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Original Article

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Disruption of the Diurnal Cortisol Hormone Pattern by Pesticide Use in a Longitudinal Study of Farmers in Thailand

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Abstracts

Alteration of the hypothalamic–pituitary–adrenal (HPA) axis hormones has been associated with a range of chronic metabolic and cardiovascular health conditions. This study evaluated whether type of farming (organic versus conventional) or the number of self-reported days of spraying pesticides in the past 8 months was associated with diurnal cortisol levels. Salivary cortisol levels were measured four times a day (waking, 30 min after waking, 6 h after waking and bedtime) longitudinally, 8 months apart during three rounds of data collection. Pesticide using (conventional) and organic farmers were recruited to participate. Pesticide use in the previous 8 months was determined as the number of spray days for each type of pesticide used (herbicide, insecticide, fungicide) from self-reported questionnaires. Estimates of cortisol levels at four time points, the cortisol awakening response (CAR), and the diurnal cortisol slope (DCS) were estimated with a longitudinal mixed model that accounted for the non-linearity of cortisol levels across the day. Conventional farmers had significantly lower cortisol levels at waking than organic farmers (3.39 versus 3.86 ng ml^{-1}), 30 min after waking (5.87 versus 6.96 ng ml^{-1}), 6 h after waking (1.62 versus 1.88 ng ml^{-1}), and lower diurnal cortisol slope (−2.26 versus −2.51 ng ml^{-1}). Farmers who frequently applied herbicides (90th percentile of the number of spray days in the past 8 months) had significantly lower waking, 30 min after waking, 6 h after waking, bedtime and diurnal cortisol slopes compared with those with no spray days of herbicide in the past 8 months (organic and some of the conventional farmers). Those who frequently applied insecticides in the past 8 months had significantly lower bedtime levels and diurnal cortisol slopes, compared with those with no spray days of insecticide in the past 8 months. There were no significant differences in cortisol hormones between those who frequently applied

What's important about this paper?

Salivary cortisol levels reflect hypothalamic–pituitary–adrenal (HPA) axis activity, and dysregulating the HPA axis has been associated with variety of adverse cardiometabolic outcomes. There is little known about how pesticide exposures affect the HPA axis, so this study measured salivary cortisol levels four times a day, longitudinally for three rounds, 8 months apart among organic and conventional farmers. The diurnal cortisol slopes of the conventional farmers were significantly flatter than those of organic farmers, indicating dysregulation of the HPA axis.

fungicides and those who did not spray fungicides. Repeated pesticide use appears to be disrupting the HPA axis and depressing the normal diurnal cortisol rhythm among conventional Thai farmers.

Keywords: agriculture, cortisol, fungicide, herbicide, hypothalamic–pituitary–adrenal axis, insecticide, organic, pesticide

Introduction

Pesticides are widely applied to prevent or treat infestations of weeds, insects, or fungi. In Thailand, pesticide importation has been increasing annually, putting the country among the top ten for annual pesticide consumption per capita (tons imported) (Pretty and Bharucha, 2015). Several endocrine-disrupting chemicals (EDCs) have been associated with changes in human cortisol levels (Kido *et al*., 2013; Mustieles *et al*., 2018). Exposure to EDCs has been linked with the development of obesity and metabolic syndrome in animals, especially when exposures occur early in life (World Health Organization, 2012). Changes in hypothalamic–pituitary–adrenal (HPA) axis cortisol levels have been linked with increased risk of type 2 diabetes, obesity, metabolic syndrome, and cardiovascular mortality (Björntorp and Rosmond, 2000; Brunner *et al*., 2002; Kumari *et al*., 2011; Champaneri *et al*., 2012, 2013; Hackett *et al*., 2014). Previously, we have reported that among the cohort examined in this paper, conventional farmers had significantly higher abnormal body mass index (BMI), waist circumference, body fat%, triglyceride, total cholesterol, and low-density lipoprotein than organic farmers (Kongtip *et al*., 2018, 2020). To date, there has been little work examining HPA axis cortisol levels among workers exposed to pesticides (Cecchi *et al*., 2012).

