Occupational Medicine
doi:10.1093/occmed/kqy006

Physiological reactions to long-term fishing in the Barents Sea

B. Netterstrøm[1], Å. M. Hansen[2,3], D. Isaacson[4], A. M. Simonsen[4] and P. Weihe[4]

[1]Department of Occupational and Environmental Medicine, University Hospital, Bispebjerg, Copenhagen, Denmark, [2]Department of Public Health, University of Copenhagen, Copenhagen 1499, Denmark, [3]National Research Centre for the Working Environment, Copenhagen 2100, Denmark, [4]Department of Occupational Medicine and Public Health, The Faroese Hospital System, Torshavn 100, Faroe Islands.

Correspondence to: B. Netterstrøm, Department of Occupational and Environmental Medicine, University Hospital, Bispebjerg, Bispebjerg Bakke 23, 2400 Copenhagen N, Denmark. Tel: +45 20437778; e-mail: Bone@dadlnet.dk

Background	Fishing in distant waters for months may induce physiological stress.
Aims	To assess the physiological stress reactions in fishermen working for 2–3 months continuously in 6-h shifts on trawlers in the Barents Sea.
Methods	The crew of five trawlers fishing in the Barents Sea from January to April 2004 were invited to participate. In the week before and 5–7 days after the trip, the following measures were collected: salivary cortisol four times a day, 24-h urinary cortisol, blood pressure, heart rate, serum cholesterol, serum high-density lipoprotein (HDL-cholesterol), HbA_{1c} (glycosylated haemoglobin) and weight. In addition, 24-h urinary cortisol, blood pressure and heart rate were measured three times. A questionnaire on health, social conditions and work environment was obtained after the trip.
Results	In total, 136 men agreed to participate. Full data were obtained for 96 fishermen (70%). A significant decrease in salivary and urinary cortisol was found during the trip. Adjustment for age, body mass index, smoking, shift work schedule and time of day for sample collection did not change this finding. Systolic and diastolic blood pressure declined significantly and remained significantly lower after the trip compared to before the trip. Serum cholesterol/HDL ratio declined significantly, whereas triglycerides, HbA_{1c} and weight were unchanged.
Conclusions	Working up to 3 months on 6-h shifts, 84 h a week, with moderate physical activity, even in artificial light and cold weather on a ship, did not result in increased physiological stress.
Key words	Blood pressure; cortisol; fishing; shift work.

Introduction

Fishing is a potentially risky occupation. This implies an elevated risk of developing cancer, including smoking-related cancer [1,2], injuries and cardiovascular disease [2,3]. This excess risk may be due to life style factors as suggested by Hjarnoe and Leppin [4]. In line with this, an increased risk of metabolic syndrome has been found among Danish seafarers [5]. Other factors, e.g. shift work, could, however, contribute to this.

In the Faroe Islands, the large deep-sea trawlers in distant waters, that is, the Barents Sea, use shift work, mainly 6 h on and 6 h off work, leaving fishermen with 12 h rest, except when the catch is excessive or when equipment is damaged and needs to be repaired. The work is characterized by intermittent high physical demands in cold and rough weather conditions when working on the trawl deck [6]. This alternates with work on the factory deck, which includes operating the head-and-cut machine, sorting and cleaning the fish and packing blocks of fish for freezing. The tasks are, however, fixed for each fisherman, and life on board the ship is scheduled and, to a certain extent, predictable [7].

One of the most problematic factors about shift work is that it disrupts circadian rhythms, causing sleep disturbance for many workers [8]. Research has shown that at least 75% of the population working in shifts suffers from sleep disruption [9]. The circadian rhythm is our 24-h body clock located in the hypothalamus region of the brain. In the morning, the hypothalamus increases the amount of cortisol to prepare the body for the activities of the coming day. Burch *et al*. found higher reports of fatigue and lower mental well-being in shift workers, with lower melatonin

levels during sleep and at work [10]. Further, fishermen were found to suffer more from sleepiness when at sea, compared to when ashore (82% compared to 35%) [11]. Fishermen working at sea with a standard working week of 84 h or more may suffer from fatigue. However, Wadsworth *et al*. found that fishermen adapted to the requirements of life at sea quite quickly, as fatigue increased more slowly after the first week, but that it took the fishermen a full week to recover after returning to land [12].

