 6 presents the prospective association between the proportion of employees who witnessed workplace bullying at baseline and the risk of newly-onset depression after 2 years. The results did not support that witnessing bullying at the work-unit level predicts newly-onset depression, neither in the crude nor in the adjusted analysis. The risk of depression was estimated according to the exposure groups with 1-20 % witnesses, 21-30 % and >30 %. The adjusted odds ratios for depression were 0.91 [95% CI: 0.51-1.64], 0.81 [95% CI: 0.43-1.53] and 0.89 [95% CI: 0.46-1.73], respectively. The hierarchical logistic regression produced essentially the same results (adjusted odds ratio 0.91 (95% CI: 0.51-1.64), 0.92 (95% CI: 0.50-1.73 and 1.17 (95% CI: 0.63-2.20, respectively)

To control for participants who changed their job between baseline and follow-up, we adjusted the sensitivity analysis for participants who at follow-up answered they had changed work-unit. The analysis did not change the results. According to the exposure groups the OR for depression was 1.20 [95% CI: 0.66 - 2.12], 1.16 [95% CI: 0.62 - 2.20] and 1.24 [95% CI: 0.65 - 2.40], respectively. All work units with 0-10 employees were removed from the analysis. This did not change our main result in any of the exposure groups; 0-20%: 0.86 [95% CI: 0.35- 2.10], 21-30 %: 0.83 [95 % CI: 0.32 – 2.12], > 30%: 0.75 [95% CI: 0.23-2.50]. We also separated the analysis between men and women. Results were unchanged. For the three exposure groups the OR for men were

0.75 [95% CI: 0.20 - 3.60], 1.40 [95% CI: 0.26 - 7.34] and 1.73 [95% CI: 0.33 - 9.13]) and for women the OR were 0.96 [95% CI: 0.53 -1.75], 0.81 [95% CI: 0.42-1.56] and 1.02 [95% CI: 0.52-2.0].

With respect to the risk of SCAN verified depression according to work unit proportion of witnessing bullying the ORs from low to high prevalence were 0.88 [95% CI: 0.49- 1.60], 0.80 [95 % CI: 0.41 – 1.54] and 1.10 [95% CI: 0.57-1.92] and the corresponding cohort specific ORs were for the Prisme cohort 0.92 [95% CI: 0.50 - 1.70], 1.04 [95% CI: 0.53 - 2.04] and 1.21 [95% CI: 0.65 - 2.30] and for the WBH cohort 0.50 [95% CI: 0.10 - 2.30], 0.40 [95% CI: 0.01 - 1.89] and 0.40 [95% CI: 0.05 - 3.3].

In the Prisme cohort we controlled for personality traits by adjusting for extraversion and neuroticism. This did not change the main result. According to the exposure groups the OR were 0.93 [95% CI: 0.47 – 1.83], 1.08 [95% CI: 0.51 – 2.25] and 1.46 [95% CI: 0.75 – 2.86] respectively. In the WBH cohort we controlled for personality traits with the SOC scale**.** This did not change the main result. When controlling for SOC the OR for depression after 2 years were 0.72 [95% CI: 0.10 – 5.20], 0.46 [95% CI: 0.06 – 3.61] and 0.58 [95% CI: 0.06 – 5.32] respectively.

DISCUSSION

We observed a strong prospective association between self-labelled bullying at the workplace the past 6 months and the occurrence of depression two years later among employees without depression at baseline. Furthermore, the risk of depression was related to the severity of bullying as the risk was markedly higher in participants reporting weekly or daily bullying than among participants with less frequent bullying. The risk was not attenuated when accounted for by several potential confounders. On the contrary, when we measured bullying at the work-unit level as a non-self-reported measurement by percentage of witnesses, we did not observe an increased risk of newly-onset depression.

The results regarding self-labelled bullying corroborate the findings from the only two prospective studies of workplace bullying and depression that to the best of our knowledge have been published so far (10, 11). The first study addressed the risk of self-reported physician diagnosed depression among 5,432 healthcare workers who reported workplace bullying at baseline and at follow-up after two years (OR 4.81 [CI 95% 2.46-9.40] (10)). In this study the self-labelling of workplace bullying and depression were not separated in time since information on both was obtained at follow-up. In the second study (11) of 5,701 eldercare workers in Denmark the risk of depression ascertained by the MDI was substantially increased in employees free of previous depression who reported workplace bullying two years earlier. Thus, the findings of the two earlier studies and of the present are consistent and the observed associations were very strong with regard to the risk of depression among people reporting bullying at work. The present study contributes to the two earlier studies by the use of a standardised interview to establish the diagnosis of major depression as opposed to use of questionnaires. Findings are also consistent with results of several earlier cross-sectional studies some of which include other employees than health- and eldercare workers (2, 33, 34). Furthermore, in the present study participants came from a wide range of occupations, whereas the two previous studies were restricted to health care workers (10, 11). Thus, our study showed that the association between self-labelled bullying and risk of depression is not limited to specific occupations. Finally, an array of larger and smaller work-units is included in the current study. When we controlled for the work-unit size the results did not change.

To our knowledge, this is the first study of depression in relation to workplace bullying where the outcome was ascertained by standardized interviews. Previous studies have measured depression by self-administrated depression checklists only (10, 11). In our study, the outcome was defined by MDI inventory only in the WBH

cohort. However, in a Danish validation study, the sensitivity and specificity of the MDI algorithms of moderate to severe depression according to ICD-10-DCR were analysed by using the SCAN as the index of validity; the sensitivity was 0.86 and the specificity 0.86 indicating a satisfactory agreement between the two instruments (17). Furthermore, our sensitivity analysis leaving out the MDI cases revealed essentially the same results for respondents reporting frequently bullying, where as we also discovered a significant increased OR among respondents reporting occasionally bullying.

Our goal was to identify as many of the participants with clinical depression as possible while keeping a high specificity. The selection of participants for SCAN interviews was based on screening criteria, which according to earlier analyses had a sensitivity of 84 % and a specificity of 96 %. However, the design did not allow for detection of new cases of depression that vanished during the follow-up period. Moreover, since the questionnaires were filled in three to six months before the SCAN interviews were completed, some cases were probably not identified because of remission before the interview was carried out. However, incomplete ascertainment of depression is hardly related to the exposure under study and is therefore primarily expected to reduce the statistical power of the study but not to result in bias.

The participation rate for the initial survey was below 50%. In both cohorts the respondents differed from the non-respondents both by gender, age and social class (16). Obviously such a low participation rate raises concern regarding selection bias. Using registry data also available for non-respondents we compared risk of sick leave and use of antidepressant medicine according to work-unit-measures of the psychosocial environment (also available for non-respondents) in the sample of respondents and the entire sample also including non-respondents and observed no substantial differences in risk estimates (16). We believe that the low participation in both cohorts mostly is caused by inconvenience related to the high demands to participate including repeated sampling of saliva and lengthy SCAN interviews. In any case, differential participation in the baseline surveys is less likely to cause biased estimates in the follow-up analyses as loss to follow-up was limited. Loss to follow-up was most pronounced in young males and in low socio-economic classes which may bias analyses of the association between self-reported bullying and risk of depression if the drop-out is differential with respect to both the exposure and the outcome. Comparing baseline characteristics of respondents and non-respondents do not indicate bias related to loss of follow-up (Data not shown).

Due to the lack of independence between self-reported exposure and self-reported outcome it is difficult to state causality. In occupational health it is a primary goal to obtain knowledge on adverse working environments that can be targeted by preventive means to improve health. For this purpose the individual perception of the working environment is useful to the extend it is a reliable reflection of the working environment. The perception of other people's behaviors and intentions are strongly dependent on the individual norms, attitudes, preconceptions and social factors. A behavior that one individual perceives as bullying behavior may be perceived as normal (in the literary sense) by managers and colleagues (1). Even more important, perception of adverse behavior of others and susceptibility to develop mental disorders may share common determinants – for instance linked to personality characteristics (35). The very strong cross-sectional and longitudinal associations between individual perception of bullying and depression with relative risks in the range of 5-10 that has been reported in earlier studies (10, 11) indicate that common-method bias because of lacking independence of exposure and outcome variables may be important. For that reason it is from a preventive occupational health point of view needed to get independent measures of exposures. In this paper we use witnessing of bullying among employees in work units as such a measure. The interest is not whether witnessing of bullying increase the risk of depression among witnesses. The prevalence of employees witnessing bullying is used to define a bullying work environment independently of individuals that perceive themselves as victims of bullying. If bullying is causing depression among the bullied we will expect that the risk of depression is higher in work-units with a high proportion of witnesses as an indication of a higher and/or more agreed occurrence of bullying behavior. Is this assumption reasonable? First, bullying could be hidden to the majority of employees and in the extreme known only by the victim and the perpetrator. This might in particular be an issue in large work-units, but risk estimates were close to unity in both large and small work units. Second, the proportion of witnesses may not reflect the number of subjects that are exposed to bullying behavior and for that reason cause bias towards null. This is probably the most serious limitation of our approach and unfortunately we are lacking information on the number of persons that employees consider bullied in each work-unit. However, the risk of depression was the same in work units with no witnesses of work place bulling at all compared to workplaces with at least one witness. Third, in small work units a large group of employees might engage in bullying behavior against one single scapegoat and this particular scenario where most witnesses are perpetrators themselves would hardly be reflected by our questionnaire. Although research indicates that there is an overlap between self-labelled bullying and bullying reported by witnesses (36) is seems obvious that there is a need to develop more reliable methods for measuring

of work place bullying. Thus, this approach does not include employees' personal experiences of being bullied, but represents a supplement to the traditional approach.

Conclusion

In this study the perception of frequent workplace bullying is a strong predictor of development of a depressive disorder within a two year period. However, a bullying work environment defined by a high proportion of employees witnessing bullying is not predictive of depression. The findings of this study provide new perspectives for the understanding of workplace bullying and options for preventive actions. From a preventive and an occupational health point of view it is crucial to understand the mechanisms which explain the association between perceived bullying and depression. Considering the strong association between the individual perception of bullying and risk of depression we expected to find an association between prevalence of witnesses reporting bullying and risk of depression among employees working in high-risk units. The discrepant findings of this study may therefore indicate a need for more studies applying non-self-reported assessments. Furthermore, there is a need to refine and validate tools to measure workplace bullying in order to enable studies that reliably can separate effects attributable to individual and environmental characteristics.

Table 1 Source- and study populations and outcome ascertainment by cohort and year of data collection

Year	Source population	Respondents		Study population[a]	Invited to SCAN	Participated in SCAN		Prevalence of depression				New cases of depression			
			%	N			%	SCAN	%	MDI[b]	%	SCAN	%	MDI[b]	%
The Prisme Cohort															
2007	10 036	4351	43		904[c]	595	66	97	2.2	-	-	-	-	-	-
2009	4508	3204	71		1088[d]	562	52	78	2.4	-	-	63	2.0	-	-
2011	4489	3278	73		225[e]	142	63	49	1.4	-	-	41	1.3	-	-
				3196											
The WBH Cohort															
2006	7358	3123	42		-	-	-	-	-	100	3.2	-	-	-	-
2008	3707	2237	60		-	-	-	-	-	65	3.0	-	-	52	2.3
2011	3707	2210	60		430[e]	182	42	33	-	-	-	31	1.4	-	-
				2002											
Total		18 403		**5198**	2647	1481		422				187			

a) Included respondents with one or two follow-ups (see Table 1: Overview of included and excluded participants)
b) Measured by the MDI
c) Screening criteria for SCAN Interview 2007 were depressive-, burnout- and stress symptoms and a random sample
d) Screening criteria for SCAN interview 2009 were depressive-, burnout and stress symptoms, cases of depression from 2007, high psycho-social demands and a random sample
e) Screening criteria for SCAN interview 2011 were depressive- and anxiety symptoms, self-reported workplace bullying or negative acts and a random sample. In the Prisme cohort the number of invited are relatively lower because the random sample primarily were invited from the WBH cohort.

Table 2 Overview of included and excluded participants

Cohort		Round 1 baseline	Round 2	Round 3 follow-up	Included participants	Excluded participants	Courses of events
Prisme	Participants responding 2 out of 3 rounds	2007		2009	469		469
		2009		2011	52		52
	Participants responding 3 out of 3 rounds	2007	2009	2011	2675		5350
	Participants responding 1 out of 3 rounds					688	
	Participants responding 2 out of 3 rounds with four years follow-up	2007		2011		547	
WBH	Participants responding 2 out of 3 rounds	2006		2008	406		406
		2008		2011	349		349
	Participants responding 3 out of 3 rounds	2006	2008	2011	1247		2494
	Participants responding 1 out of 3 rounds only					1090	
	Participants responding 2 out of 3 rounds with four years follow-up	2006		2011		615	
	Total number of participants				5198	2940	9120

Overview of partcipants included in the study. Partcipants responding at only one occasion and participants with four years follo-up time were excluded. Dataanalysis is based on observations with only two-year follow-up periods. This overview shows baseline and follow-up year. Participants who responded at all three occasions are occuring twice in the dataanlysis with different baseline and follow-up information depending on the wave.