Limited information is available on the effect of EDC exposure on adrenal function. The adrenal gland controls the stress response, blood pressure, and electrolyte homeostasis (Martinez-Arguelles and Papadopoulos, 2015). The enzymes involved in the steroid biosynthesis pathway are important targets for the actions of EDCs and may result in impaired reproduction, alterations in (sexual) differentiation, growth, and development as well as the development of certain cancers (Sanderson, 2006). The literature on pesticides and the HPA axis cortisol levels is limited to lab studies of fish (Cericato *et al*., 2008; Koakoski *et al*., 2014; Pandya *et al*., 2018), organophosphate (OP) poisoning patients (Güven *et al*., 1999), and pregnant women in Argentina who were found to have significantly higher levels of cortisol during the OP pesticide spraying season than during the pre-spraying season (Cecchi *et al*., 2012). The steroidogenic pathway leading to synthesis of aldosterone and cortisol is shown in Fig. 1. Cholesterol is transported to the mitochondria via a carrier protein called steroidogenic acute regulatory protein (StAR) and it is cleaved by the CYP11A1 enzyme to pregnenolone and then by the CYP17A1 enzyme to 17α-OH-Pregnenolone. Pregnenolone is then transformed to progesterone and eventually aldosterone. 17α-OH-Pregnenolone is transformed to 17α-OH-Progesterone and eventually to cortisol (Ullerås *et al*., 2008; Martinez-Arguelles and Papadopoulos, 2015). Endocrine disruptors, such as aminoglutethimide that are used to treat Cushing's disease by suppressing cortisol (and aldosterone) production, are reported to inhibit the CYP11A1 enzyme, the first step of cortisol synthesis (Johansson *et al*., 2002; Ullerås *et al*., 2008). Etomidate, an anesthetic that is a potent inhibitor of cortisol and aldosterone secretion, has been shown to directly inhibit CYP11A1 and CYP11B1 (Wagner *et al*., 1984). Although no universal mechanism has been identified for the impact of EDC pesticides on the HPA axis, the pesticides lindane and Roundup® (glyphosate) have been shown to inhibit steroidogenesis and the expression of the StAR cholesterol carrier protein needed for production of cortisol and aldosterone (Walsh and Stocco, 2000; Walsh *et al*., 2000).

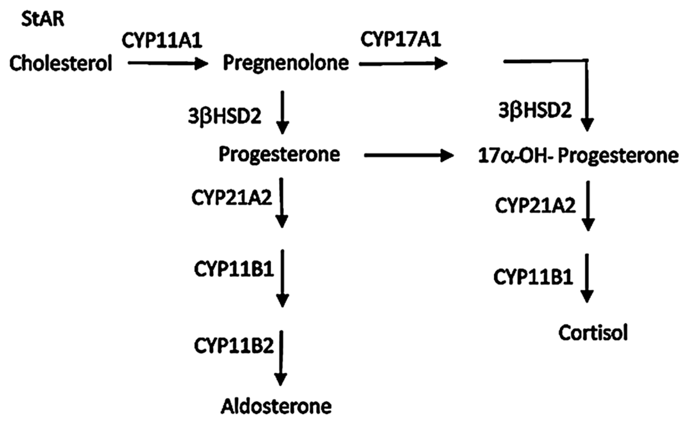

Figure 1. Steroidogenic pathway leading to the synthesis of aldosterone and cortisol. 3βHSD, 3β-hydroxysteroiddehydrogenase; StAR, steroidogenic acute regulatory protein.

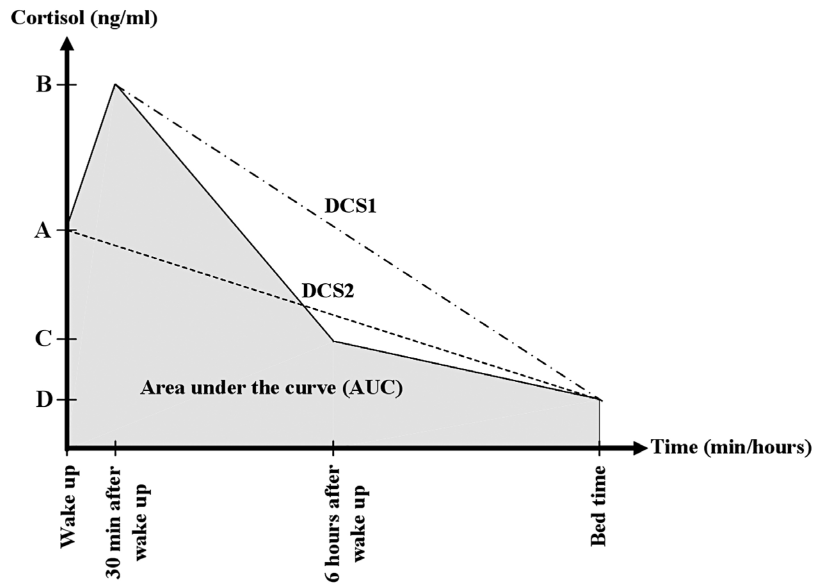

Figure 2. Cortisol metrics. (A) Cortisol level at waking. (B) Cortisol level at 30 min after waking. (C) Cortisol level at 6 h after waking. (D) Cortisol level at bedtime. The cortisol-awakening response (CAR) = cortisol level at 30 min after waking (B) – cortisol level at waking (A). Diurnal cortisol response (DCS1) using peak to bedtime = cortisol level at bedtime (D) – cortisol level at 30 min after waking (B). Diurnal cortisol response (DCS2) using waking to bedtime = cortisol level at bedtime (D) – cortisol level at waking (A).