A previous study investigated the course and duration of neuroendocrine recovery after a 2-week period in which workers either worked 12-h day shifts or 12-h night shifts [13]. The results showed that day shift workers showed a significantly lower cortisol concentration only upon awakening on their first day off. Compared to day workers, night shift workers had a flatter profile on the first day off and a lower cortisol concentration upon awakening on the fourth day. Another study examined how different work schedules (day shift, night shift and rotating shift) affect the cortisol rhythm and the resetting of the rhythms among off-shore workers [14]. The study found that participants adapted to night shifts within a week regardless of schedule, but recovery from night shifts took longer.

We assumed that continuously working on a 2- to 3-month fishing trip in deep winter in Arctic waters may be an exhausting experience for the crew due to rough weather, lack of daylight, shift work and general workload [15]. The physiological effect of this strenuous work may be elevation of blood pressure [16], even hypertension [17] and changes in metabolism [18]. In particular, measurements of cortisol may be a marker of the physiological stress that fishermen might experience [19]. To our knowledge, no studies have investigated to what extent cortisol is affected by a fishing trip of 2- to 3-month duration.

Methods

The study population consisted of the crew of five trawlers heading for the Barents Sea from January until April 2004.

To obtain a biochemical measure of stress load in this study, we intended to measure cortisol in saliva and urine before, during and after the fishing trip. We also decided to make simultaneous blood pressure and heart rate measurement and to measure blood markers of the metabolic system before and after the trip. The saliva samples were collected during the working day. Saliva samples were collected in Salivette® polyester tubes at awakening and at 6, 12 and 18 h after awakening. Written instructions emphasized that swabs should be kept in the mouth until thoroughly soaked and to fill in the exact time of sampling carefully when collecting each saliva sample [20]. The samples were stored at –20°C on board the trawlers and at –80°C after returning to the Faroes. They were analysed within 12 months.

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 (Spectria Cortisol Coated Tube RIA, Orion Diagnostica, Espoo, Finland), according to the manufacturer's specifications. The sample volume was 150 µl, the range of the standard solutions prepared was 1.0−100.0 nmol/l and the incubation time was 30 min at 37°C [21].

To show equivalence between different runs, natural saliva samples (5.9 and 18.5 nmol/l) were used as control materials and analysed 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 precision of the analytical methods remained stable [22]. The performance of the methods has been further validated by participation in interlaboratory comparison schemes [23].

As with the saliva tests described previously, urine from the cohort was also tested on 5 days: 1 day prior to the fishing trip, 3 days during the trip and 5–7 days after the trip had concluded. On each of the days, the urine was collected over a period of 24 h, and three quantities were measured: (i) cortisol, (ii) creatinine and (iii) cortisol–creatinine ratio. The samples were stored at –20°C on board and at –80°C on land until they were analysed within 12 months. To standardize results, creatinine was measured in urine [24].

The assay used for the determination of cortisol in urine was a competitive RIA (Coat-a-count kit) purchased from DPC (Diagnostic Products Corporation). The creatinine method was based on the well-known Jaffe's reaction and measured on a COBAS Mira autoanalyser (Roche Diagnostic Systems, Basel, Switzerland). We used commercial matrix reference materials for cortisol and creatinine: Lyphochek® Quantitative Urine Control Normal [1] and Lyphochek® Quantitative Urine Control Abnormal [2] (Bio-Rad Laboratories A/B, Herlev, Denmark).

The between-day analytical variation for urinary cortisol was estimated to be 15%. To show equivalence between different runs, natural urine samples (110.4 and 552.0 nmol/l) were analysed together with the urine samples. The analytical method for cortisol and creatinine was evaluated by a method evaluation function design according to Christensen *et al*. [23] to estimate the random and systematic effects. The between-day analytical variation for creatinine measurements was 2%. The detection limit for the analytical method was 13 nmol/l for cortisol [24].

Blood pressure and heart rate were measured by a semi-automatic device [Digital Blood Pressure Monitor Model US-779 (A&D Instruments LTD, UK)]. The

measurement for before and after the trip was done by one of the authors (P.W.) and, during the trip, by the fishermen themselves. During the trip, one of the officers on duty instructed the crew in correct blood pressure measurement. Blood pressure and heart rate were measured three times a day for 3 days during the trip and at the clinical examination before and after the trip. Samples in blood were drawn at the clinical examination and analyses for glycosylated haemoglobin (HbA_{1c}) and lipids at the National Hospital of the Faroes as routine analyses. Data on health, occupational history and private life issues were collected in questionnaires at the same time.