Table 3 Distribution of participants according to percentage of employees witnessing bullying past 6 months by cohort and size of the work units (upper part) and distribution of employees that perceive that they have been bullied by percentage of employees witnessing bullying in their work unit (lower part)

	0%			**1-20 %**			**21 -30 %**			**>30 %**			**All**		
	Work units	Persons	%	Work units	Persons	%	Work units	Persons	%	Work units	Persons	%	Work units	Persons	%
The Prisme study															
Size of work units															
0-10 employees	171	513	30.8	42	321	19.3	32	268	16.1	101	563	33.8	346	1665	100.0
11-20 employees	4	58	7.2	28	451	56.0	12	226	28.0	3	71	8.80	47	806	100.0
>20 employees	1	39	6.2	8	341	54.2	4	204	32.4	1	45	7.2	14	629	100.0
The WBH Cohort															
Size of work units															
0-10 employees	-	-	-	1	10	7.8	5	46	36.0	8	72	56.3	14	128	100.0
11-20 employees	2	28	17.4	4	85	52.8	2	48	29.8	0	0	-	6	161	100.0
>20 employees	-	-	-	16	1066	62.2	10	561	32.7	2	86	5.0	28	1713	100.0
Sum	**178**	**638**		**99**	**2274**		**65**	**1353**		**115**	**837**		**469**	**5102**[a]	**100.0**
Distribution of employees reporting workplace bullying within exposure groups															
Never		586	98.9		2034	94.60		1173	92.73		715	86.88			
Occasionally		4	0.68		106	4.93		80	6.32		97	11.79			
Frequently		2	0.34		10	0.47		12	0.95		11	1.34			

[a] Ninety six subjects could not be identified with their work-unit and are therefore missing in this table

Tabel 4 Characteristics of respondents across groups categorized by percentage of employees witnessing bullying

	Percentage witnessing bullying in work units								
	0% (n=638)		**0-20 % (n=2274)**		**20-30 % (n=1353)**		**>30 % (n=837)**		
	n	**%**	**n**	**%**	**n**	**%**	**n**	**%**	***P***
Cohort									
The Prisme Cohort	610	19.67	1113	35.90	698	22.51	715	21.90	<.0001
The WBH Cohort	28	1.39	1161	57.99	655	32.71	158	7.89	
Sex									
Female	511	13.22	1783	46.15	1006	26.04	563	14.57	<.0001
Male	127	9.96	491	38.51	347	27.22	310	24.31	
Age groups									
<30 years	15	9,38	63	39,38	62	38,75	20	12,50	0.0002
30-39 years	136	12,57	506	46,77	291	26,89	149	13,77	
40-49 years	173	12,25	608	43,06	354	25,07	277	19,62	
50-59 years	242	13,20	821	44,79	478	26,08	292	15,93	
>60 years	72	11,06	276	42,40	168	25,81	135	20,74	
Higher education									
< 3 years	145	9,91	616	42,11	391	26,73	311	21,26	<.0001
3-4 years	400	13,73	1286	44,13	792	27,18	436	14,96	
> 4 years	86	12,03	348	48,67	161	22,52	120	16,78	
Living alone									
No	525	12,40	1890	44,63	1091	25,76	729	17,21	0.230
Yes	113	12,96	370	42,43	251	28,78	138	15,83	
Body mass index									
<18,5 kg/m2	17	27,42	4	6,45	24	38,71	17	27,42	0.006
18,5-25.0 kg/m2	393	13,17	1329	44,55	801	26,85	460	15,42	
>25 kg/m2	228	11,32	881	43,72	512	25,41	394	19,55	
Smoking									
Smoker	79	10,39	308	40,53	203	26,71	170	22,37	<.0001
Ex-smoker	220	12,00	791	43,15	503	27,44	319	17,40	
Never smoked	334	13,34	1157	46,22	635	25,37	377	15,06	
Alcohol									
<14 (Female)/<21 (Male) units of alcohol	612	12,54	2160	44,27	1277	26,17	830	17,01	0.530
>14 (Female)/>21 (Male) units of alcohol	26	10,04	114	44,02	76	29,34	43	16,60	
Family income									
< 500.000	134	20,49	233	35,63	159	24,31	128	19,57	0.029

500-899.999	304	18,70	577	35,49	375	23,06	370	22,76	
>=900.000	74	19,17	135	34,97	68	17,62	109	28,24	
Life events									
No	558	19,65	1002	35,29	631	22,23	648	22,82	0.805
Yes	52	17,51	111	37,37	67	22,56	67	22,56	
Family depression									
Yes	149	17,65	288	34,12	201	23,82	206	24,41	0.177
No	404	20,16	717	35,78	443	22,11	440	21,96	
Unknown	43	18,30	92	39,15	41	17,45	59	25,11	
Earlier depression									
Yes	79	17,06	161	34,77	102	22,03	121	26,13	0.337
No	449	18,10	899	36,24	558	22,49	575	23,18	
Workplace bullying									
No	586	13,00	2034	45,12	1173	26,02	715	15,86	<.0001
Occasionally	4	1,39	106	36,93	80	27,87	97	33,80	
Frequently	2	5,71	10	28,57	12	34,29	11	31,43	

Table 5 Risk of newly-onset depression by self-reported workplace bullying

	Number of observations	New cases of depression by SCAN/MDI	%	OR	OR adj[a].	95 % CI
Depression						
Perceived workplace bullying						
Never	7864	113	1.4	1.0	-	-
Occasionally	460	16	3.5	2.47	2.17	1.11-4.23
Frequently	52	6	11.5	8.94	9.63	3.42-27.10

[a]Adjusted for age, gender, earlier depression, depressive symptoms, family history of depression, higher education, alcohol, smoking

Table 6 Risk of newly-onset depression by percentage of employees witnessing bullying

	Percentage witnessing workplace bullying	Number of observations	New cases of depression/ depressive symptoms by SCAN/MDI	%	OR	OR adj[a].	95 % CI
Depression							
	0	1159	19	1.6	1.0	-	-
	1-20 %	3872	63	1.6	0.99	0.91	0.51-1.64
	21-30 %	2276	34	1.5	0.91	0.81	0.43-1.53
	>30 %	1539	29	1.9	1.15	0.89	0.46-1.73

[a]Adjusted for age, gender, earlier depression, depressive symptoms, family history of depression, higher education, alcohol, smoking

Reference List

1. Einarsen S, Hoel H, Zapf D, Cooper CL. The concept of bullying and harassment at work: The European Tradition. In: Einarsen S, Hoel H, Zapf D, Cooper CL, eds. *Bullying and Harassment in the Workplace*. Boca Raton: CRC Press; 2011:3-39.

2. Hauge LJ, Skogstad A, Einarsen S. Relationships between stressful work environments and bullying: Results of a large representative study. *Work Stress.* 2007;21:220-242.

3. Mikkelsen EG, Einarsen S. Bullying in Danish work-life: Prevalence and health correlates. *Eur J Work Org Psychol.* 2001;10:393-413.

4. Ortega A, Hogh A, Borg V. Bullying, absence and presenteeism in Danish elderly care sector: A one-year follow-up study. June 4-6, Montreal, Canada: Sixt International Conference on Workplace Bullying; 2008:84-86.

5. Einarsen S, Gemzøe ME. Individual effects of exposure to bullying at work. In: Einarsen S, Hoel H, Zapf D, Cooper CL, eds. *Bullying and Emotional Abuse in Workplace: International Perspectives in research and practice*. London: Taylor & Francis; 2003:127-144.

6. Hogh A, Mikkelsen EG, Hansen AM. Individual consequences of workplace bullying / mobbing. In: Einarsen S, Hoel H, Zapf D, Cooper C, eds. *Workplace Bullying: Development in Theory, Research and Practice. CRC Press, Taylor & Francis*. 2011.

7. Moayed FA, Daraiseh N, Shell R, Salem S. Workplace Bullying: a systematic review of risk factors and outcomes. *Theoretical Issues in Ergonomics Science.* 2006;7:311-327.

8. Mikkelsen EG, Iversen GF. Bullying at work: Perceived effects on health, well-being and present job situation. In: Liefooghe APD, Hoel H, eds. London: Birkbeck, University of London; 2002:31.

9. O'Morre M, Seigne E, McGuire L, Smith M. Bullyign at work. Victims of bullying at work in Ireland. *Journa of Occupational Health and Safety.* 1998;16:569-574.

10. Kivimäki M, Virtanen M, Vartia M, Elovainio M, Vahtera J, Keltikangas-Jarvinen L. Workplace bullying and the risk of cardiovascular disease and depression. *Occup Environ Med.* 2003;60:779-783.

11. Rugulies R, Madsen IE, Hjarsbech PU, et al. Bullying at work and onset of a major depressive episode among Danish female eldercare workers. *Scand J Work Environ Health.* 2012;38:218-227.

12. Kasl SV. Measuring job stressors and studying the health impact of the work environment: an epidemiologic commentary. *J Occup Health Psychol.* 1998;3:390-401.

13. Zapf D, Dormann C, Frese M. Longitudinal studies in organizational stress research: a review of the literature with reference to methodological issues. *J Occup Health Psychol.* 1996;1:145-169.

14. Hogh A, Hansen AM, Bloch C, et al. Mobning og negativ adfærd på arbejdspladsen [Bullying and negative behaviour in the workplace]. Copenhagen: The National Research Centre for the Working Enviroment; 2009.

15. Kolstad HA, Hansen AM, Kaergaard A, et al. Job strain and the risk of depression: is reporting biased? *Am J Epidemiol.* 2011;173:94-102.

16. Kaerlev L, Kolstad HA, Hansen AM, et al. Are risk estimates biased in follow-up studies of psychosocial factors with low base-line participation? *BMC Public Health.* 2011;11:539.

17. Bech P, Rasmussen NA, Olsen LR, Noerholm V, Abildgaard W. The sensitivity and specificity of the Major Depression Inventory, using the Present State Examination as the index of diagnostic validity. *J Affect Disord.* 2001;66:159-164.

18. World Health Organization. The ICD-10 Classification of Mental and Behavioural Disorders. Diagnostic criteria for research. Geneva, Switzerland: 1993.

19. Wing J.K, Sartorius N, Ustun T.B. Diagnosis and clinical measurement in psychiatry. A reference manual for SCAN. *WHO.* 1998.

20. Celik C. *Computer assisted personal interview application for the Schedules for Clinical Assessment in Neuropsychiatry, version 2.1, and diagnostic algorithms for WHO ICD 10 chapter V DCR and for Diagnostic and Statistical Manual for Mental disorders, version IV.* Geneva: WHO; 2005.

21. Christensen KS, Fink P, Toft T, Frostholm L, Ørnbøl E, Olesen F. A brief case-finding questionnaire for common mental disorders: the CMDQ. *Family Practice.* 2005;22:448-457.

22. Twisk JW. *Applied Multilevel Analysis: A practical Guide for Medical Researchers.* New York: Cambridge University Press; 2006.

23. Dai J, Li Z, Rocke DM. Hierarchical logistic regression modelling with SAS GLIMMIX. 2006.

24. Hasin DS GRSFGB. Epidemiology of major depressive disorder: Results from the national epidemiologic survey on alcoholism and related conditions. *Arch Gen Psychiatry.* 2005;62:1097-1106.

25. Burcusa SL, Iacono WG. Risk for recurrence in depression. *Clinical Psychology review.* 2007;27:959-985.

26. Andersen I, Thielen K, Nygaard E, Diderichsen F. Social inequality in the prevalence of depressive disorders. *Journal of Epidemiology and Community Health.* 2009;63:575-581.

27. Boden JM, Fergusson DM, Horwood LJ. Cigarette smoking and depression: tests of causal linkages using a longitudinal birth cohort. *The British Journal of Psychiatry.* 2010;196:440-446.

28. Kessler RC. The Effects of Stressful Life Events on Depression. *Annu Rev Psychol.* 1997;48:191-214.

29. Eysenck HJ. A Short Questionnaire for the Measurement of two Dimensions of Personality. *J Appl Psychol.* 1958;42:14-17.

30. Francis L.J, Lewis C.A, Ziebertz H. The short-form revised Eysenck personality Questionnaire (EPQ-S): A german edition. *Social Behavior and Personality.* 2006;34:197-204.

31. Setterlind S, Larsson G. The stress profile: A psychosocial approach to measuring stress. *Stress Med.* 1995;11:85-92.

32. Antonovsky A. The structure and properties of the sense of coherence scale. *Soc Sci Med.* 1993;36:725-733.

33. Niedhammer I, David S, Degioanni S. Association between workplace bullying and depressive symptoms in the French working population. *J Psychosom Res.* 2006;61:251-259.

34. Vie TL, Glaso L, Einarsen S. How does it feel? Workplace bullying, emotions and musculoskeletal complaints. *Scand J Psychol.* 2012;53:165-173.

35. Zapf D, Einarsen S. Individual Antecedents of Bullying: Victims and Perpetrators. In: Einarsen S, Hoel H, Zapf D, Cooper CL, eds. *Bullying and Harassment in the Workplace*. Boca Raton: CRC Press; 2011:177-200.

36. Nielsen MB, Notelaers G, Einarsen S. Measuring Exposure to Workplace Bullying. In: Einarsen S, Hoel H, Zapf D, Cooper CL, eds. *Bullying and Harassment in the Workplace*. Boca Raton: CRC Press, Taylor & Francis Group; 2011:149-174.

PAPER II

Is workplace bullying related to changes in levels of cortisol in saliva?

A longitudinal study among Danish employees

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ABSTRACT

It is well established that acute psychosocial stressors may trigger a physiological response including substantial increase of cortisol secretion, but the physiological adjustment following exposure to psychosocial stressors over longer time periods is less explored. Workplace bullying is considered a strong psychosocial stressor. The aim of this two year follow-up study was to investigate if perception of being bullied at work is related to subsequent change in morning and evening saliva cortisol concentrations and if cortisol concentrations changed after discontinuance of workplace bullying.

Methods: Participants came from two Danish cohort studies, the PRISME cohort (n=4489) and the Workplace Bullying and Harassment Cohort (n=3707). All participants with a two year follow-up period and complete records were included. At baseline, and follow-up, exposure to bullying was measured by a single question on bullying (preceded by a definition). Two saliva samples to measure cortisol were collected during a work-day (30 minutes after awakening and at 8 p.m.). The reference group consisted of non-bullied respondents at baseline and follow-up.

Results: Results did not indicate any statistical significant change in saliva cortisol, neither when participants changed their self-labeling from never bullied at baseline to being bullied at follow-up, nor when participants reported discontinuance of bullying after two years. All analyses were adjusted for the potential confounders (education, smoking, alcohol and BMI).

Conclusion: This longitudinal controlled study addressing the impact of long-term workplace bullying on saliva cortisol does not corroborate earlier reports of cross-sectional associations between perception of bullying and saliva cortisol.