Evaluation of HPA axis cortisol levels has changed over time from one-time serum levels to evaluation of the diurnal cortisol rhythm that is typically characterized by high levels upon waking, a substantial increase in cortisol concentration in the 30–45 min after waking (cortisol awakening response or CAR), and a subsequent decline over the remainder of the day (Adam and Kumari, 2009) (Fig. 2). Adam and Kumari (2009) have suggested that the diurnal cortisol slope (negative slope from waking to bed) and CAR are the most meaningful metrics related to the physiological stress response and disease outcomes. The size of the CAR has been correlated with several psychosocial processes and health outcomes (Adam *et al.*, 2006; Nater *et al.*, 2008). An abnormal CAR (heightened or blunted) has also been associated with type 2 diabetes (Bruehl *et al.*, 2009; Champaneri *et al.*, 2012) and BMI (Champaneri *et al.*, 2013). In a meta-analysis, a flattened DCS was significantly associated with cancer, depression, inflammation/immune outcomes, obesity, and other physical and mental health outcomes (Adam *et al.*, 2017).

The objective of this longitudinal study is to examine the impact of pesticide spraying on the neuroendocrine HPA axis cortisol hormone levels among Thai conventional and organic farmers.

Materials and methods

Study population and data collection

Conventional farmers were recruited with the help of the local health-promoting hospital/primary care unit, public health volunteers and community leaders in Phitsanulok province and Nakorn Sawan province. Organic farmers were recruited by community leaders in five sub-districts in Yasothorn province. These were all small family farms primarily growing vegetables, sugarcane, and rice. Inclusion criteria included one male or female farmer per family aged 18 years or older, without treatment or diagnosis of diabetes, high blood pressure, thyroid, or heart disease. To be eligible, conventional farmers had to report that they sprayed any type of pesticide as part of their farm work. Typically spraying is done with a motorized backpack sprayer and pesticides include a range of herbicides, insecticides, or fungicides. The organic farmers in the study were certified through the Foundation of Organic Agriculture Certification of Thailand (ACT), which gained legal status in 2001. They define "organic" as agricultural products which have been processed without any chemical that can harm plants, animals, or the environment. The process of certification requires an application with production plan forms, inspection, and review of the inspection report to address any non-compliance. Once the inspection report and corrective actions are approved, the certification is received. This study was approved by the Ethical Review Committee for Human Research, Faculty of Public Health, Mahidol University (MUPH 2015–146). The data were collected at 8, 16, and 24 months after initial recruitment into the study using questionnaires consisting of farmer personal characteristics, self-reported stress, home and demographic information, self-reported health problems, agricultural activities, and history of pesticide use. The physical health examination was conducted to collect weight and height, and BMI was calculated. Normal BMI was classified according to the WHO cutoff point of <18.5–24.9 kg m^{-2} (normal range) and ≥25 kg m^{-2} (overweight) (World Health Organization, 2004).

Saliva sample collection and analysis

At the time of the physical health examination, the saliva samples for cortisol were collected. After explanation and training at the local clinic, farmers were provided with four saliva collection tubes labeled 1–4 in a sealed plastic bag and a small booklet describing the passive drool saliva collection procedure. The saliva samples were collected four times per day. The first saliva sample was then collected the next morning upon waking and the second sample at the typical peak of the CAR (30 min after waking). We defined waking as 'as soon as you open your eyes and you are ready to get up and won't go back to sleep'. Subjects put the samples in the refrigerator until they took them to the local primary care health clinic (PCU) at around 7 am where the questionnaire data were collected. Subjects collected their 6 h after waking sample on site at the PCU. At bedtime, they collected the sample and put it in the refrigerator overnight and the subject brought the sample to the PCU the next morning. For the half an hour before each saliva sample collection, subjects were instructed not to eat, smoke, or brush their teeth. Subjects who were unable to provide samples for all four time points were asked to repeat the sample collection the next day. After collection from the subjects, saliva samples were kept at −20°C until analysis. Saliva samples were thawed and centrifuged at 2000 rpm for 10 min and the clear saliva solution was used for analysis by ELISA at Faculty of Public Health, Mahidol University using a saliva cortisol test kit (DRG International, Inc., 2018). The limit of detection (LOD) of cortisol level was 0.09 ng ml^{-1}. Cortisol levels lower than the detection limit were replaced by $\mathrm{LOD}/\sqrt{2}$(Hornung and Reed, 1990).

The cortisol hormone levels were right skewed and did not fit a normal distribution, so were transformed by taking the natural log (ln) of the levels. To address the issue of outliers, we utilized a winsorizing approach (Stalder *et al.*, 2016). We calculated the mean ± 3 standard deviations (SD) of the log value for each individual cortisol level. Cortisol levels higher than the mean + 3 SD were replaced with the value of the mean + 3 SD.

Pesticide exposure

Our primary exposure metric was the classification of conventional versus organic farmers. However, at each round, we collected information on agricultural activities and pesticide use during the past 8 months (two growing seasons) using a questionnaire. Thus, we were also able to use the self-reported frequency of spraying each type of pesticide (herbicides, fungicides, or insecticides) during the preceding 8 months as our secondary exposure metric.