Chi-square tests were used for the discrete demographic variables and *t*-tests for the continuous. Paired sample *t*-tests were conducted for all dependent physiological variables. Cortisol data were logarithmic transformed.

A multilevel regression analysis using the Proc mixed procedure in SAS version 9.4 was established to test for differences in concentration of cortisol in saliva and urine before, during and after fishing in the Barents Sea. We used repeated measure by included the fishermen in the random statement.

Due to abnormal (skewed) distributions and increasing variances, concentrations of cortisol in saliva were analysed on a logarithmic scale. The models included work schedules [two levels (1 = work: 6:00–12:00 and 18:00–24:00; 2 = work: 0:00–06:00 and 12:00–18:00)], before, during and after fishing in the Barents Sea (five levels: 1 = before, 2 = after 2 weeks, 3 = after 1½ months, 4 = a few days before end of trip, 5 = 7 days after the trip) and time of sampling (four levels: 1 = at awakening, 2 = +6, 3 = +12 and 4 = +18 h after awakening). The effect of the exact sampling time was evaluated by including the variable exact time of sampling (linear and squared) as continuous independent variables. All samples were included. The initial model was adjusted for age, smoking and body mass index (BMI).

To test for differences in urinary cortisol before, during and after fishing in the Barents Sea (five levels: 1 = before, 2 = after 2 weeks, 3 = after 1½ months, 4 = a few days before end of trip, 5 = 7 days after the trip), we calculated the ratio between cortisol and creatinine and analysed the ratio on a logarithmical scale. The model was adjusted for age, smoking and BMI. For blood pressure and heart rate data, a mean value of the three measures before and after the trip was calculated as for the nine measurements during the trip.

P values below 0.05 were considered statistically significant. The study was approved by the local scientific ethical committee (Visindasidsemievnd Føroya).

Results

The study population consisted of 185 male crew members, of whom 136 agreed to participate. The mean age of the participants on each ship was 33–42 years and the mean duration as a deep sea fishermen was 9–15 years. Reporting excellent health ranged from 50 to 76% highest at the ship with the lowest mean age. All trawlers operated in the same waters in the Barents Sea. They produced frozen cod fillets. A part of the crew specialized in working on the trawl deck, another part specialized in filleting the cod in a semi-automatic factory under deck. Other job roles included navigators, engineers and the cooking staff.

Calculations from their personal identification number showed that the mean age of the 49 non-participants was non-significantly lower than the mean of the participants. However, full data were obtained from only 96 men, in the form of two questionnaires, blood samples and measurement of blood pressure at a clinical examination in the week before and 5–7 days after the trip. Demographics of these and the drop-outs are shown in Table 1.

The drop-outs were younger, and there were more singles and smokers compared to the participants. With respect to experience as fishermen, no differences between the two groups were found.

Table 2 shows the mean values of the physiological variables and *P* values obtained by the paired sample *t*-tests. Salivary cortisol collected during the early morning (0:00–6:00) rose significantly during

Table 1. Demographics and health-related factors among 96 participants and 40 drop-outs on five different trawlers before working for 3 months at the Barents Sea, January to April 2004

	Full data, *n* = 96	Drop-outs, *n* = 40	*P* value
Age, mean (SD), range	41.7 (12.0), 17–65	37.5 (13.2), 16–66	<0.05
Years as sea man, mean (SD), range	17.9 (12.7), 1–47	15.1 (12.7), 1–37	NS
Married, %	72	52	NS
Smokers, %	46	69	NS
Intake of alcohol more than once a week, %	11	23	NS
Self-reported health excellent, %	80	78	NS
BMI, kg/m^2, mean (SD)	27.7 (4.9)	27.1 (5.1)	NS
Systolic blood pressure, mm Hg, mean (SD)	144.2 (21.2)	144.4 (21.2)	NS

NS, non-significant.