INTRODUCTION

During the last few decades workplace bullying has come more into focus. Workplace bullying is often found in settings with a poor psychosocial working environment and research have stated that exposure to bullying at work has a marked effect on employees health and well-being [1-3]. In occupational epidemiology workplace bullying has been defined by Einarsen and co-workers (1996) [4] as *harassing, offending, socially excluding or negatively affecting someone's work. In order for the label bullying to be applied to a particular activity, interaction or process, the bullying behaviour have to occur repeatedly and regularly over a longer period (e.g. six months)* [1]. While it is well established that exposure to acute stressors increases the cortisol secretion [5] the response to long term stressors is less studied [6]. McEwen (2008) has suggested that a dysregulation of cortisol can lead to dysregulation of multiple other physiological systems, including the immune and cardiovascular system, metabolic function and personal behavior [7, 8]. Being bullied involves the loss of control, a lack of predictability, and negative feedback – all of which are central factors in the regulation of emotion and/or physiological arousal. Dickerson and Kemney [9] contribute with a theoretical model on threats to one's social self and states that a social threat can be accompanied by a specific set of psychological and physiological responses, including a cortisol increase. So far four cross-sectional studies on workplace bullying and cortisol have been published [10-13]. These studies investigated exposure to workplace bullying and cortisol secretion and suggested a lower cortisol secretion in bullied persons compared to non-bullied persons. The first study to be published was a pilot study that investigated bullying and cortisol was by Kudielka and Kern [13] among twelve women and four men. The study indicated a flattened curve in the diurnal profile; however there were no significant differences between a work day and a day off**.** Hansen et al. [10] included employees (n=437) from different occupations. In a group of bullied persons (n=22) they found a 30 % (CI 1-50%) lower cortisol concentration at awakening and 7 % (CI -2; 82 %) lower cortisol concentration 45 minutes after awakening and no significant changes in the evening**.** In this study the bullied persons had a high rate of self-reported health complaints compared to the non-bullied persons which could indicate that bullied persons might have developed more chronic symptoms than non-bullied persons. Another study by Hansen and co-workers [11] reported that frequently bullied persons in generally had 24.8 % (factor: 0.752 CI 0.62; 0.91) lower cortisol concentrations compared to the occasionally bullied and the reference group. Hogh et al. [12] found significantly reduced levels of cortisol in relation to reporting of two out of four factors of negative acts (a well known measurement of exposure to bullying behaviour). A significant reduction in cortisol was found in persons who were exposed to person-related negative acts such as direct harassment (12 %) and intimidating behavior (10 %) but not in persons subjected to work related negative acts. The studies by

Kudielka [13], Hansen (2006, 2011) and Hogh (2012) were able to discover associations between exposure to workplace bullying and changes in cortisol concentrations in cross sectional designs. Measuring cortisol concentrations and exposure to workplace bullying in a longitudinal design has to date not been published. As a contribution to existing research the present study is going deeper in investigating workplace bullying and cortisol in a longitudinal design. We investigated exposure to workplace bullying and changes in cortisol concentrations in two longitudinal designs. The aim of the study was to test if changes in morning and evening cortisol occurred after exposure to workplace bullying and if cortisol level changes after discontinuance of workplace bullying, both within a two years follow-up time.

METHODS

Design & population

This follow-up study is based on the Workplace Bullying and Harassment Cohort (WBH) and the PRISME cohort. In both cohorts participants were investigated three times with approximately two years between the examinations; the WBH cohort in was examined in 2006-2008 and enrolled employees from public and private workplaces throughout Denmark. The cohort was established in in 2006 (*n*=7358, response rate 42 %) and re-examined in 2008 (*n*=3707, response rate 60%) [14]. The PRISME cohort was examined in 2007-2009. The PRISME cohort comprised hospital and civil-service employees from workplaces in the Central Denmark Region. The PRISME cohort was established in 2007 (*n*=10 036, response rate 45 %) and re-examined in 2009 (*n*=4489, response rate 72 %) [15-17]. In 2011 WBH- and the PRISME cohort were combined into a joint study (*n*=3707, response rate 60 %, *n*=4489, response rate 73 %, respectively). At all occasions participants received an extensive questionnaire measuring workplace bullying, social and family life, education level and a kit for measurement of saliva cortisol in the morning and evening.

Data

Among the 8118 respondents we excluded 2920 (WBH *n*=1705; PRISME *n*=1215) respondents because they either participated only at one occasion or if their follow-up time was more than approximately two years (for details see figure 2). Both cohorts were measured at three occasions and all participants had therefore possibility to contribute up to three times during the study period. Among our 5198 respondents we identified a total of 9120 follow-up periods with two years between measurements. Because some respondents participated at tree occasions, these respondents were included in the analysis with two courses. For example, a respondent who participates in 2007, 2009 and 2011 will be included with two courses; one course with baseline in 2007 and follow-up in 2009 and a second course with baseline in 2009 and follow-up in 2011. The same concept is used for respondents participating at three occasions in the WBH cohort and leaves us a total of 9120 courses in this study.

For the analysis of morning cortisol we completed restrictions among our 9120 courses; we excluded cortisol values higher than 100 nmol/l and included only morning samples delivered between 04 and 10 am. For the evening sample we excluded cortisol values higher than 100 nmol/l and included only samples delivered between 6 pm and 02 am. Lastly, participants were excluded if they did not answer the question on exposure to workplace bullying at both baseline and

follow-up. After these restrictions we ended up with 3913 morning samples and 4206 evening samples at baseline and 3814 morning samples and 4050 evening samples at follow-up.

For the analysis of saliva cortisol relative to onset of bullying we compared participants who changed status from *never bullied* at baseline to *occasionally* (n=200, 2.26 %) or *frequently bullied* (n=29, 0,33%) at follow- up with participants who were either bullied at baseline or follow-up (n=8,625). For analyses of cortisol following discontinuance of cortisol we compared participants, who changed status from *occasionally bullied* (n=230 (2.58 %)) or *frequently bullied* (n=30 (0.34 %)) to not bullied at follow-up with participants who did not perceive themselves as bullied at either baseline or follow-up (n=2769, 97%)

Measures of self-labelled workplace bullying

Questions on bullying was preceded by a description of bullying similar to the definition by Einarsen et al [4]; *Bullying occurs when one or more persons repeatedly over a longer period, are exposed to unpleasant or negative acts or behaviours at work, that it is difficult to defend one-self against*. Then we asked *have you been exposed to bullying at your current workplace within the last 6 months?* The response categories were *never*, *now and then*, *monthly*, *weekly* and *daily*. Based on these categories we constructed a three-level exposure variable with the following categories: *Never bullied*, *Occasional bullying* (comprising the response categories *now and then* and *monthly*) and *Frequent bullying* (comprising the response categories *weekly* and *daily*).

Collection of saliva samples

In the first two waves 2006/2007 and 2008/2009 saliva samples were collected in Salivette® containing a cotton swab and filled in a short questionnaire on sleep, medication, and intake of alcohol at the day of sampling. A written instruction emphasized that swabs should be kept in the mouth until thoroughly saturated. Samples were to be stored in a refrigerator until they were returned by mail. The samples were stored at -20 ºC and analyzed within six months. For both cohorts, at all occasions, respondents were instructed to provide two saliva samples, the first sample was provided in the morning 30 minutes after awakening and the second sample in the evening at 20:00. In 2011 the participant collected saliva samples by spitting directly into Salivette® without swabs following the same procedure as described above.

Laboratory analyses

In 2006/2007 and 2008/2009 the determination of cortisol in saliva was carried out with a competitive radioimmunoassay (RIA) designed for quantitative in vitro measurement of cortisol in serum, plasma, urine and saliva, the Spectria Cortisol Coated Tube RIA, purchased from Orion Diagnostica, Espoo, Finland, according to the manufacturer's specifications. The sample volume was 150 ml, the range of the standard solutions prepared was 1.0—100.0 nmol/l and the incubation time was 30 min at 37°C. The specifications given by the manufacturer were a sensitivity of twice the standard deviation of the zero binding value in saliva (0.8 nmol/l), a bias of 10% (3—15%), an intra-assay variation of 5.4% and an inter-assay variation of 7.3%. Cross-reactivity to cortisone was <0.2%. A 1470 Wizard Gamma Counter (Wallac, Turku, Finland) was used for measurement of radioactivity. A method evaluation of certified reference material in water performed by our laboratory showed no bias of the method and recovery being 97% [95% CI: 94.0—100.9]. The limit of detection (LOD) was 1.59 nmol/l. Between-run coefficients of variation were 19% at 11.5 nmol/l and 16% at 49.2 nmol/l [18]. Concentrations below the limit of detection were assigned a random value between 0 and LOD extracted from a uniform distribution. Concentrations above100 nmol/l were considered outliers and deleted from the sample. To show equivalence between different runs, natural saliva samples (5.9 nmol/l and 18.5 nmol/l) were used as control materials and analyzed together with the samples. Westgard control charts were used to document that the trueness and the precision of the analytical methods remained stable [19]. The performance of the methods has been further validated by participation in interlaboratory comparison schemes [18, 20].

In 2011 we used a newly developed method: cortisol was extracted from saliva described by Jensen et al. [21]. The samples were left to thaw at room temperature for approximately 45 min and centrifuged at 3500 g for 10 min. Liquid-liquid extraction of 200 µl saliva with 1 ml ethyl acetate, evaporated to dryness under nitrogen flow and re-dissolve in 200 µL 10 % methanol (MeOH) were carried out as described by Jensen et al. D-4-cortisol was used ad internal standard. The calibration range were 0.5 – 90.0 nmol/L.

Cortisol were determined by injecting a volume of 25 µL into an Agilent 1200 HPLC (Agilent technologies, Santa Clara, CA, USA) equipped with a C18 2.1x50 mm 2.6 µm Kinetex column and a Krud-katcher ultra filter (Phenomenex, Torrance, CA). The mobile phase consisted of a 2 mM aquatic solution of ammonium acetate with 0.1 % (v/v) formic acid (A) and MeOH with 2 mM ammonium acetate and 0.1% (v/v) formic acid (B). A linear gradient was run over 3 min from 10 % to 100 % B and maintained at 100% MeOH for 1,5 min, followed by 2 min of equilibration at

10% MeOH resulting in a total run time of 6.5 min. The flow rate was 0.5 mL/min and the temperature of the auto sampler and column oven was 8°C and 40°C, respectively. Detection of cortisol was performed by a mass spectrometer, an Agilent 6460 QQQ (Agilent technologies, Santa Clara, CA) equipped with a jet stream ESI ion source, was operated in the positive ion mode as described by Jensen et al [21]. The flow and temperature of the dry and sheath gases were: 11 L/min, 350 ºC, 8 L/min and 400 ºC, respectively. The nebulizer gas pressure was 50 psi and the capillary voltage was 4,5 kV. The quantification was achieved by using low-energy collision induced tandem mass spectrometry in the multiple reaction monitoring mode. A single precursor ion – product ion transition was measured for each hormone and its internal standard. The transitions were: m/z 363.2→m/z 121.1 for cortisol; m/z 367.2→m/z 121.2 for D-4-cortisol. Intra-day precision was 8.5% for a concentration of 13 nmol/L and 7.8% for a concentration of 65 nmol/L. Inter-day precision was 14.3% for a concentration of 13 nmol/L and 13.4% for a concentration of 65 nmol/L. The LOD for cortisol was found to be 0.27 nmol/L [21]. To show equivalence between different runs, natural saliva samples (2.5 nmol/l and 11.9 nmol/l) were used as control materials and analyzed together with the samples. Westgard control charts were used to document that the analytical method remained under analytical and statistical control − in other words, that the trueness and the precision of the analytical methods remained stable [19]. The performance of the methods has been further validated by participation in interlaboratory comparison schemes [18, 20].

Statistics

We used multilevel mixed-effects linear regression to examine changes in cortisol by the onset as well as discontinuance of workplace bullying. We stratified all analyses by cohort (PRISME- and WBH), but also performed pooled analyses. Crude risk estimates were adjusted by a fixed set of covariates selected a-priori based on current knowledge [22]: smoking (never, ex-smoker, smoker), weekly alcohol consumption (≤14 [for females] and ≤21 [for males]), BMI (<18.5/18.5-25/>25) (continuous or categorical) and years of education beyond primary or high school (<3/3-4/>4). Moreover we included sample time, sample time squared (to account for the non-monotonic diurnal variation of saliva cortisol) and time of awakening. We took account of the interdependence of up to two observational units per participant by using a random effects model. Supplementary we tested for effect modification between cohort and perceived workplace bullying on salivary cortisol concentration. All analyses were performed in STATA 12.

RESULTS

Crude morning and evening saliva cortisol values for participants in the PRISME- and the WBH cohort are presented in Table 1. Supplementary analysis for effect modification between cohort and perceived workplace bullying on salivary cortisol concentration showed the relationship between bullying and cortisol to be non-significant, neither the relationship between cohort and cortisol was significant (data not shown). We found no interaction between occasionally bullying and cohort, whereas the interaction between frequently bullying and cohort was significant (p = <0.001). Thus, the association between bullying and cortisol was significantly different for the two cohorts. In both the analysis of onset workplace bullying and discontinuance of workplace bullying were overrepresented by women with 37 % (35 % in WBH- and 39 % in the PRISME cohort) (Table 2 and Table 4). Characteristic of participants in both analysis are presented in Table 2 and 4, respectively, with illustrations of participants who change category in lifestyle factors from baseline to follow-up.

Results from analysis of onset of workplace bullying (Table 3) show crude values estimated as difference from baseline to follow-up. The adjusted analysis shows no statistical significant change in cortisol when respondents are changing their self-labelling from *never bullied* to *occasionally bullied* or *frequently bullied* compared to those who remained *never bullied*. The results indicate no change in morning or evening cortisol in the analysis of onset workplace bullying at follow-up among those who were not bullied at baseline. In a supplementary analysis cohorts were pooled and no statistically significant were found (*morning samples* for occasionally bullied: -0.08 (95 % CI: -1.77;1.62) and frequently bullied: 0,01 (95 % CI -4,41;4,43) and *evening samples* for occasionally bullied: 0.30 (95 % CI: -0.45;1.02) and frequently bullied: 1.20 (95 % CI -0.73;3.10)) .