Statistical analysis

Descriptive analysis of the demographic characteristics used chi-square test, Fisher's exact test, and independent *t*-test in SPSS for Windows, version 23 (IBM Thailand Co., Ltd., Bangkok, Thailand). Since our cortisol measurements varied across the day and we had up to three rounds of repeated measurements on each subject, we utilized a linear mixed model for repeated measures that included random intercepts for subjects to estimate the mean log concentration at each time point as well as for the CAR and DCS. We calculated DCS using the two most common methods, from peak (30 min after waking) to bedtime (DCS1), and from waking to bedtime (DCS2) (Adam and Kumari, 2009; Adam *et al.*, 2017). We included covariates that have been found to be associated with cortisol levels (baseline age and gender) (Hajat *et al.*, 2010; Karlamangla *et al.*, 2013). We evaluated other potential covariates including BMI, depression, and stress and included them if they changed the exposure parameter estimate by more than 10% (BMI). The resulting models included BMI, gender, and age at baseline. To model the time-varying cortisol levels within the day and allow for a non-linear fit, we used indicator variables for the time period of measurement and an interaction term for the exposure variable and the times within the day. Estimates of cortisol levels for each time period and for the DCS and CAR were generated using SAS Proc Mixed and Proc PLM (Version 9.4 SAS Institute, Cary, NC) using the mixed model described above.

Results

The number of overall saliva samples was 4988, which includes 435 subjects (1740 saliva samples) for the first round, 412 subjects (1648 saliva samples) for the second round and 400 subjects (1600 saliva samples) for the third round, with 47% of samples from conventional farmers and 53% from organic farmers. Thus, between the first and second rounds, 23 (5.3%) of the participants were lost to follow-up, and we lost 12 (2.9%) of the participants between the second and third rounds. At baseline, the average age of the organic farmers was higher than that of conventional farmers (Table 1). The conventional farmers were primary male (74.2%), but the organic farmers were equally distributed between males and females. The organic farmers reported higher educational levels than the conventional farmers. They reported similar average working hours in agriculture (hours per week). Conventional farmers were significantly more likely to report current smoking and alcohol consumption than were organic farmers. The frequency of self-reported stress symptoms and depression in the past 2–4 months was not significantly different between the two groups. Conventional farmers were significantly more likely to have an abnormal BMI than were organic farmers.

Our primary analysis compared the diurnal cortisol levels and pattern of organic and conventional farmers. A mix model that accounted for a non-linear fit in cortisol levels over the day after controlling for repeated measures, BMI, gender and age at baseline, estimated cortisol levels for organic and conventional farmers and compared the difference to see if it was significantly different from zero. We found that cortisol hormone levels were significantly lower for conventional farmers at waking, 30 min after waking, and 6 h after waking (Table 2). In addition, the DCS slope, calculated either from waking or from peak, was significantly reduced among conventional farmers. When the estimated values in Table 2 are exponentiated, they represent an average reduction for conventional farmers of −12% at waking, −16% at 30 min post-waking, and −14% at 6 h post-waking. The estimated natural log cortisol levels at the four measured time points during the day are illustrated in Fig. 3.

Although organic farmers had zero spray days for pesticides, some conventional farmers also reported no spray days of insecticides (12%), herbicides (9%), or fungicides (54%). The median number of spray days of each type of pesticide in the previous 8 months was low, but there was a wide range in the reported frequency (Table 3). Therefore, as part of a secondary analysis we examined reported days of spraying different types of pesticides for the whole cohort to see whether there was an association between days of spraying in the past 8 months and the cortisol diurnal pattern and levels.

Using the distribution of spray days for the whole cohort, the mixed model was used to estimate cortisol at waking, 30 min after waking, at bedtime, as well as the CAR, and DCS from waking and from peak for the 10th percentile of reported days of spraying (which was zero for all pesticide types for the whole cohort). These estimates were then compared with the cortisol estimates using the 90th percentile of reported days of spraying each pesticide type and the difference in levels compared to see whether it was significantly different from zero (Table 4).