Table 2. Means of physiological measures in fishermen working at the Barents Sea for 3 months in winter 2004 before, during and after the trip

	Before	During	After			
	Mean (SD)	Mean (SD)	Mean (SD)	*P* before/after	*P* before/during	*P* during/after
Salivary cortisol, nmol/l (0–6 a.m.)	3.56 (3.2)	5.10 (3.2)	2.25 (2.1)	NS	<0.05	<0.05
Salivary cortisol, nmol/l (6–12 a.m.)	10.44 (7.5)	7.14 (3.8)	12.24 (7.1)	NS	<0.01	<0.01
Salivary cortisol, nmol/l (12–18 p.m.)	6.22 (4.3)	6.28 (2.9)	4.68 (3.6)	NS	NS	<0.05
Salivary cortisol, nmol/l (18–24 p.m.)	4.64 (8.7)	4.68 (2.6)	3.43 (3.7)	<0.01	NS	<0.05
24-h urinary cortisol (micromol)/creatinine (mol)	7.30 (4.8)	5.61 (3.3)	6.44 (4.6)	<0.05	<0.01	NS
Systolic BP, mm Hg	133.2 (13.4)	132.7 (9.9)	131.6 (11.4)	<0.05	NS	NS
Diastolic BP, mm Hg	76.9 (10.4)	74.0 (8.7)	73.7 (10.6)	<0.01	<0.01	NS
Heart rate, beats/min	67.7 (8.8)	66.5 (9.4)	66.8 (9.3)	NS	<0.05	NS
Weight, kg	88.4 (14.6)		88.3 (13.9)	NS		
Hba_1C, %	5.19 (0.5)		5.21 (0.3)	NS		
Cholesterol/HDL	4.26 (1.2)		4.04 (1.0)	<0.01		
Triglyceride, mmol/l	1.32 (0.8)		1.24 (0.7)	NS		

'During' is the mean of measurements in three different days. Paired samples *t*-tests. BP, blood pressure; HDL, high-density lipoprotein; NS, non-significant.

the trip (three measures) but declined to an insignificant level after the trip, compared to the level before. The opposite pattern was seen for samples collected from 6:00 to 12:00, whereas saliva cortisol collected later in the day was significantly lower after the trip compared to the level before and during the trip.

Table 3 shows the results of the General Linear Model (GLM) analysis of salivary cortisol, where adjustment for working schedule, time of sample collection, age, smoking and BMI were conducted. During the trip, salivary cortisol declined significantly at the first two sample collections, and the level was almost the same after, compared to the level before the trip. Time of collection and age was significantly negatively associated with salivary cortisol, whereas working schedule, smoking and BMI were not.

In accordance with this, Figure 1 shows that the morning peak was established after the trip. It appears that a flattened diurnal curve occurred during the trip.

The urinary cortisol showed a significant decline during the trip (three measures) and a tendency to a lower level after the trip compared to the level before (Table 2). Table 3 shows the GLM analysis. In general, urinary cortisol showed a tendency to decline during the trip and, after adjustment for work schedule, time, age, smoking and BMI, this association was significant ($P < 0.001$). The data divided in the two work schedule groups are graphically shown in Figure 2. It appears that the decline in urinary cortisol was more pronounced among those with work schedule 1 (normal hours). The decline from before until after the trip was significant ($P < 0.01$). Those with work schedule 2 (night shifts) had the same cortisol level after the trip as before. The difference between the two schedules during and after the trip was significant (Table 3).

Table 3. Results of multilevel regression analyses of salivary and urinary (24 h) cortisol/creatinine adjusted for work schedule, time of sampling, date of sampling, BMI, age and smoking

Effect	Estimate	SE	*t*-value	*P* value
Salivary cortisol				
Intercept	1.373	0.222	6.17	<0.001
Before fishing	−0.063	0.074	−0.86	NS
During 1	−0.201	0.074	−2.70	<0.01
During 2	−0.272	0.075	−3.62	<0.001
During 3	−0.095	0.076	−1.26	NS
After	0			
Work schedule 1	−0.097	0.047	−2.19	NS
Work schedule 2	0			
Time 1	0.111	0.128	0.44	NS
Time 2	0.164	0.106	1.18	NS
Time 3	0.018	0.083	−0.17	NS
Time 4	0			.
Urinary cortisol				
Intercept	−43.77	23.16	−1.89	NS
Before fishing	0.453	0.152	2.97	<0.001
During 1	0.306	0.121	2.53	<0.05
During 2	0.189	0.094	2.00	<0.05
During 3	0.069	0.071	0.97	NS
After	0			
Work schedule 1	−0.165	0.25	−6.57	<0.001
Work schedule 2	0			

Work schedule 1: 6:00–12:00 and 18:00–24:00; work schedule 2: 0:00–06:00 and 12:00–18:00. NS, non-significant.