Results of analysis for discontinuance of workplace bullying (Table 5) shows crude values estimated as difference from baseline to follow-up. The adjusted analyses show a significant decrease in the cortisol morning sample among occasionally bullied in the PRISME cohort compared to the participants that remained *never bullied*, however no significant change were discovered in other samples. In the pooled analysis no statistically significant results were found (*morning samples* for occasionally bullied: -0.94 (95 % CI: -2.55;0.66) and frequently bullied: -0.50 (95 % CI -4.93;3.93) and *evening samples* for occasionally bullied: 0.32 (95 % CI: -0.38;1.02) and frequently bullied: 0.50 (95 % CI -1.55;2.55)).

DISCUSSION

The longitudinal analyses indicated no significant within-person change in saliva cortisol concentrations neither as a result of newly onset perception of being bullied at the workplace past 6 months nor as a result of discontinuance of workplace bullying. To our knowledge this is the first observational study that examines cortisol response in a longitudinal design following changes in perceived bullying exposure.

Considering our a priori hypothesis we need to deliberate if bias can explain the unexpected null findings. First, our study has few cases of frequently bullied persons: 18 persons with newly onset bullying weekly or more frequently and 19 persons who discontinued frequent bullying. Forty frequently bullied persons (0.4 %) participated only once and were thereby excluded. Obviously this limits the statistical power but numbers are not much different from the number of frequently bullied in the few earlier cross-sectional studies [10-13] and the number of newly onset or discontinued occasionally bullied counts in the hundreds. Nevertheless we acknowledge that the study has poor power to examine possible effects of rare cases of very severe bullying. Second, non-differential misclassification of exposure may cause bias towards the null. We used self-labelling to measure exposure to workplace bullying, which may be distorted by the emotional- and cognitive factors [23]. Third, 40 persons (0.4 %) of the frequently bullied participants at baseline did not participate at follow-up. Hence, if the loss to follow-up is differential with regard to change in cortisol concentrations findings may be biased as well. However, this seems hypothetical since the saliva cortisol concentrations at baseline were similar among follow-up participants and non-participants (data not shown).

The earlier studies provide indications that cortisol secretion is lower in bullying targets after exposure to workplace bullying [10-13]. Kudielka & Kern's (2004) pilot study on cortisol and exposure to workplace bullying examined twelve consecutive women and four men recruited through 8 months, who were bullied according to the *Leymann Inventory of Psychological Terrorization*, a measurement comprised of 45 defined bulling activities, where a victim of bulling is defined if a person experiences one out of the 45 activities during the last six months. Cortisol day profiles were measured with seven samples. The diurnal cortisol profile tended to flatten in terms of a lower peak in the morning on work days compared with days-off although differences were not significant. Furthermore, it can be questioned if effects of bullying on the HPA axis will change from day-to-day hypothesized by this study. Hansen et al. (2006) measured exposure to self-labelled bullying at the workplace by a single question among employees from different Swedish occupations (n=437) and collected 3 cortisol samples on a workday. In the group of bullied persons

(irrespective of frequency, some 5 % in this population) they found 30 % (CI 1-50 %) lower cortisol in the morning and a 7 % (CI -2; 82 %) lower cortisol concentration 8 hours after awakening (14:00) in comparison with non-bullied employees**.** In their third (45 minutes after awakening) and last (20:00) sample of cortisol no significant difference was found. In this study Hansen and co-workers used one question to distribute respondents as exposed and non-exposed by a dichotomized response category. In another sample Hansen et al. (2011) measured exposure to self-labelled workplace bullying in Danish workplaces by a single item (preceded by a definition) among 1944 employees and included a reference group of 1783 persons. Cortisol concentrations were measured three times during a workday; at awakening, 30 minutes after awakening and at 20:00 in the evening. The authors found that frequently bullied (1.1 % of the sample; weekly or more frequent) had lower raw cortisol values at awakening, after 30 minutes and at 20:00 (24.8 %) compared to occasionally bullied persons and the reference group. However, no statistically significant difference was found in the cortisol awakening response or in the decline during the day. Hogh and co-workers (2012) investigated exposure to bullying at work and cortisol concentrations in 684 employees from public and private workplaces in Denmark. The negative acts questionnaire was used to investigate if exposure to negative acts subsequent provides poor physical and psychological well-being. Cortisol samples were collected in line with the study by Hansen et al. (2006). Hogh et al. (2012) found that employees reporting exposure to person related acts, such as direct harassment and intimidating behavior, but not work-related acts, had a lowered level of cortisol per score of negative behavior (12 % ($p=0.001$) and 10 % ($p=0.030$), respectively). However; the group of respondents who report $\geq$1.5 score (the high exposure group) represents approximately 25% of the study population and could indicate to cover more than persons labelling themselves as bullied. Further, it is unclear what the cortisol reduction of 12 % and 10 % cover in this study; it seems to indicate a difference in the morning peak approximately 30 minutes after awakening, but not at awakening and in the evening.

Overall, these four studies indicated a possible association between exposure to workplace bullying and lower cortisol concentrations, but results are not entirely consistent because of different measures of exposure that classify highly different proportions of study populations as bullied and thus hardly measuring the same outcome. The four studies included different measurements of bullying; Leymann Inventory of Psychological Terrorization, negative act questionnaire and self-labelling which may define different population subgroups – and the prevalence of bullying according to the negative acts definition is much more prevalent (in the range of 15 % [24]) than the prevalence according to the self-labeling method, in this study 2-3 %.

In conclusion, our longitudinal study tested cortisol and exposure to workplace bullying in a strong design with focus on the change in persons with newly onset or discontinued perception of bullying at work. Our follow-up study indicated no association between exposure to bullying and cortisol concentrations, either in the direct pathway from not being bullied at baseline to being bullied at follow-up or in the retrospective direction from being bullied at baseline to discontinuance of bullying at follow-up.

Figure 1 Illustration of Analysis

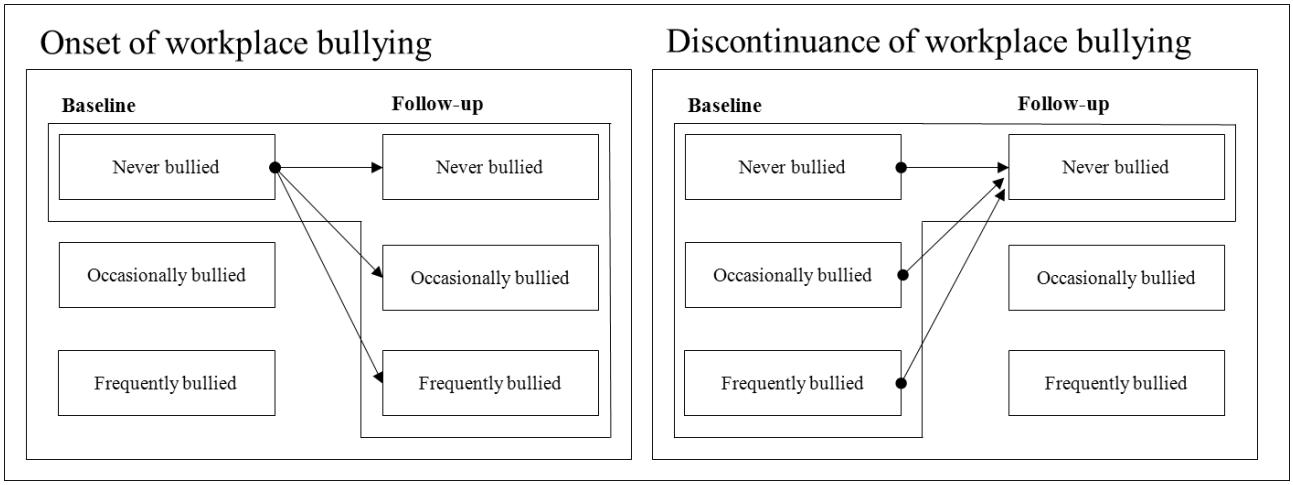

Figure 2 Flowchart

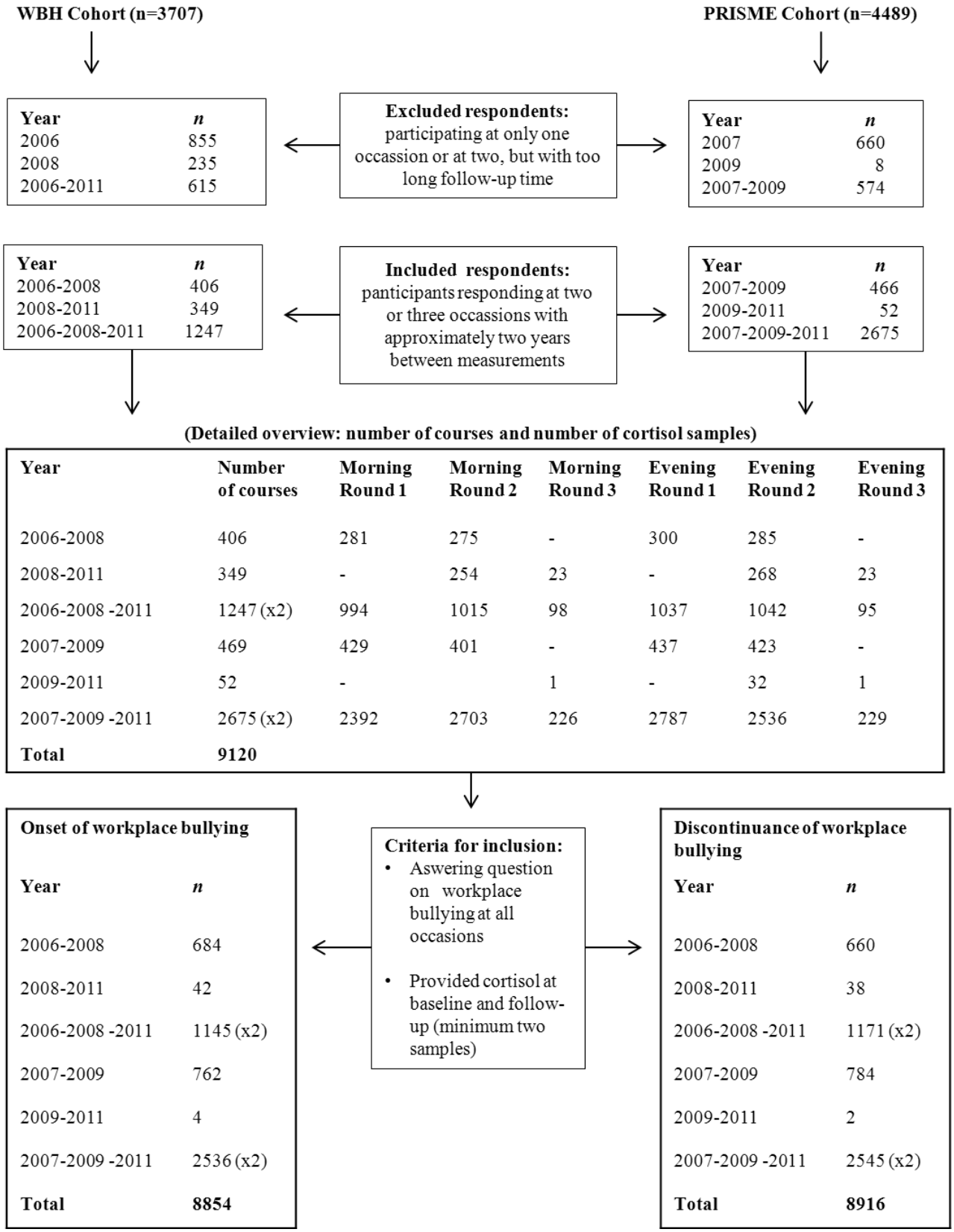

Table 1 Cortisol concentrations for morning and evening samples in the PRISME- and the WBH cohort in cross-sectional design. Samples are distributed according to respondents self-labeling of workplace bullying at all three occasions

Cohort	**Not bullied**			**Occasionally bullied**			**Frequently bullied**		
Year									
Sample	***n***	**Median**	**5-95 percentile**	***n***	**Median**	**5-95 percentile**	***n***	**Median**	**5-95 percentile**
PRISME									
2007									
Morning	3326	11.30	(3.30;25.20)	250	12.35	(2.40;31.79)	38	11.80	(3.19;34.60)
Evening	3620	1.40	(0.40;5.40)	291	1.39	(0.40;5.40)	42	1.45	(0.40;4.40)
2009									
Morning	2210	13.70	(4.20;29.70)	125	14.29	(3.50;27.10)	14	15.65	(7.50;31.50)
Evening	2459	1.40	(0.40;6.00)	148	1.49	(0.50;4.70)	18	1.30	(0.40;9.70)
2011									
Morning	171	15.04	(3.89;39.65)	32	15.55	(4.33;41.64)	12	19.52	(6.56;43.47)
Evening	173	2.82	(2.10;8.94)	35	2.87	(2.10;5.92)	11	2.66	(2.10;3.52)
WBH									
2006									
Morning	1456	11.80	(3.90;25.20)	147	12.69	(3.10;25.60)	22	8.39	(3.30;14.10)
Evening	2027	1.20	(0.40;4.10)	204	1.10	(0.30;3.60)	29	1.00	(0.30;2.60)
2008									
Morning	1327	15.35	(5.80;30.10)	100	15.00	(4.95;28.50)	11	8.40	(2.60;17.39)
Evening	1514	1.50	(0.50;5.60)	114	1.70	(0.50;7.10)	13	1.70	(0.20;9.59)
2011									
Morning	106	16.98	(3.99;34.33)	21	17.08	(4.24;26.10)	4	10.10	(2.79;33.48)
Evening	104	2.74	(2.09;5.42)	23	2.69	(2.10;4.17)	3	2.71	(2.58;2.764)

Table 2: Characteristics of respondents included in *Onset of workplace bullying* distributed according to self-labelling at follow-up