We found a significant reduction in cortisol levels at waking, 30 min after waking, and 6 h after waking, when farmers had frequently applied herbicide (90th percentile = 12 days of spraying in past 8 months) compared with when farmers did not report spraying herbicides (10th percentile = 0 days). However, bedtime levels were significantly increased when farmers had frequently applied herbicides compared with those who had reported not spraying herbicides. When the estimated

Table 1. Characteristic of conventional farmers (n = 213) and organic farmers (n = 225) at baseline

Variables	Total (n)	Conventional farmers, n (%)	Organic farmers, n (%)	P-value
Age				
Minimum–maximum	438	18–69	28–79	0.004*,§
Mean (SD)		50.21 (11.1)	53.20 (10.3)	
Sex				
Male	273	158 (74.2)	115 (51.1)	<0.001*,†
Female	165	55 (25.8)	110 (48.9)	
Educational level				
Below elementary	18	14 (6.6)	4 (1.8)	0.035*,†
Elementary	247	122 (57.3)	125 (55.6)	
High school	157	72 (33.8)	85 (37.8)	
Bachelor or higher	16	5 (2.3)	11 (4.9)	
Marital status				
Single	34	21 (9.9)	13 (5.8)	0.030*,†
Married	373	184 (86.4)	189 (84.0)	
Widowed/divorced	31	8 (3.7)	23 (10.2)	
Agricultural work time (h week^{-1})				
Mean (SD)	438	26.9 (13.8)	28.8 (17.2)	0.227§
Alcohol intake				
Current drinker	227	136 (63.8)	91 (40.4)	<0.001*,†
Non-drinker	211	77 (36.2)	134 (59.6)	
Smoking				
Current smoker	93	57 (26.8)	36 (16.0)	0.006*,†
Non smoker	345	156 (73.2)	189 (84.0)	
Any stress symptom in past 2–4 weeks				
Yes	237	114 (53.5)	123 (54.7)	0.087†
No	201	99 (46.5)	102 (45.3)	
Depression reported in the past 3 months				
Yes	11	4 (1.9)	7 (3.1)	0.410†
No	427	209 (98.1)	218 (96.9)	
BMI (kg m^{-2})				
Normal (<18.49–24.99)	289	122 (57.3)	167 (74.2)	<0.001*,†
Abnormal (>25.00)	148	90 (42.3)	58 (25.8)	
Missing	1	1 (0.4)		

†P-value from chi-square test for the difference between conventional and organic farmers.
§P-value from t-test for the difference between conventional and organic farmers.
*P-value < 0.05.

values in Table 4 are exponentiated, they represent a reduction of −9% for waking, −11% for 30 min after waking, −8% for 6 h after waking, and an increase of +11% in the bedtime cortisol levels. The DCS estimates for both peak (DCS1) and waking (DCS2) were significantly reduced, representing a flattening of the slope, for farmers who frequently applied herbicides.

For insecticides, there was a significant 7% increase in bedtime cortisol levels when farmers had frequently applied insecticide (90th percentile = 24 days of spraying in the past 8 months) compared with when farmers did not report spraying insecticides (10th percentile = 0 days). The DCS1 calculated from the peak was significantly reduced, representing a flattening of the slope, for farmers who frequently applied insecticides, but the DCS2 calculated from waking showed no significant difference.

For fungicides, there were no significant changes in cortisol measures in the comparison of those who frequently applied fungicides (90th percentile = 8 days of spraying in the past 8 months) and those who did not report spraying fungicides (10th percentile = 0 days).

We also conducted a sub-analysis for only the conventional farmers using the distribution of their spray

Table 2. Predicted average natural log cortisol levels for organic and conventional (pesticide-using) farmers from a repeated-measures linear model using three rounds of data collection

	Estimated cortisol at waking (ng ml^{-1}) (95% CI)	Estimated cortisol 30 min after waking (ng ml^{-1}) (95% CI)	Estimated cortisol 6 h after waking (ng ml^{-1}) (95% CI)	Estimated cortisol at bedtime (ng ml^{-1}) (95% CI)	Estimated cortisol awakening response (CAR)[a] (ng ml^{-1}) (95% CI)	Estimated diurnal cortisol slope (DCS1)[b] (ng ml^{-1}) (95% CI)	Estimated diurnal cortisol slope (DCS2)[c] (ng ml^{-1}) (95% CI)
Organic farmers	1.35 (1.27, 1.42)	1.94 (1.86, 2.01)	0.63 (0.55, 0.70)	−0.57 (−0.65, −0.50)	0.59 (0.51, 0.67)	−2.51 (−2.58, −2.43)	−1.92 (−1.84, −2.00)
Conventional farmers	1.22 (1.15, 1.29)	1.77 (1.69, 1.84)	0.48 (0.41, 0.55)	−0.50 (−0.57, −0.43)	0.54 (0.46, 0.63)	−2.26 (−2.35, −2.18)	−1.72 (−1.64, −1.80)
Difference (95% CI on difference of conventional and organic estimates)	−0.13 (−0.22, −0.03)*	−0.17 (−0.27, −0.07)*	−0.15 (−0.24, −0.05)*	0.08 (−0.02, 0.17)	−0.04 (−0.16, 0.07)	0.25 (0.13, 0.36)*	0.20* (0.09, 0.32)

The number of overall saliva samples was 4988, which includes 435 subjects (1740 saliva samples) for the first round, 412 subjects (1648 saliva samples) for the second round, and 400 subjects (1600 saliva samples) for the third round, with 47% conventional farmers and 53% organic farmers. Models use a random intercept for subject, fixed effects for BMI, gender and age at baseline, index variables for the time periods within the day and an interaction term for the exposure variable and the times within the day. Use of index allows non-linear fit. Estimates are for males, using average BMI (23.88) and average age at baseline (51.72). CI, confidence interval.