Table 2 also gives the mean values of blood pressure and metabolic variables. Both systolic and diastolic blood pressure and serum cholesterol/high-density lipoprotein ratio decreased significantly from before

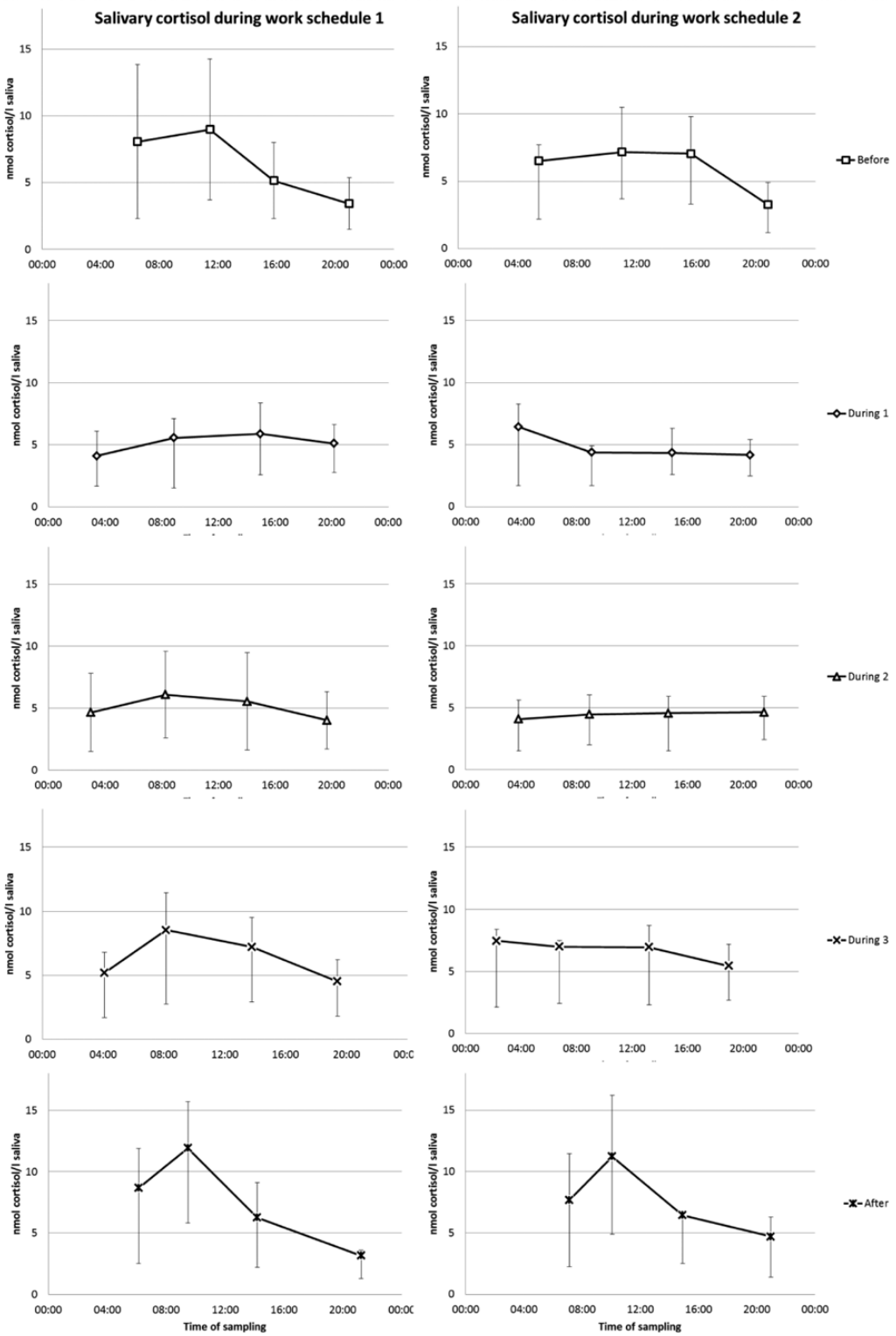

Figure 1. Salivary cortisol in 96 fishermen working at the Barents Sea for 3 months in winter 2004. Means and 25 and 75 percentiles.

until after the trip. Diastolic blood pressure and heart rate decreased during the trip. No differences between the two work schedule groups could be detected. Weight, HbA_{1c} and serum triglyceride remained unchanged.