	PRISME						WBH					
	Never		Occasionally		Frequently		Never		Occasionally		Frequently	
	n	**%**	***n***	**%**	***n***	**%**	***n***	**%**	***n***	**%**	***n***	**%**
Age												
-34.99	476	8.15	12	0.21	1	0.02	159	5.27	5	0.17	1	0.03
35-44.99	616	10.55	27	0.46	3	0.1	361	11.97	14	0.46	2	0.07
45-54.99	907	15.54	44	0.75	13	0.2	445	14.75	30	0.99	1	0.03
55-	371	6.35	24	0.41	3	0.1	263	8.72	13	0.43	2	0.07
Gender												
Male	582	9.97	34	0.58	7	0.1	429	14.22	19	0.63	4	0.13
Female	2187	37.46	95	1.63	14	0.2	1000	33.16	52	1.72	4	0.13
Smoking												
Current	400	6.85	25	0.43	2	0.03	241	7.99	15	0.50	0	-
Former	998	17.09	44	0.75	9	0.15	476	15.78	28	0.93	2	0.07
Never	1352	23,16	49	0,84	10	0,17	704	23,34	26	0,86	5	0,17
Changes from baseline to follow-up												
Current smoker to former smoker	100	1.71	4	0.07	0	-	43	1.43	5	0.17	0	-
Former smoker to current smoker	58	0.99	2	0.03	1	0.02	26	0.86	0	-	0	-
Never smoked to former smoker	37	0.63	1	0.02	0	-	12	0.40	0	-	0	-
Never smoked to current smoker	2	0.03	2	0.03	0	-	5	0.17	0	-	0	-
BMI												
Underweight (-18.5)	74	1.27	3	0.05	0	-	22	0.73	2	0.07	0	-
Normal weigth (18.5-24.99)	1791	30.68	68	1.16	14	0.24	778	25.80	36	1.19	3	0.10
Overweight (25-)	904	15.48	58	0.99	7	0.12	581	19.26	29	0.96	5	0.17
Changes from baseline to follow-up												
Underweight to normal weigth	33	0.57	0	-	0	-	13	0.43	0	-	0	-
Underweigth to overweight	10	0.17	2	0.03	0	-	0	-	0	-	0	-
Normal weight to underweight	27	0.46	1	0.02	0	-	7	0.23	0	-	0	-
Normal weight to overweight	159	2.72	8	0.14	1	0.02	66	2.19	7	0.23	0	-
Overweight to underweigth	9	0.15	0	-	0	-	0	-	0	-	0	-
Overweight to normal weigh	106	1.82	5	0.09	2	0.03	37	1.23	1	0.03	1	0.03
Education level												
Low (<3 years)	413	7.07	19	0.33	2	0.03	543	18.00	33	1.09	3	0.10
Middle (3-4 years)	1826	31.28	68	1.16	9	0.15	543	18.00	16	0.53	3	0.10
High (>4 years)	329	5.64	11	0.19	0	-	213	7.06	6	0.20	0	-
Changes from baseline to follow-up												
From low education to middle education	43	0.74	0	-	0	-	34	1.13	3	0.10	0	-
From low education to high education	3	0.05	1	0.02	0	-	5	0.17	0	-	0	-

From middle education to high education	18	0.31	1	0.02	1	0.02	16	0.53	0	-	0	-
Alcohol												
Units of alcohol <14 (women) / <21 (men)	2644	45.29	123	2.11	20	0.34	1333	44.20	67	2.22	7	0.23
Units of alcohol >14 (women) / >21 (men)	125	2.14	6	0.10	1	0.02	96	3.18	4	0.13	1	0.03
Changes from baseline to follow-up												
from less than <14 to >14 / <21 to >21	57	0.98	1	0.02	0	-	36	1.19	1	0.03	0	-
from less than >14 to <14 / >21 to <21	59	1.01	3	0.05	0	-	45	1.49	1	0.03	0	-

Table 3 Analysis of onset of workplace bullying: cortisol differences and differences between exposure groups

	Never Bullied to Never bullied			**Never Bullied to Occasionally bullied**			**Never Bullied to Frequently bullied**			**Diff. between reference group and *Occasionally bullied***			**Diff. between reference group and *Frequently bullied***		
	n	**diff**	**(95 % CI)**	***n***	**diff**	**(95 % CI)**	***n***	**diff**	**(95 % CI)**	**β**	**β***	**(95 % CI)**	**β**	**β***	**95 % CI)**
Prisme															
Morning	2088	2.07	(1.65;2.50)	87	1.54	(-0.36;3.43)	16	1.57	(-3.70;6.84)	-0.54	-0.55	(-2.67;1.58)	-0.50	-0.70	(-5.44;4.09)
Evening	2223	0.25	(0.05;0.44)	105	0.39	(-0.03;0.80)	16	0.84	(-0.45;2.14)	0.14	0.32	(-0.62;1.26)	0.60	1.27	(-1.02;3.56)
WBH															
Morning	781	3.04	(2.39;3.70)	44	3.96	(1.17;6.74	2	8.75	(-2.05;19.55)	0.91	0.49	(-2.31;3.30)	5.71	5.68	(-6.83;18.19)
Evening	951	0.64	(0.41;0.87)	51	0.91	(-0.06;1.89	4	0.78	(-1.25;2.79)	0.31	0.20	(-0.87;1.27)	0.14	0.40	(-3.21;4.01)

**Adjusted analyses controlled for confounders (smoking, alcohol, BMI, education) only in cases only with explored changes in variables from baseline to follow-up*

Table 4: Characteristics of respondents included in *Discontinuance of workplace bullying* distributed according to self-labeling at follow-up

	PRISME						WBH					
	Never		Occasionally		Frequently		Never		Occasionally		Frequently	
	n	**%**	***n***	**%**	***n***	**%**	***n***	**%**	***n***	**%**	***n***	**%**
Age												
-34.99	476	8.10	21	0.36	2	0.03	159	5.23	10	0.33	0	-
35-44.99	616	10.48	28	0.48	5	0.09	361	11.88	15	0.49	4	0.13
45-54.99	907	15.44	46	0.78	5	0.09	445	14.64	32	1.05	0	-
55-	371	6.31	24	0.41	4	0.07	263	8.65	15	0.49	4	0.13
Gender												
Male	582	9.90	38	0.65	3	0.05	429	14.11	34	1.12	0	-
Female	2187	37.22	111	1.89	17	0.29	1000	32.89	47	1.55	10	0.33
Smoking												
Current	400	6.81	34	0.58	1	0.02	241	7.93	17	0.56	4	0.13
Former	998	16.98	57	0.97	7	0.12	476	15.66	32	1.05	2	0.07
Never	1352	23.01	54	0.92	12	0.20	704	23.16	31	1.02	4	0.13
Changes from baseline to follow-up												
Current smoker to former smoker	100	1.70	8	0.14	0	-	43	1.41	6	0.20	2	0.07
Former smoker to current smoker	58	0.99	2	0.03	0	-	26	0.86	5	0.16	0	-
Never smoked to fomer smoker	37	0.63	5	0.09	0	-	12	0.39	2	0.07	0	-
Never smoked to current smoker	2	0.03	0	-	0	-	5	0.16	0	-	0	-
BMI												
Underweight (-18.5)	74	1.26	2	0.03	0	-	22	0.72	1	0.03	1	0.03
Normal weigth (18.5-24.99)	1791	30.48	80	1.36	9	0.15	778	25.59	29	0.95	6	0.20
Overweight (25-)	904	15.38	67	1.14	11	0.19	581	19.11	49	1.61	2	0.07
Changes from baseline to follow-up												
Underweight to normal weigth	33	0.56	1	0.02	0	-	13	0.43	1	0.03	0	-
Underweigth to overweight	10	0.17	1	0.02	0	-	0	-	0	-	0	-
Normal weight to underweight	27	0.46	2	0.03	0	-	7	0.23	0	-	0	-
Normal weight to overweight	159	2.71	10	0.17	2	0.03	66	2.17	4	0.13	1	0.03
Overweight to underweigth	9	0.15	1	0.02	0	-	0	-	1	0.03	0	-
Overweight to normal weigh	106	1.80	5	0.09	2	0.03	37	1.22	1	0.03	1	0.03
Education level												
Low (<3 years)	413	7.03	24	0.41	4	0.07	543	17.86	43	1.41	0	-
Middel (3-4 years)	1826	31.08	94	1.60	13	0.22	543	17.86	18	0.59	4	0.13
High (>4 years)	329	5.60	12	0.20	1	0.02	213	7.01	11	0.36	6	0.20
Changes from baseline to follow-up												
From low education to middel education	43	0.73	2	0.03	1	0.02	34	1.12	2	0.07	0	-
From low education to high education	3	0.05	0	-	0	-	5	0.16	0	-	0	-
From middel education to high education	18	0.31	2	0.03	0	-	16	0.53	0	-	0	-
Alcohol												

Units of alcohol <14 (women) / <21 (men)	2644	45.00	139	2.37	20	0.34	1333	43.85	79	2.60	10	0.33
Units of alcohol >14 (women) / >21 (men)	125	2.13	10	0.17	0	-	96	3.16	2	0.07	0	-
Changes from baseline to follow-up												
from less than <14 to >14 / <21 to >21	57	0.97	3	0.05	0	-	36	1.18	2	0.07	1	0.03
from less than >14 to <14 / >21 to <21	79	1.34	6	0.10	0	-	45	1.48	2	0.07	0	-

Table 5 Analysis of discontinuance of workplace bullying: cortisol differences and differences between exposure groups

	Never bullied to Never bullied			**Occasionally bullied to Never Bullied**			**Frequently bullied To Never Bullied**			**Diff, betwee reference group and *Occasionally bullied***			**Diff, between reference group and *Frequently bullied***		
	n	**diff**	**(95 % CI)**	***n***	**diff**	**(95 % CI)**	***n***	**diff**	**(95 % CI)**	**β**	**β ***	**(95 % CI)**	**β**	**β ***	**95 % CI)**
Prisme															
Morning	2088	2.07	1.65-2.50	107	0.24	-1.69-2.17	12	-1.38	-6.21-3.46	-1.83	-1.95	(-3.87;-0.03)	-3.45	-4.07	(-9.57;1.43)
Evening	2223	0.25	0.05-0.44	119	0.80	-0.30-1.89	12	1.03	-1.55-3.62	0.27	0.37	(-0.52;1.27)	0.79	0.78	(-1.98;3.53)
WBH															
Morning	781	3.04	2.39-3.70	40	4.51	1.65-7.37	6	9.82	1.87-17.77	1.47	1.12	(-1.78;4.02)	6.77	6.19	(-1.06;13.44)
Evening	951	0.64	0.41-0.87	57	0.81	0.09-1.55	7	0.39	-0.28-1.05	0.22	0.11	(-0.92;1.14)	-0.25	0.31	(-2.63;2.70)

**Adjusted analyses controlled for confounders (smoking, alcohol, BMI, education) only in cases only with explored changes in variables from baseline to follow-up*

Reference List

1 Einarsen S, Hoel H, Zapf D, *et al.* The concept of bullying and harassment at work: The European Tradition. In: Einarsen S, Hoel H, Zapf D, *et al.*, eds. *Bullying and Harassment in the Workplace*. Boca Raton: CRC Press 2011:3-39.

2 Hauge LJ, Skogstad A, Einarsen S. The relative impact of workplace bullying as a social stressor at work. *Scand J Psychol* 2010.

3 Niedhammer I, David S, Degioanni S. Association between workplace bullying and depressive symptoms in the French working population. *J Psychosom Res* 2006;**61** (2):251-9.

4 Einarsen S. *Bullying and Harassment at work: Epidemiological and psychosocial aspects*. Bergen: Department of Psychosocial Science, Faculty of Psychology, University of Bergen 1996.

5 Dickerson SS, Kemeny ME. Acute Stressors and Cortisol Responses: A Theoretical Integration and Synthesis of Laboratory Research. *Psychol Bull* 2004;**130** (3):355-91.

6 Karlson B, Lindfors P, Roberto R, *et al.* Psychosocial Work Stressors and saliva Cortisol. In: Kristenson M, Garvin P, Lundberg U, eds. *The Role of Saliva Cortisol Measurement in Health and Disease*: Bentham Science Publisher 2011:43-66.

7 Marques AH, Silverman MN, Sternberg EM. Evaluation of stress systems by applying noninvasive methodologies: measurements of neuroimmune biomarkers in the sweat, heart rate variability and salivary cortisol. *Neuroimmunomodulation* 2010;**17** (3):205-8.

8 McEwen BS. Central effects of stress hormones in health and disease: Understanding the protective and damaging effects of stress and stress mediators. *Eur J Pharmacol* 2008;**583** (2-3):174-85.

9 Dickerson SS, Gruenewald TL, Kemeny ME. When the social self is threatened: shame, physiology, and health. *J Pers* 2004;**72** (6):1191-216.

10 Hansen AM, Hogh A, Persson R, *et al.* Bullying at work, health outcomes, and physiological stress response. *J Psychosom Res* 2006;**60** (1):63-72.

11 Hansen AM, Hogh A, Persson R. Frequency of bullying at work, physiological response, and mental health. *J Psychosom Res* 2011;**70** (1):19-27.

12 Hogh A, Hansen AM, Mikkelsen EG, *et al.* Exposure to negative acts at work, psychological stress reactions and physiological stress response. *J Psychosom Res* 2012;**73** (1):47-52.

13 Kudielka BM, Kern S. Cortisol day profiles in victims of mobbing (bullying at the work place): preliminary results of a first psychobiological field study. *J Psychosom Res* 2004;**56** (1):149-50.

14 Hansen AM, Hogh A, Garde AH, *et al.* Workplace bullying and sleep difficulties: a 2-year follow-up study. *Int Arch Occup Environ Health* 2013.

15 Grynderup MB, Kolstad HA, Mikkelsen S, *et al.* A two-year follow-up study of salivary cortisol concentration and the risk of depression. *Psychoneuroendocrinology* 2013;**38** (10):2042-50.

16 Kolstad HA, Hansen AM, Kaergaard A, *et al.* Job strain and the risk of depression: is reporting biased? *Am J Epidemiol* 2011;**173** (1):94-102.

17 Grynderup MB, Mors O, Hansen AM, *et al.* Work-unit measures of organisational justice and risk of depression--a 2-year cohort study. *Occup Environ Med* 2013;**70** (6):380-5.