[a]CAR = change from waking to 30 min after waking.

[b]DCS1 = change from 30 min after waking to bedtime.

[c]DCS2 = change from waking to bedtime.

*P-value < 0.05 for t-test of whether difference of estimates for conventional minus organic farmers are significantly different from 0.

days to estimate cortisol levels at the 10th and 90th percentile levels (see Supplementary Table S1, available at *Annals of Occupational Hygiene* online). For herbicides, none of the differences in cortisol levels were significant. However, all differences were in the same direction as the full cohort, albeit smaller. For insecticides, the only significant difference for the full cohort was for bedtime levels. For the subcohort of conventional farmers, the estimated difference between the 10th and 90th percentile at bedtime was non-significant but in the same direction (90th percentile levels were higher). We did however find a significant difference (0.13 ng ml^{-1}) between the 6-h cortisol levels, with the 90th percentile of insecticide spray days (0.52 ng ml^{-1}) significantly higher than the 10th percentile (0.39 ng ml^{-1}) for the subcohort of conventional farmers.

Discussion

Cortisol is also known to play a regulatory role in appetite, metabolism, fat deposition, and visceral adiposity (Epel *et al.*, 2001; Rosmond, 2005). Therefore, determining whether specific stressors activate or repress the cortisol system could delineate the conditions contributing to the onset or exacerbation of certain health outcomes. This is the first study to examine the relationship between current use of pesticides and salivary cortisol metrics. We hypothesized that conventional farmers would have a more dysregulated HPA axis than organic farmers, and the more times a farmer sprayed in a season, the more their cortisol would be affected. In the comparison of cortisol hormones between conventional and organic farmers, we found that cortisol hormone levels were significantly lower for conventional farmers for waking, 30 min after waking, and 6 h after waking. In addition, we compared estimated cortisol levels for farmers with no spray days of specific pesticide types (organic and some of the conventional farmers) to those with frequently applied pesticides (90th percentile of days of spraying in the past 8 months for each type of pesticide). We found that for herbicides, there was a significant reduction in cortisol levels when farmers had frequently applied herbicides at waking, 30 min after waking, and 6 h after waking. Bedtime levels were significantly increased when farmers had frequently applied herbicides. Although there were no significant differences between farmers who did not spray and those who frequently applied fungicides, there was a significant increase in bedtime cortisol levels when farmers had frequently applied insecticides. The blunting of cortisol response or hypocortisolism was originally identified with chronic psychosocial stressors (Miller *et al.*, 2007). Björntorp and Rosmond (2000) describe significantly lower morning cortisol levels among men with high stress and/or pathologically 'burned-out' cortisol secretion that resulted in lower variability in cortisol levels across the day. Healthy pregnant women living in small towns in Argentina, where they could be exposed to the spray drift of OP pesticides from pear and apple farms, were found to have significantly lower serum cortisol levels during the spraying period, compared with their levels during a pre-spraying period (Cecchi *et al.*, 2012). However, in a cohort of adults in six US cities exposed to air pollution, no association with cortisol levels was found with exposures to particulate matter < 2.5 µm in diameter (PM2.5), nitrogen dioxide, or nitrogen oxides, except a borderline increase in waking cortisol with higher NO_2 levels (Hajat *et al.*, 2019). In animal studies, cortisol levels were found to be lower in exposed fish than controls, when they were exposed to chlorpyrifos insecticide in a progressive dose and duration design (Oruç, 2010).

The diurnal cortisol slopes (DCS) of the conventional farmers were also significantly flatter than those of organic farmers. Farmers who frequently sprayed herbicides and insecticides, but not fungicides, in the past 8 months had a significantly reduced DCS1 (peak to bedtime) compared with those who did not spray these types of pesticides. However, the DCS2 from waking to bedtime for insecticides did not show a significant reduction. Flattened diurnal slopes have been identified as a superior predictor of both psychosocial stress and potential HPA axis dysregulation relative to other measures of cortisol, AUC or CAR (Saxbe, 2008; Adam and Kumari, 2009). Chronic exposure to psychosocial stress, due to disadvantageous race and socioeconomic status, was found to be associated with a flattened DCS, most commonly as a result of lower peak and/or higher bedtime levels (Cohen *et al.*, 2006; Hajat *et al.*,

Table 3. Distribution of days of pesticide spraying reported per 8-month period

Type of pesticide sprayed	Spray days per 8-month period for full cohort of organic and conventional farmers (*n* = 1318 reports over 3 rounds)		Spray days per 8-month period for conventional farmers only (*n* = 643 reports over three rounds)	
	% with 0 spray days	Median (10–90%) [minimum–maximum]	% with 0 spray days	Median (10–90%) [minimum–maximum]
Insecticide	56	0 (0–24) [0–96]	12	4 (0–24) [0–96]
Herbicide	54	0 (0–12) [0–50]	9	4 (2–16) [0–50]
Fungicide	77	0 (0–8) [0–96]	54	0 (0–24) [0–96]