Discussion

Cortisol excretion measured in saliva showed a flattened diurnal curve, which developed into a normal curve with a peak in the morning during the trip. No difference was

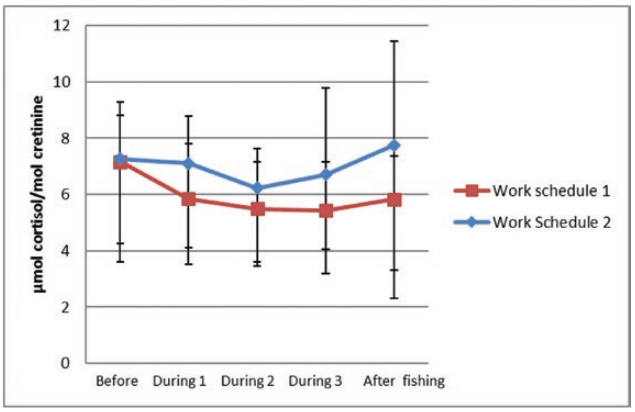

Figure 2. Urinary cortisol before, during and after the trip in 96 fishermen working at the Barents Sea for 3 months in winter 2004. Means and 25 and 75 percentiles.

found between the two work time schedules. The total cortisol excretion decreased during the trip, a result that was supported by the measurements of urinary cortisol. This was most pronounced among those with normal working hours. In addition, the coronary risk profile assessed by measurement of blood pressure and lipids improved. In this context, it should be noted that the blood sugar level and weight among the participants remained the same. This might seem surprising because the working conditions might lead to both physical and psychological stress, which might affect at least the blood pressure level. As stated by Høye *et al.* [6], the urinary catecholamine excretion and heart rate in fishermen during long-line bank fishing were not as high as in coastal fishermen.

It is noticeable that the cortisol levels found in this study were inside the 95% confidence intervals for both saliva cortisol (1.1–35.7 nmol/l) [21] and urinary cortisol (1.1–10.5 nmol/l) [24].

The interpretation of this could be that working without diurnal variation of sunlight in fixed-schedule and normally well-arranged work with predictable tasks was much less stressful than we expected. All the results from this study suggest that working for 2–3 months on 6-h shifts with no regular personal contact with family and other non-work-related relations, with moderate physical activity even in artificial light and cold weather on a ship, did not increase the physiological stress reaction. Rather the opposite was the case. Regular intake of healthy food, which is the recommendations on the ships, and no alcohol intake might contribute to this too.

The strength of this study is the repeated collections of saliva and urinary samples and blood pressure measurements in a longitudinal design, where the fishermen served as their own controls. We were even able to assess the effect in two work time schedules. To our knowledge, no previous studies have been able to collect this kind of data for assessment of physiological stress reactions in the hypothalamic–pituitary–adrenal (HPA) axis and sympathetic nervous system in a relatively large population.

The main limitations are that it was not possible to collect samples at fixed times and that we could not control for the accuracy of time of collection. However, we ensured that the time of sampling was noted and adjusted for in the statistical analyses. It was not possible to assess awakening cortisol response with samples at awakening and half an hour later as recommended. This was due to different work time schedules and the tasks each fisherman was engaged in at the time for delivering saliva samples. We do not know when the fishermen slept, but were told that most men slept twice during their leisure time. The consequence might be more blurred results, and comparison with other studies on shift work in similar working conditions at sea is not possible [13,15]. The decrease in 24-h urinary cortisol does, however, support the findings for salivary cortisol and might be seen as a more robust measure of the effect of the HPA-axis.

The participation rate was low (53%). Data were not complete for 40 out of 136 fishermen, which gave a participation rate of 70% of those who agreed to participate. The data given in the material section indicate that there might be a healthy worker effect, as those from whom we obtained full data were often married and less frequent smokers. In addition, long distance fishing in the Faroe Islands is a profitable job with high esteem in the population, which in itself might lead to selection of the most fit.

Key points

- Fishermen working away for up to 3 months on 6-h shifts doing 84 h a week in artificial light and cold weather may be subjected to physiological stress.
- Measurements of cortisol, blood pressure and blood lipids did not show an increase in physiological stress.
- Regular working hours, regular healthy meals, predictable tasks and social well-being on board as well as healthy worker effect may account for this.

Funding

The study was funded by the Fisheries Research Fund of the Faroe Islands and Amerada Hess Faroes.

Conflicts of interest

None declared.

References

1. Oldenburg M, Harth V, Manuwald U. Comparison of hospitalization among German coastal and deep sea fishermen. *Int Arch Occup Environ Health* 2015;**88:**751–757.