18 Hansen AM, Garde AH, Christensen JM, *et al.* Evaluation of a radioimmunoassay and establishment of a reference interval for salivary cortisol in healthy subjects in Denmark. *Scand J Clin Lab Invest* 2003;**63** (4):303-10.

19 Westgard JO, Barry PL, Hunt MR, *et al.* A multi-rule Shewhart chart for quality control in clinical chemistry. *Clin Chem* 1981;**27** (3):493-501.

20 Garde AH, Hansen ÅM, Nikolajsen TB. An inter-laboratory comparison for determination of cortisol in saliva. *Accred Qual Assur* 2003;**8** (1):16-20.

21 Jensen MA, Hansen AM, Abrahamsson P, *et al.* Development and evaluation of a liquid chromatography tandem mass spectrometry method for simultaneous determination of salivary melatonin, cortisol and testosterone. *J Chromatogr B Analyt Technol Biomed Life Sci* 2011;**879**:2527.

22 Garde AH, Persson R, Hansen AM, *et al.* Effects of lifestyle factors on concentrations of salivary cortisol in healthy individuals. *Scand J Clin Lab Invest* 2009;**69** (2):242-50.

23 Nielsen MB, Notelaers G, Einarsen S. Measuring Exposure to Workplace Bullying. In: Einarsen S, Hoel H, Zapf D, *et al.*, eds. *Bullying and Harassment in the Workplace*. Boca Raton: CRC Press, Taylor & Francis Group 2011:149-74.

24 Nielsen MB, Matthiesen SB, Einarsen S. The impact of methodological moderators on prevalence rates of workplace bullying. A meta-analysis. *Journal of Occupational and Organizational Psychology* 2010;**83** (4):955-79.

PAPER III

An examination of cortisol reactivity and recovery during low level exercise among workplace bullied persons

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ABSTRACT

Background: People exposed to workplace bullying report more health problems than those who report not being bullied, and exposure to workplace bullying is associated with poor mental health, depression and anxiety. Few studies have used the cortisol response to examine the mechanisms involved in the relationship between workplace bullying and mental distress. These studies have focused on diurnal cortisol responses and they suggest that there is lower cortisol secretion in bullied persons compared to non-bullied persons. To our knowledge, no studies have examined cortisol reactivity and recovery in bullied versus non-bullied people according to uniform physical activity in a laboratory context.

Aim: The aim of this study was to investigate reactivity and recovery of the HPA-axis measured by saliva cortisol induced by a standardized bicycle exercise challenge test among workplace bullied and non-bullied persons.

Method: Participants of the PRISME Cohort (n=4489, response rate 73 %) and the Workplace Bullying and Harassment Cohort (WBH) (n=3707, response rate 59 %) were invited to answer a survey in 2011 (n=5488, response rate 66 %). Based on four predefined screening criteria, a group of 718 respondents was invited to a test session with an interview, a clinical examination and a cycling test (n=351, participation rate 48 %). The participants were divided into four groups depending on the screening and their self-report of workplace bullying, namely: those *Never Bullied*, those bullied *Sometimes* or *Monthly*, those bullied *Weekly* or *Daily*, and a random sample. A short interview about each participant's physical fitness was conducted before the cycling test to determine his/her workload during the test (Åstrand's cycling test). Measurements of saliva cortisol were conducted three times: (*time 1*) at the start of the cycling test, (*time 2*) at the end of the cycling test, and (*time 3*) two hours after the cycling test.

Results: Our analysis of saliva cortisol among bullied and non-bullied persons indicated an increase in saliva cortisol between *time1* and *time 2* among the most frequently bullied persons in the morning-sample. However, this was not supported by the analyses of afternoon-samples and may be a finding by chance.

Conclusion: The results of this study do not indicate that workplace bullying impacts HPA axis activity as measured by saliva cortisol in an exercise challenge test, but the reliability of the latter needs further investigation with respect to level of exercise and timing of samples.

INTRODUCTION

The definition by Einarsen et al. (1996) expresses that workplace bullying occurs when a person is repeatedly and regularly (for example six months) exposed to negative behaviour from one or several others, in situations where he or she, for different reasons, have difficulty defending him or herself against these actions [1]. Workplace bullying is often found in settings with poor psycho-social working environments, and it is particularly reported in relations to workplaces with a negative and stressful atmosphere [1]. According to the definition by Einarsen, the prevalence of workplace bullying varies depending on the occupation, culture and the country [2] and type of assessment [3]. Summarised by Einarsen et al [4] the prevalence of workplace bullying varies from 2 through 17 %. For example in a study among 1657 employees in the Royal Norwegian Navy 2,5 % reported being bullied at work [5] whereas another study among 7694 employees in the general French working population showed a prevalence of 11 % for men and 13 % for women [6].

For the bullied person(s) this can create severe psychological and physiological health consequences [7]. As an explanation for the consequences that can develop after exposure to negative psycho-social stressors, the psycho-biological research points to the cascades of mechanisms that are produced by activity in several biological systems [8]**.** Some researchers have noted that the biological changes may be an underlying mechanism contributes to an understanding of poor physical health as a consequence of long-term exposure to an unhealthy working environment [9]. To some extent, this has been investigated by studying people's experiences of workplace bullying [7, 10-12]. People exposed to workplace bullying report more physical health problems than those who report not being bullied [7] and bullying has further been associated with poor mental health [13], depression [14, 15] and anxiety [16]. Only four studies have used the cortisol response to examine the mechanisms involved in the relationship between workplace bullying and mental distress [7, 10-12]; these studies focused on diurnal cortisol response and suggested a lower cortisol secretion in bullied persons compared to non-bullied persons. One study examined day-to day variation within bullied subjects and three studies applied a cross-sectional design.

Disturbances in the HPA-axis are often measured by changes in a person's cortisol levels. However, it is unclear whether the hormonal changes are part of what causes a disease, or if causes of disease changes the endocrine system. Physiological stress has an impact on various biological systems, including the HPA-axis [8, 17]. Being bullied involves the loss of control, a lack of predictability, and negative feedback [18] – all of which are central factors in the regulation of emotion and/or physiological arousal. In this sense, cortisol may provide information about an individual's ability to adjust various external demands [19]. According to Dickerson and Kemeny [20] acute stressors were found to increase cortisol reactivity. They propose that uncontrollable tasks which involved a social evaluative threat produces a larger cortisol change and results in a longer recovery time compared to uncontrollable tasks without a social evaluative threat.

Studies on cortisol reactivity and recovery have been conducted by exposing humans to social stressors such as The Trier Social Stress Test (TSST) [21]. Studies in people diagnosed with depression have shown a blunted cortisol response compared to healthy control groups [22]. In addition people with other forms of mental distress have shown decreased cortisol levels during the TSST compared to healthy controls [23]. In test with persons with self-rated anxiety

symptoms, Balodis et al. (2010) found a higher reactivity in this group during TSST [24]. With use of TSST, Hamilton et al. [25] found no difference in cortisol reactivity and recovery between bullied versus non-bullied young adolescents.

Physical exercise is also known to activate the HPA axis [26] and may be a simple un-expensive alternative to TSST and therefore more feasible to apply in large-scale epidemiological field studies. To our knowledge, no studies have examined cortisol reactivity and recovery in bullied versus non-bullied people according to uniform physical activity in a laboratory context. The aim of this study was to investigate *reactivity and recovery of the HPA-axis measured by saliva cortisol induced by a standardised bicycle exercise challenge test following workplace bullied versus non bullied persons.*

METHOD

POPULATION

In January 2011, a questionnaire (the Modena Project) was sent by mail to 8,196 participants in two cohorts of Danish employees; the PRISME Cohort is comprised of civil-service employees from the central region of Denmark (n=4351, response rate 43 % at baseline in 2007) [27] and the Workplace Bullying and Harassment Cohort (WBH) (n=3123, response rate 42 % at baseline in 2006) includes employees from different workplaces throughout Denmark [12]. The total response rate to the Modena questionnaire was 66 % in 2011 (n=5488). The questionnaire measured different aspects with regard to the respondents' health status, working conditions, social- and family life, level of education and their history of depression, mental distress and workplace bullying. All participants received information and completed a written consent. The test sessions were conducted between April and July 2011. The study was conducted in accordance with the declaration of Helsinki and was proved by the Ethical Committee of Copenhagen.

Selection into study population

Participants for the cycle test were selected by the respondents' exposure to workplace bullying, symptoms of depression and symptoms of anxiety. Furthermore we draw a random sample; the four groups were overlapping. 1) *Bullying* (n=354) was assessed with a short version (11 items) of the Negative Acts Questionnaire (NAQ) and the self-labeling method. The screening criteria were a) experiencing of at least one out of eleven negative acts weekly or more frequent, or b) experiencing of workplace bullying once a month or more frequent during the past 12 months. Assessment of the self-labeling method was completed with the question *Have you been subjected to bullying within the past six months?* 2) *Depressive symptoms* (n=65) were measured using six questions from the Common Mental Disorder Questionnaire (CMDQ) [26]. The participants answered the six questions on a 5-point scale and according to their experiences during the previous four weeks; answers ranged from *Not at all*, *Sometimes*, *Occasionally*, *Frequently* to *Very often*. Scores were dichotomized (negative coded as 0 and positive coded as 1) with a cut-off between *Sometimes* and *Occasionally*, 3) *Anxiety symptoms* (n=47) were measured by using three items from the CMDQ [26]. Anxiety symptoms were also based on the previous four weeks and were answered on a 5-point scale, ranging from *Not at all*, *Sometimes*, *Occasionally*, *Frequently* to *Very often*. These scores were also dichotomized (negative coded as 0 and positive coded as 1) with a cut-off between *sometimes* and *occasionally*, 4) *A random sample* (n=400) was selected through the statistical software program SAS.

Based on these four criteria, a group of 718 respondents was invited to a test session with an interview, clinical examination and cycling test (See Figure 1). Among the 351 responding cohort members (48%), 41 persons were excluded for the following reasons: pregnancy (n=1); chronic heart disease (n=12); fever and flue (n=2); musculoskeletal disorders (n=7); and asthma (n=1); participants who did not complete the test (n=11) and errors in the saliva sample (n=7). A total of 310 participants (43 %) completed the study. Participants exposed to workplace bullying (n=137) were screened into the bullied sample by the self-labelling method (n=69) or by NAQ (n=68). The bullied sample was further divided into three groups depending on their self-report of workplace bullying on the test day, namely: those *Never Bullied* (group A), those bullied *Sometimes* or *Monthly* (group B) and those bullied *Weekly* or *Daily* (group C).

ASSESSMENT OF WORKPLACE BULLYING

On the test day workplace bullying was measured by the self-labelling method preceded by the definition by Einarsen (1996) [1]. The definition was followed by the question *Have you been exposed to bullying at your current workplace within the last 6 months*? Participants answered on a five-point scale between *Never*, *Sometimes*, *Monthly*, *Weekly* and *Daily*.

CLINICAL EXAMINATION

The clinical examinations took place at both Aarhus University Hospital (n= 241) and Bispebjerg University Hospital (n= 110), and were conducted by trained psychology- or medical students. All information was provided by the participants themselves and was written in a logbook. The examination consisted of an information session followed by a questionnaire, which assessed the same questions they each had answered three to six months before the test session. This was followed by a blood test, measurements of height, weight, waist and hip ratio, 15 minutes of heart-rate variability and an interview about mental health. A short interview about each participant's physical fitness was conducted just before the cycling test to determine his/her workload during the test. Åstrand's cycling test was completed by taking three samples of cortisol in saliva. Just before the cycling test, each participant was asked if he/she had smoked tobacco, eaten or been physically active during the last hour prior to arriving at the laboratory. The test sessions were held between 8:05 Am and 7:15 Pm. Due to the well-known diurnal variation in cortisol the population were divided into two groups; those who perform the cycling test before and after 12:00 Am.

CYCLING TEST

Åstrand's standardised method was used to estimate aerobic fitness (maximal oxygen uptake: VO2-max) during a sub-maximal workload as determined by a bicycle ergometer (Monark, model Ergomedic 874E) [27]. The initial workload was estimated based on each participant's age and usual activity level; it was typically 30-90 watts at a cadence of 60 revolutions per minute (rpm). Heart rates were measured using a heart-rate monitor (Polar Electro OY, Kempele, Finland). The workload was increased if a subject's heart rate was less than 110 beats per minute (bpm) within the first minute. The goal was to achieve a heart rate that would be 60 % of maximal heart-rate reserve capacity, and at least 120 bpm. If the heart rate had reached a steady state (i.e., difference < 5 bpm) between the fifth and sixth minute of the test, the test was terminated and the final heart rate was registered. Otherwise, the subject continued with the test until a steady state was reached, with a maximum duration of 10 minutes. Subsequently, the workload and corresponding heart rate were used to estimate VO2-max using the Åstrand-Rhyming nomogram with a correction for age [27]. Each participant's VO2-max was normalised to her/his bodyweight (ml O2.min-1.kg-1).

CORTISOL IN SALIVA DURING THE CYCLING TEST

Measurements of saliva cortisol were conducted three times in relation to the cycling test; (*time 1*) at the start of the cycling test, (*time 2*) at the end of the cycling test, and (*time 3*) two hours after the cycling test. Saliva samples collected at time 1 and 2 were stored at -80 degrees Celsius at the National Research Center of Occupational Health (NRCWE) before the analysis. Saliva from sample 3 was sent by mail to NRCWE by the participants themselves later, and was stored in the same freezer until analysis.

LABORATORY ANALYSIS

Sample preparation

All saliva samples were stored at -20°C until analysed. At the day of analysis, the samples were left to thaw at room temperature for approximately 45 min and centrifuged at 3500 g for 10 min. Liquid-liquid extraction of 200 µl saliva with 1 ml ethyl acetate , evaporated to dryness under nitrogen flow and re-dissolve in 200 µL 10 % methanol (MeOH) were carried [28]. D-4-cortisol was used ad internal standard. The calibration range were 0.5 – 90.0 nmol/L.