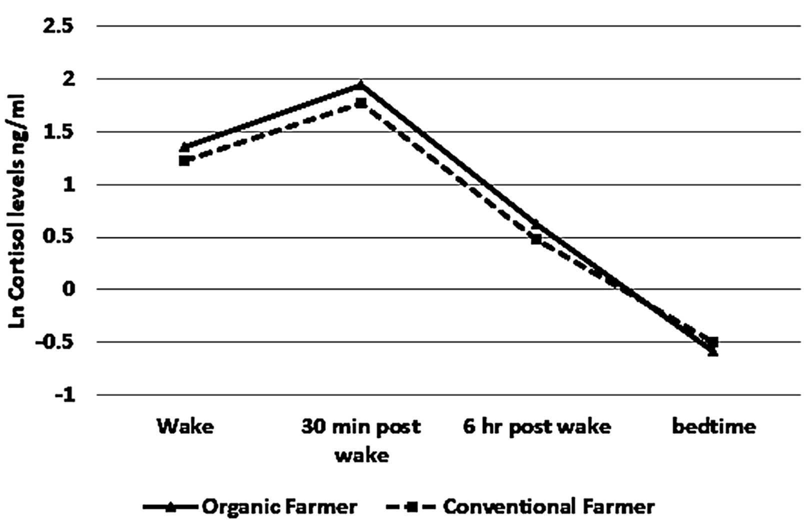

Figure 3. The predicted average natural log cortisol levels for conventional and organic farmers from a repeated-measures random intercept model for each cortisol measurement time after controlling for average BMI, male gender, and average age at baseline.

Table 4. Predicted log cortisol levels for spray days in previous 8 months for herbicides, insecticides, and fungicides from a repeated-measures linear model using three rounds of data collection (*n* = 1247 person-days of saliva samples resulting in 4988 samples)

		Estimated cortisol at waking (ng ml^{-1}) (95% CI)	Estimated cortisol 30 min after waking (ng ml^{-1}) (95% CI)	Estimated cortisol 6 h after waking (ng ml^{-1}) (95% CI)	Estimated cortisol at bedtime (ng ml^{-1}) (95% CI)	Estimated Cortisol Awakening Response (CAR)[a] (ng ml^{-1}) (95% CI)	Estimated diurnal cortisol slope (DCS1)[b] (ng ml^{-1}) (95% CI)	Estimated diurnal cortisol slope (DCS2)[c] (ng ml^{-1}) (95% CI)
Herbicide spray days in previous 8 months	E10: Estimate for 10th percentile of reported spray days of exposure (95% CI)	1.31 (1.25, 1.37)	1.89 (1.83, 1.95)	0.58 (0.52, 0.64)	−0.58 (−0.64, −0.51)	0.58 (0.51, 0.64)	−2.46 (−2.40, −2.53)	−1.88 (−1.95, −1.82)
	E90: Estimate for 90th percentile of reported spray days of exposure (95% CI)	1.22 (1.15, 1.30)	1.77 (1.70, 1.84)	0.50 (0.42, 0.57)	−0.48 (−0.56, −0.41)	0.55 (0.45, 0.64)	−2.25 (−2.16, −2.34)	−1.70 (−1.80, −1.61)
	Difference of E90–E10 estimates (95% CI)	−0.09* (−0.17, −0.00)	−0.12* (−0.20, −0.04	−0.08* (−0.16, 0.00)	0.09* (0.01, 0.17)	−0.03 (−0.14, 0.07)	0.22* (0.11, 0.32)	0.18* (0.07, 0.29)
Insecticide spray days in previous 8 months	E10: Estimate for 10th percentile of reported spray days of exposure (95% CI)	1.28 (1.22, 1.34)	1.85 (1.79, 1.91)	0.53 (0.47, 0.59)	−0.57 (−0.51, −0.63)	0.57 (0.51, 0.63)	−2.42 (−2.48, −2.36)	−1.85 (−1.91, −1.79)
	E90: Estimate for 90th percentile of reported spray days of exposure (95% CI)	1.27 (1.19, 1.35)	1.82 (1.74, 1.90)	0.59 (0.51, 0.67)	−0.49 (−0.5, −0.41)	0.55 (0.46, 0.65)	−2.32 (−2.41, −2.22)	−1.76 (−1.86, −1.66)
	Difference of E90–E10 estimates (95% CI)	−0.01 (−0.09, 0.07)	−0.02 (−0.10, 0.06)	0.06 (−0.03, 0.14)	0.08* (0.00, 0.16)	−0.02 (−0.12, 0.08)	0.11* (0.003, 0.21)	0.09 (−0.02, 0.19)
Fungicide spray days in previous 8 months	E10: Estimate for 10th percentile of reported spray days of exposure (95% CI)	1.29 (1.23, 1.34)	1.86 (1.80, 1.91)	0.54 (0.49, 0.60)	−0.55 (−0.61, −0.50)	0.57 (0.51, 0.63)	−2.41 (−2.47, −2.35)	−1.84 (−1.90, 1.78)
	E90: Estimate for 90th percentile of reported spray days of exposure (95% CI)	1.27 (1.21, 1.33)	1.84 (1.78, 1.89)	0.55 (0.50, 0.61)	−0.54 (−0.60, −0.49)	0.57 (0.51, 0.63)	−2.38 (−2.44, −2.32)	−1.81 (−1.87, −1.75)
	Difference of E90–E10 estimates (95% CI)	−0.02 (−0.05, 0.01)	−0.02 (−0.05, 0.01)	0.01 (−0.02, 0.04)	0.01 (−0.02, 0.04)	−0.003 (−0.04, 0.04)	0.03 (−0.01, 0.07)	0.03 (−0.01, 0.06)