2. Poulsen TR, Burr H, Hansen HL, Jepsen JR. Health of Danish seafarers and fishermen 1970–2010: what have register-based studies found? *Scand J Public Health* 2014;**42:**534–545.
3. Carter T, Jepsen JR. Exposures and health effects at sea: report on the NIVA course: maritime occupational medicine, exposures and health effects at Sea Elsinore, Denmark, May 2014. *Int Marit Health* 2014;**65:**114–121.
4. Hjarnoe L, Leppin A. Health promotion in the Danish maritime setting: challenges and possibilities for changing lifestyle behavior and health among seafarers. *BMC Public Health* 2013;**13:**1165.
5. Møller Pedersen SF, Jepsen JR. The metabolic syndrome among Danish seafarers. *Int Marit Health* 2013;**64:**183–190.
6. Høye EU, Sandsund M, Heidelberg CT, Aasmoe L, Reinertsen RE. Thermophysiological responses and work strain in fishermen on deep-sea fishing vessels. *Int Marit Health* 2016;**67:**104–111.
7. Rodahl K, Vokac Z. Work stress in long-line bank fishing. *Scand J Work Environ Health* 1977;**3:**154–159.
8. Bøggild H, Knutsson A. Shift work, risk factors and cardiovascular disease. *Scand J Work Environ Health* 1999;**25:**85–99.
9. Parkes KR. Age, smoking, and negative affectivity as predictors of sleep patterns among shiftworkers in two environments. *J Occup Health Psychol* 2002;**7:**156–173.
10. Burch JB, Yost MG, Johnson W, Allen E. Melatonin, sleep, and shift work adaptation. *J Occup Environ Med* 2005;**47:**893–901.
11. Gander P, van den Berg M, Signal L. Sleep and sleepiness of fishermen on rotating schedules. *Chronobiol Int* 2008;**25:**389–398.
12. Wadsworth EJ, Allen PH, Wellens BT, McNamara RL, Smith AP. Patterns of fatigue among seafarers during a tour of duty. *Am J Ind Med* 2006;**49:**836–844.
13. Merkus SL, Holte KA, Huysmans MA *et al.* Neuroendocrine recovery after 2-week 12-h day and night shifts: an 11-day follow-up. *Int Arch Occup Environ Health* 2015;**88:**247–257.
14. Harris A, Waage S, Ursin H, Hansen AM, Bjorvatn B, Eriksen HR. Cortisol, reaction time test and health among offshore shift workers. *Psychoneuroendocrinology* 2010;**35:**1339–1347.
15. Harris A, Waage S, Ursin H, Eriksen HR. Saliva cortisol levels in construction workers in the Arctic (78°N). *Int J Circumpolar Health* 2011;**70:**542–551.
16. al'Absi M, Arnett DK. Adrenocortical responses to psychological stress and risk for hypertension. *Biomed Pharmacother* 2000;**54:**234–244.
17. Rosenthal T, Alter A. Occupational stress and hypertension. *J Am Soc Hypertens* 2012;**6:**2–22.
18. Juster RP, Sindi S, Marin MF *et al*. A clinical allostatic load index is associated with burnout symptoms and hypocortisolemic profiles in healthy workers. *Psychoneuroendocrinology* 2010;**35:**1436–1442.
19. Kristenson M, Garvin P, Lundberg U. Why this book? In: Kristenson M, Garvin P, Lundberg U, eds. *The Role of Salivary Cortisol Measurement in Health and Disease*. Stockholm, Sweden, 2012; 1–20.
20. Hansen AM, 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:**448–458.
21. Hansen AM, Garde AH, Christensen JM, Eller NH, Netterstrøm B. 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:**303–310.
22. Westgard JO, Barry PL, Hunt MR, Groth T. A multi-rule Shewhart chart for quality control in clinical chemistry. *Clin Chem* 1981;**27:**493–501.
23. Christensen JM, Poulsen OM, Anglov T. Method evaluation, quality control, and external quality assurance systems of analytical procedures. In: Seiler H, ed. *Handbook on Metals in Clinical and Analytical Chemistry*. New York: Marcel Dekker Inc., 1994; 45–61.
24. Hansen AM, Garde AH, Christensen JM, Eller NH, Netterstrøm B. Reference intervals and variation for urinary epinephrine, norepinephrine and cortisol in healthy men and women in Denmark. *Clin Chem Lab Med* 2001;**39:**842–849.