Determination of cortisol

A volume of 25 µL was injected into an Agilent 1200 HPLC (Agilent technologies, Santa Clara, CA, USA) equipped with a C18 2.1x50 mm 2.6 µm Kinetex column and a Krud-katcher ultra filter (Phenomenex, Torrance, CA). The mobile phase consisted of a 2 mM aquatic solution of ammonium acetate with 0.1 % (v/v) formic acid (A) and MeOH with 2 mM ammonium acetate and 0.1% (v/v) formic acid (B). A linear gradient was run over 3 min from 10 % to 100 % B and maintained at 100% MeOH for 1,5 min, followed by 2 min of equilibration at 10% MeOH resulting in a total run time of 6.5 min. The flow rate was 0.5 mL/min and the temperature of the auto sampler and column oven was 8°C and 40°C, respectively. Detection of cortisol was performed by a mass spectrometer, an Agilent 6460 QQQ (Agilent technologies, Santa Clara, CA) equipped with a jet stream ESI ion source, was operated in the positive ion mode as described by Jensen et al. (2011) [28]. The flow and temperature of the dry and sheath gases were: 11 L/min, 350 ºC, 8 L/min and 400 ºC, respectively. The nebulizer gas pressure was 50 psi and the capillary voltage was 4,5 kV. The quantification was achieved by using low-energy collision induced tandem mass spectrometry (CDI-MS/MS) in the multiple reaction monitoring (MRM) mode. A single precursor ion – product ion transition was measured for each hormone and its internal standard. The transitions were: m/z 363.2→m/z 121.1 for cortisol; m/z 367.2→m/z 121.2 for D-4-cortisol.

To show equivalence between different runs, natural saliva samples (2.5 nmol/l and 11.9 nmol/l) were used as control materials and analyzed together with the samples. Westgard control charts were used to document that the analytical method remained under analytical and statistical control − in other words, that the trueness and the precision of the analytical methods remained stable [29]. The performance of the methods has been further validated by participation in interlaboratory comparison schemes [30, 31].

STATISTICS

Cortisol reactivity was defined as the difference in saliva cortisol concentration between (*time 1*) the start of the cycling test and (*time 2*) the end of the cycling test (some 10 minutes). Recovery was defined as the difference in cortisol between (*time 2*) the end of the cycling test and (*time 3*) saliva cortisol 2 hours after the end of the cycling test Although the distribution of saliva cortisol was skewed towards the left our results are based on crude cortisol concentrations since results were essentially the same in analyses based upon the logarithmic transformation of cortisol concentrations. One single outlier was deleted from the concentrations on saliva cortisol before the statistical analyses were run. Differences in cortisol reactivity and recovery between the groups of bullied classified according to frequency of bullying and the group of non-bullied participants were investigated by a one-way ANOVA and were carried out for the participants who conducted the cycling test before 12:00 (morning sample) and after 12:00 (afternoon sample),

respectively. All analyses were adjusted for age, gender and sample time of the day. SAS Statitical software (version 9.2; SAS Institute, Inc., Cary, NC, USA) was used for statistical analysis.

RESULTS

CYCLING EXERCISE AS A CHALLENGE TEST

Exercise characteristic of the cycling test showed that the females were overrepresented in both the morning- (72 %) and afternoon sample (64 %). Mean age among respondents were in the morning sample 53 years and 49 for the afternoon sample (Table 1). During the short standardised cycling test the reactivity was 0.17 unit nmol/l (±1.13 SD) and the mean recovery was 0.78 unit nmol/l (±3.59 SD) within the random sample. Results were separated into the morning sample (n=68, 72% females) and the afternoon sample (n=105, 64 % females). In the morning group, there was no significant increase in cortisol between *time 1* and *time 2* (p=0.665, 95 % CI -0.346-0.222), whereas there was a significant decrease between *time 2* and *time 3* (p=0.018, 95 % CI 0.1818-1.8288). In the afternoon group, there was no significant increase in cortisol between *time 1* and *time 2* (*p*=0.053, 95 % CI -0.483-0.003), nor was there a decrease of cortisol between *time 2* and *time 3* (*p*=0.202, 95 % CI -0.341-1.5807).

Exercise characteristics were explored for both the morning- and afternoon sample within the random sample (Table 2). Results showed no significant differences for gender, age, workload and maximal oxygen uptake, whereas the aerobic fitness level in the morning sample (*p*=0.03) and the heart rate at steady state in the afternoon sample (*p*=0.04) were significant.

REACTIVITY AND RECOVERY BETWEEN BULLIED AND NON-BULLIED PERSONS

To investigate reactivity and recovery between bullied and non-bullied persons the sample were split in a morning- and an afternoon sample. The bullied sample was distributed into three groups according to their bullied status within the past 12 months (baseline) and on the test day (See Figure 1). Three exposure groups were distributed as follows: group A: bullied at baseline and answering *Never Bullied* on the test day (morning sample n=40 / afternoon sample n=45); group B: bullied at baseline and answering *Sometimes*- or *Monthly Bullied* on the test day (morning sample n=20 / afternoon sample n=21); group C: bullied at baseline and answering *Weekly*- or *Daily Bullied* on the test day (morning sample n=4 / afternoon sample n=7). The random-sample (morning sample n=68 / afternoon sample n=105) was used as reference group (See figure 1).

Morning sample

Differences in cortisol concentrations was in the crude analysis not significant different between the groups. This was the case for both sample 1 (*p*= 0.5089), sample 2 (*p*=0.7788) and sample 3 (*p*=0.6969). The adjusted analysis was controlled for gender, age and sample time of the day and showed a significant difference in cortisol concentration between the groups for sample 1 (*p*=0.0015) and sample 2 (*p*=0.0014). Sample time of the day indicated to have a significant influence on sample 1 (*p*=<.0001) and sample 2 (p=<.0001), whereas no significant result was seen for sample 3.

Cortisol reactivity increased within the reference group (0.06 unit nmol/l, (Std.D 1.07)), whereas group A, B and C each had a decrease in the mean cortisol levels (group A: -0.12 unit nmol/l (Std.D 1.20), group B: -0.07 unit nmol/l (Std.D 1.31), group C: -1.15 unit nmol/l (Std.D 1.14)). There was no significant differences in reactivity between the reference group, group A and group B, whereas group C was significant different from the reference group (p=0.029). Cortisol recovery mean values increased within all four groups (Reference group: 1.01 unit nmol/l (Std.D 2.86), group A: 1.04 unit nmol/l (Std.D 2.84), group B: 0.30 unit nmol/l (Std.D 1.26), group C: 0.72 unit nmol/l (Std.D 2.04)) and no significant differences were found between the groups (Table 4).

Afternoon sample

Differences in cortisol concentrations were in the crude analysis not significantly different between the groups. This was the case for both sample 1 (p= 0.5404), sample 2 (p=0.2674) and sample 3 (p=0.6788). The adjusted analysis was controlled for gender, age and sample time of the day and showed no significant difference in cortisol concentration between the groups for either sample 1 (p=0.0732), sample 2 (p=0.1176) or sample 3 (p= 0.8532).

Cortisol reactivity increased within the reference group (0.24 unit nmol/l (Std.D 1.17) and group C (0.27 unit nmol/l (Std.D 0.55) whereas a decrease was found in group A (-0.31 unit nmol/l (Std.D 0.71) and B (-0.15 unit nmol/l (Std.D 0.50). There was no significant differences in reactivity between the reference group, group B and C, whereas group A was significant different from the reference group (p=0.003).Cortisol recovery increased within all four groups ((Reference group: 0.62 unit nmol/l (Std.D 4.03), group A: 0.82 unit nmol/l (Std.D 2.66), group B: 0.25 unit nmol/l (Std.D 0.50), group C: 2.26 unit nmol/l (Std.D 3.75)) and no significant differences were found between the groups (Table 5).

DISCUSSION

To our knowledge, the present study is the first to examine reactivity and recovery of the HPA-axis among bullied and non-bullied people utilising low-level exercise. According to Kudielka [32] the investigation of cortisol mechanisms in humans is a challenging task. Most researchers know that cortisol mechanisms which are studied in laboratory settings vary across study designs. They can be influenced by a large range of variables, such as meals, smoking, drinking, pharmacological stimulation, physical activity, etc. Since our study uses a simple method to activate the HPA-axis, we were able to complete many tests in a standardised and controlled test set-up. However, our results show that the study design would benefit from some adjustments.

Compared to previous studies using TSST, the reactivity and recovery were less pronounced in our study. This can be attributed to adjustments that we can recommend for future studies. For example, the first saliva cortisol was measured at the start of the cycling test, about one hour after the participant arrived at the laboratory. All the participants, however, had received an intensive clinical examination before the cycling test, and it can be argued that the cortisol levels observed were due to this examination and not the cycling test itself. According to Balodis et al. [24], cortisol levels are stable after 30 minutes in the laboratory, and we could have controlled for this bias if the participants' cortisol levels had been measured when they first entered the laboratory. Furthermore, the time span between the pre and post exercise sample of about 10 minutes may be too short a period. Additional samples in the post exercise period might have revealed more pronounced changes of cortisol over time [33].

Another issue that should be considered is the physical activation in the Åstrand cycling test. A recent study indicated that a 70% physical-activity load is needed to elicit a significant cortisol response [32], whereas our cycling test was designed to achieve 60 % of physical-activity load. Our test paradigm, however, did not activate the HPA-axis with a significant increase between time 1 and time 2, either in the morning or the afternoon sample, but we found that the sample time of the day has a significant influence. From what we know about the diurnal cortisol rhythm [32, 34], it can be argued that laboratory tests on cortisol response should be conducted only in the afternoon to avoid being influenced by an increase in cortisol levels due to the cortisol awakening response [35, 36]. This relationship is supported by the non-significant increase in our samples; here the diurnal cortisol response that occurs in the morning may have played a role.

Our analysis of saliva cortisol among bullied versus *Non-bullied* people indicated an increase in saliva cortisol among the most *Frequently Bullied* persons in comparison with *Non-bullied* examined in the morning, but this result was not corroborated by the analyses of afternoon-samples and may be finding by chance. The investigation of biomarkers within the bullying research is new and only a couple of studies have investigated people exposed to bullying in relation to a biological response [10, 11]. There have been suggestions that bullied people have a lower cortisol response on a diurnal basis [10, 11]. Studies on the cortisol responses in humans are contributing to a better understanding of the relationship between psycho-social environment and health. Many confounding factors and routines are needed when using saliva cortisol as biomarker [37, 38], and the present study took variables such as age, gender and time of day into consideration. Other variables, such as information about menstrual cycles and genetic factors, were not controlled for

but are not confounding results of within person variation across short time spans [32]. Using the baseline information, participants were grouped by the established screening criteria, followed by a follow-up status that formed the classification for exposure groups. As such, we may assume that participants who indicated that they were bullied at baseline and again on the test day can be classified as people who have been exposed to bullying.

In conclusion, the results of this study do not indicate that workplace bullying impacts HPA axis activity as measured by saliva cortisol in an exercise challenge test, but the reliability of the latter needs further study with respect to level of exercise and timing of samples.

CONFLICTS OF INTERESTS

All authors contributing to this article declare that they have no conflicts of interest.

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Figure 1 Flowchart

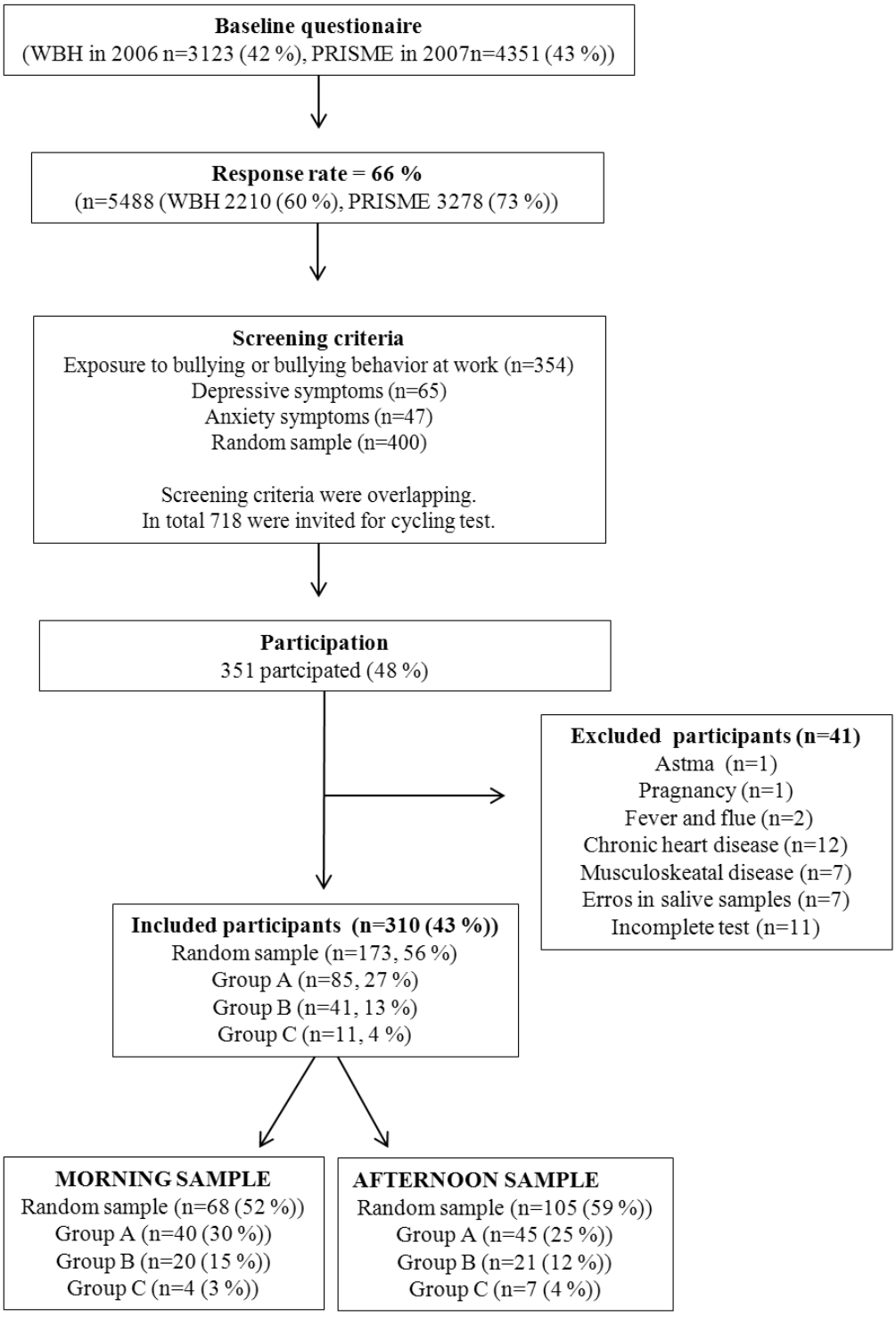

Table 1 Exercise characteristics of random sample (n=173)