Models use a random intercept for subject, fixed effects for BMI, gender and age at baseline, index variables for the time periods within the day and an interaction term for the exposure variable and the times within the day. Use of index allows non-linear fit. Estimates are for males, using average BMI (23.88) and average age at baseline (51.72). CI, confidence interval.

[a]CAR = change from waking to 30 min after waking.

[b]DCS1 = change from 30 min after waking to bedtime.

[c]DCS2 = change from waking to bedtime.

*P-value < 0.05 for t-test of whether difference of E90 and E10 is significantly different from zero.

2010; Karlamangla *et al.*, 2013; Novak *et al.*, 2017). A cross-sectional study of adults exposed to traffic related air pollution found a decrease in the DCS with higher ambient exposures to NO_2 (Hajat *et al.*, 2019).

Limitations of the study include reliance on subjects to comply with saliva collection protocols and timing at waking, 30 min after waking, and bedtime. Of particular importance is the timing of the wakeup and 30 min after wakeup samples. If sampling after wakeup is delayed 15 min or more, the CAR estimate can be significantly impacted (Stalder *et al.*, 2016). We did not have a method to verify the reported time of sample collection by the subjects. In terms of exposure metrics, many of our organic farmers at one time used pesticides, thus they may have already entered the study with HPA dysregulation. For the number of days of spraying each type of pesticide, we had to rely on self-reports of usage for each growing season in the previous 8 months. Thus, recall bias may come into play. In this study, we hypothesize that the impact of pesticide use is related to ongoing exposure, rather than acute exposure. Although most pesticides have relatively short biological half-lives, the conventional farmers are regularly exposed to pesticides, not only when they mix and spray but also when they are working in the fields touching the plants and digging in farm soil, as well as by eating fruit and vegetables with pesticides residues. However, since most pesticides have relatively short biological half-lives, a biological measure of chronic exposure is difficult to ascertain. Since cortisol levels tend to increase with age (Cauter *et al.*, 1996; Hajat *et al.*, 2010; Karlamangla *et al.*, 2013) and the organic farmers were significantly older than the conventional farmers, it is possible that the difference in organic farmers versus conventional farmers could be age related. However, the mean difference was only 3 years, and age was included in the models. Those with depression, anxiety, and post-traumatic stress disorder have been shown to have dysregulated cortisol profiles (Zorn *et al.*, 2017). However, in this cohort, we did not see a significant difference in stress symptoms or depression between organic and conventional farmers.

Conclusion

We found that conventional farmers had significantly lower cortisol levels at waking, 30 min after waking, and 6 h after waking. In looking at the types of pesticides used, we found that farmers who frequently sprayed herbicides (90th percentile of number of days spraying herbicides in the past 8 months) had significantly lower cortisol levels at waking, 30 min after waking, and 6 h after waking and significantly higher bedtime levels compared with farmers with no spray days of herbicide (organic farmers and some of the conventional farmers). There were no significant differences between farmers with no spray days and those who frequently sprayed fungicides, but with insecticides, there was a significant increase in bedtime cortisol levels for farmers who frequently sprayed insecticides. The diurnal cortisol slopes (DCS) of the conventional farmers were significantly flatter than those of organic farmers. The DCS1 (peak to bedtime) of farmers who frequently sprayed herbicides or insecticides (but not fungicides) was significantly reduced (flattened) compared with farmers who did not spray these pesticides and this was also seen for herbicides when the DCS2 was calculated from waking to bedtime. We conclude that pesticide use appears to be disrupting the HPA axis and depressing the normal diurnal cortisol rhythm in Thai farmers.

Supplementary data

Supplementary data are available at *Annals of Work Exposures and Health* online.

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Conflict of Interest

The authors declare no conflict of interest relating to the material presented in this article. Its contents, including any opinions and/or conclusions expressed, are solely those of the authors.

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