	Morning sample (n=68)				Afternoon sample (n=105)			
	n	**%**	**mean**	**Sd**	**n**	**%**	**mean**	**Sd**
Female	49	72.06	-	-	68	64.76	-	-
Male	19	27.94	-	-	37	35.24	-	-
Age	68	-	53.38	8.34	105	-	49.76	9.90
Aerob fitness	66	-	37.16	13.07	102	-	36.76	11.01
Workload	68	-	1.48	0.52	104	-	1.52	0.52
Heart rate at steady state	67	-	127.12	15.18	104	-	129.60	11.83
Maximal oxygen uptake	66	-	2.69	0.96	102	-	2.72	0.77

Table 2 Reactivity and recovery within subgroups of random sample in morning- and afternoon sample

		MORNING SAMPLE (n=68)						AFTERNOON SAMPLE (n=105)					
		Reactivity			Recovery			Reactivity			Recovery		
		Mean	Std. D	*p*	Mean	Std. D	*p*	Mean	Std. D	*p*	Mean	Std. D	*p*
Gender	**Female**	0.20	1.03	0.62	0.90	3.09	0.73	0.15	0.54	0.25	0.17	4.27	0.13
	Male	-0.32	1.12		1.32	2.10		0.42	1.88		1.82	3.07	
Age	**>40**	0.02	0.92	0.71	1.05	2.84	0.34	0.28	1.26	0.81	0.64	4.42	0.18
Years	**<40**	0.47	2.23		0.64	3.45		0.04	0.53		0.53	0.89	
Aerob fitness	**>35**	0.00	0.94	0.49	0.83	1.45	0.03	0.20	1.21	0.49	1.31	2.66	0.42
ml02/min/kg	**<35**	0.12	1.19		1.24	4.10		0.28	1.14		0.00	4.90	
Workload	**>90**	-0.14	1.25	0.66	1.21	2.16	0.21	0.39	2.02	0.63	1.62	3.50	0.61
Watt 60rpm/min	**<90**	0.24	1.12		1.06	3.54		0.26	1.14		0.04	5.35	
Heart rate at steady state	**>120**	-0.10	0.89	0.14	0.82	3.45	0.34	0.33	1.27	0.35	0.35	4.22	0.04
HR / min	**<120**	0.31	1.45		1.40	1.69		-0.10	0.75		1.62	3.67	
Maximal oxygen uptake	**>2.5**	0.21	1.26	0.39	1.60	1.79	0.43	0.20	1.18	0.49	1.05	2.06	0.80
ml 02.min-1.kg-1	**<2.5**	-0.07	0.87		0.33	3.66		0.29	1.18		-0.02	5.86	

Table 3 Exercise characteristics of the three exposure groups and reference group

	Group A			Group B			Group C			Reference group		
	n	**Mean**	**Std.D**	**n**	**Mean**	**Std.D**	**n**	**Mean**	**Std.D**	**N**	**Mean**	**Std.D**
Gender (f/m)	(62/20)			(30/11)			(6/4)			(117/56)		
Age	82	47.98	9.28	41	53.12	8.43	10	53.51	7.04	173	51.18	9.46
Aerob fitness	81	35.78	11.40	39	34.10	12.67	9	31.14	10.43	168	36.92	11.82
Workload	82	1.48	0.58	40	1.37	0.46	10	1.40	0.45	172	1.50	0.51
HR at stady state	81	129.16	13.70	40	127.45	12.48	10	132.70	16.65	171	128.62	13.25
Max oxygen uptake	81	2.72	0.77	39	2.62	0.94	9	2.30	0.78	168	2.70	0.84

Table 4 Reactivity and recovery between groups in the morning sample

			MORNING SAMPLE											
			Reactivity						Recovery					
					Mean diff						Mean diff.			
Groups	**n**	**%**	**Mean**	**Std. D**	**adj.**	**95 % CL**		***p***	**Mean**	**Std. D**	**adj.**	**95 % CL**		***p***
Reference	68	52	0.06	1.07	-	-	-	-	1.01	2.86	-	-	-	-
A	40	30	-0.12	1.20	-0.337	-0.670	0.139	0.170	1.04	2.84	0.292	0.126	2.452	0.6734
B	20	15	-0.07	1.31	-0.136	-0.569	0.440	0.645	0.30	1.26	-0.187	-0.601	2.220	0.8191
C	4	3	-1.15	1.14	-1.361	-2.481	-0.096	0.029	0.72	2.04	0.376	-1.674	4.422	0.8124

Adj. for gender, age and sample time of day

Table 5 Reactivity and recovery between groups in the afternoon sample

			AFTERNOON SAMPLE											
			Reactivity						Recovery					
					Mean diff.						Mean diff.			
Groups	**n**	**%**	**Mean**	**Std. D**	**adj.**	**95 % CL**		***p***	**Mean**	**Std. D**	**adj.**	**95 % CL**		***p***
Reference	105	59	0.24	1.17	-	-	-	-	0.62	4.03	-	-	-	-
A	45	25	-0.31	0.71	-0.571	-0.652	-0.010	0.003	0.82	2.66	0.148	-0.508	2.322	0.856
B	21	12	-0.15	0.50	-0.339	-0.569	0.372	0.188	0.25	0.50	-0.253	-1.236	2.245	0.789
C	7	4	0.27	0.55	0.180	-0.393	1.236	0.671	2.26	3.75	1.902	-0.498	5.819	0.254

Adj. for gender, age and sample time of day

Reference List

1 Einarsen S. *Bullying and Harassment at work: Epidemiological and psychosocial aspects*. Bergen: Department of Psychosocial Science, Faculty of Psychology, University of Bergen 1996.

2 Niedhammer I, Sultan-Taieb H, Chastang JF, *et al.* Exposure to psychosocial work factors in 31 European countries. *Occup Med (Lond)* 2012;**62** (3):196-202.

3 Nielsen MB, Matthiesen SB, Einarsen S. The impact of methodological moderators on prevalence rates of workplace bullying. A meta-analysis. *Journal of Occupational and Organizational Psychology* 2010;**83** (4):955-79.

4 Einarsen S, Hoel H, Zapf D, *et al.* The concept of bullying and harassment at work: The European Tradition. In: Einarsen S, Hoel H, Zapf D, *et al.*, eds. *Bullying and Harassment in the Workplace*. Boca Raton: CRC Press 2011:3-39.

5 Magerøy N. Health among navy personnel: A cross-sectional study in the royal norwegian navy. University of Bergen, Norway 2009:1-64.

6 Niedhammer I, David S, Degioanni S, *et al.* Workplace bullying and psychotropic drug use: the mediating role of physical and mental health status. *Ann Occup Hyg* 2011;**55** (2):152-63.

7 Hansen AM, Hogh A, Persson R, *et al.* Bullying at work, health outcomes, and physiological stress response. *J Psychosom Res* 2006;**60** (1):63-72.

8 McEwen BS. Central effects of stress hormones in health and disease: Understanding the protective and damaging effects of stress and stress mediators. *Eur J Pharmacol* 2008;**583** (2-3):174-85.

9 Karlson B, Lindfors P, Roberto R, *et al.* Psychosocial Work Stressors and saliva Cortisol. In: Kristenson M, Garvin P, Lundberg U, eds. *The Role of Saliva Cortisol Measurement in Health and Disease*: Bentham Science Publisher 2011:43-66.

10 Kudielka BM, Kern S. Cortisol day profiles in victims of mobbing (bullying at the work place): preliminary results of a first psychobiological field study. *J Psychosom Res* 2004;**56** (1):149-50.

11 Hansen AM, Hogh A, Persson R. Frequency of bullying at work, physiological response, and mental health. *J Psychosom Res* 2011;**70** (1):19-27.

12 Hogh A, Hansen AM, Mikkelsen EG, *et al.* Exposure to negative acts at work, psychological stress reactions and physiological stress response. *J Psychosom Res* 2012;**73** (1):47-52.

13 Lahelma E, Lallukka T, Laaksonen M, *et al.* Workplace bullying and common mental disorders: a follow-up study. *Journal of Epidemiology and Community Health* 2011.

14 Kivimäki M, Virtanen M, Vartia M, *et al.* Workplace bullying and the risk of cardiovascular disease and depression. *Occup Environ Med* 2003;**60** (10):779-83.

15 Rugulies R, Madsen IE, Hjarsbech PU, *et al.* Bullying at work and onset of a major depressive episode among Danish female eldercare workers. *Scand J Work Environ Health* 2012;**38** (3):218-27.

16 Reknes I, Pallesen S, Mageroy N, *et al.* Exposure to bullying behaviors as a predictor of mental health problems among Norwegian nurses: results from the prospective SUSSH-survey. *Int J Nurs Stud* 2014;**51** (3):479-87.

17 Lambert E, Lambert G. Stress and Its Role in Sympathetic Nervous System Activation in Hypertension and the Metabolic Syndrome. *Current Hypertension Reports* 2011:1-5.

18 Zapf D, Einarsen S. Individual Antecedents of Bullying: Victims and Perpetrators. In: Einarsen S, Hoel H, Zapf D, *et al.*, eds. *Bullying and Harassment in the Workplace*. Boca Raton: CRC Press 2011:177-200.

19 Chrousos GP, Gold PW. A healthy body in a healthy mind--and vice versa--the damaging power of "uncontrollable" stress. *J Clin Endocrinol Metab* 1998;**83** (6):1842-5.

20 Dickerson SS, Kemeny ME. Acute stressors and cortisol responses: a theoretical integration and synthesis of laboratory research. *Psychol Bull* 2004;**130** (3):355-91.

21 Kirschbaum C, Hellhammer DH, Pirke K-M. The 'Trier Social Stress Test'--a tool for investigating psychobiological stress responses in a laboratory setting. *Neuropsychobiology* 1993;**28**:76-81.

22 Burke HM, Davis MC, Otte C, *et al.* Depression and cortisol responses to psychological stress: A meta-analysis. *Psychoneuroendocrinology* 2005;**30** (9):846-56.

23 Chopra KK, Ravindran A, Kennedy SH, *et al.* Sex differences in hormonal responses to a social stressor in chronic major depression. *Psychoneuroendocrinology* 2009;**34** (8):1235-41.

24 Balodis IM, Wynne-Edwards KE, Olmstead MC. The other side of the curve: examining the relationship between pre-stressor physiological responses and stress reactivity. *Psychoneuroendocrinology* 2010;**35** (9):1363-73.

25 Hamilton LD, Newman ML, Delville CL, *et al.* Physiological stress response of young adults exposed to bullying during adolescence. *Physiol Behav* 2008;**95** (5):617-24.

26 Garde AH, Persson R, Hansen AM, *et al.* Effects of lifestyle factors on concentrations of salivary cortisol in healthy indviduals. *Scand J Clin Lab Invest* 2008.

27 Kolstad HA, Hansen AM, Kaergaard A, *et al.* Job strain and the risk of depression: is reporting biased? *Am J Epidemiol* 2011;**173** (1):94-102.

28 Jensen MA, Hansen AM, Abrahamsson P, *et al.* Development and evaluation of a liquid chromatography tandem mass spectrometry method for simultaneous determination of salivary melatonin, cortisol and testosterone. *J Chromatogr B Analyt Technol Biomed Life Sci* 2011;**879**:2527.

29 Westgard JO, Barry PL, Hunt MR, *et al.* A multi-rule Shewhart chart for quality control in clinical chemistry. *Clin Chem* 1981;**27** (3):493-501.

30 Garde AH, Hansen ÅM, Nikolajsen TB. An inter-laboratory comparison for determination of cortisol in saliva. *Accred Qual Assur* 2003;**8** (1):16-20.

31 Hansen AM, Garde AH, Christensen JM, *et al.* Evaluation of a radioimmunoassay and establishment of a reference interval for salivary cortisol in healthy subjects in Denmark. *Scand J Clin Lab Invest* 2003;**63** (4):303-10.

32 Kudielka BM, Hellhammer DH, Wüst S. Why do we respond so differently? Reviewing determinants of human salivary cortisol responses to challenge. *Psychoneuroendocrinology* 2009;**34** (1):2-18.

33 Eriksen HR, Olff M, Murison R, *et al.* The time dimension in stress response: relevance for survival and health. *Psychiatry Res* 1999;**85**:39-50.

34 Hansen ÅM, Garde AH, Persson R. Sources of biological and methodological variation in salivary cortisol and their impact on measurement among healthy adults: a review. *Scand J Clin Lab Invest* 2008;**68** (6):448-58.

35 Kristenson M, Garvin P, Lundberg U. The Role of Saliva Cortisol Measurement in Health and Disease Introduction - Why This Book? In: Kristenson M, Garvin P, Lundberg U, eds. *The Role of Saliva Cortisol Measurement in Health and Disease* 2011:3-16.

36 Wilhelm I, Born J, Kudielka BM, *et al.* Is the cortisol awakening rise a response to awakening? *Psychoneuroendocrinology* 2007;**32** (4):358-66.

37 Bellingrath S, Weigl T, Kudielka BM. Chronic work stress and exhaustion is associated with higher allostastic load in female school teachers. *Stress* 2009;**12** (1):37-48.

38 Maina G, Bovenzi M, Palmas A, *et al.* Psychosocial environment and health: Methodological variability of the salivary cortisol measurements. *Toxicol Lett* (0).