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The Effect of Body Composition and Energy Expenditure on Permethrin Biomarker Concentrations among US Army National Guard Members

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Abstract

Objective: To examine relationships between percent body fat (%BF) and total energy expenditure (TEE) on permethrin exposure among Army National Guard (ARNG) Soldiers wearing permethrin-treated uniforms.

Methods: ARNG members (n=47) participated in a nine-day study. Repeated body composition (height, weight, %BF) measurements and daily urine samples, analyzed for permethrin and N,N-diethyl-meta-toluamide (DEET) metabolites, were collected. TEE was determined via doubly labeled water protocol. Linear mixed and regression models were used for analyses.

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Conflicts of Interest: None declared

Results: Neither %BF nor TEE were significantly associated with permethrin or DEET biomarkers. However, a significant interaction effect (F=10.76; p=0.0027) between laundering history and %BF was observed; 10% higher %BF was significantly associated with 25% higher permethrin biomarker concentrations among those wearing uniforms washed ≤25 (compared to >25) times.

Conclusions: Uniform laundering history significantly affects the association between %BF and permethrin-treated uniform exposure.

Keywords

Permethrin; military; biomarkers; body composition; energy expenditure; occupational

INTRODUCTION

Permethrin is a synthetic pesticide in the pyrethroid family, derived from the naturally repellent chemicals in chrysanthemum flowers. The pesticide was first synthesized in 1973 and has been used for over 30 years in agricultural, medical, and personal applications. Permethrin is a highly effective tick and mosquito repellant and has been integrated into the garments of workers in several occupations, including forestry workers, farmers, and military personnel, to reduce the incidence of vector-borne disease. Wearing permethrin-treated clothing has been shown to reduce the frequency of tick and mosquito bites among wearers as well as the incidence of disease caused by biting insects (1–4).

Permethrin is rapidly metabolized and excreted in urine (5). Animal studies have found that permethrin persists the longest in adipose and brain tissue (6) with a half-life of 4 to 5 days (7). Also, recent studies evaluating permethrin absorption in human subjects demonstrate a mean half-life of 38.5 hours when absorbed dermally via treated clothing (5) and body mass index was found to be a significant predictor of higher urinary 3PBA levels on a population level (NHANES 1999–2002 (8)). Toxicology studies have determined that permethrin is of low toxicity to mammals when absorbed dermally (9), with acute toxic effects of localized exposure manifesting as itching, irritation, and tingling of the skin (10); little to no adverse signs or symptoms have been reported at levels commonly encountered by the general U.S. population.

In the United States Army, prior to 2013, Army personnel wore permethrin-treated uniforms only when deployed overseas to endemic areas. In 2013, the policy changed (11), and Soldiers were required to wear post-tailoring, factory-treated permethrin uniforms regardless of the Soldier's role and occupation. This policy was established in order to standardize uniform quality and to prevent Soldiers from acquiring vector-borne diseases during both training and deployment. The Army requires the permethrin concentrations of all new, unwashed uniforms to meet U.S. Environmental Protection Agency (EPA) and Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) standards, as well as their service-specific standards for treated fabric levels and efficacy requirements. Specifically, according to Army policy, permethrin concentrations for the Army Combat Uniform must fall within a range between 0.095– 0.135 mg/cm^3 and extend the 90–100% bite protection from 25 (EPA standard) initially to 50 launderings, the latter being what is considered the life of the uniform (personal communication, Ms. Melynda Perry, Textile Materials Evaluation Team).

Although not at levels above recommended exposure guidelines, prior studies among German and U.S. troops have determined that there is substantially higher permethrin exposure among Soldiers who wear permethrin-treated uniforms compared to those who do not (12–14), as determined (or evidenced by) the concentration of the primary urinary metabolites of permethrin: 3-phenoxybenzoic acid (3-PBA) and *cis*- and *trans*-3-(2,2-dichlorovinyl)-2,2-dimethyl-cyclopropane-1-carboxylic acid (*cis*-DCCA and *trans*-DCCA). Additional studies have established that occupationally relevant factors modify the amount of permethrin that a Soldier absorbs from treated uniforms. Uniform wear time (number of hours/day (14), high heat conditions (5, 15, 16), and body composition (percent body fat, %BF) and physical workload (total energy expenditure, TEE) in new Army recruits (17) have positive associations with permethrin biomarkers. However, the majority of exposure studies of military and other occupational populations published to date (18) examine permethrin absorption among individuals who have only newly been exposed to treated uniforms that have never been washed or washed only once (13–15, 17, 19). Investigations detailing permethrin biomarker concentrations among groups who have been wearing treated clothing over extended periods of time are limited (12, 13) (i.e., greater than 1 month).

As part of Army prevention practices, it is policy to apply N,N-diethyl-meta-toluamide (DEET) or other Armed Forces Pest Management Board (AFPMB) approved topical repellent to those skin areas not covered with permethrin treated clothing (20, 21), during operational or training activities in regions were insect vector presence is high. In animal research (22), dermal application of DEET in the presence of other military relevant exposures (jet fuel, gasoline) has demonstrated a protective effect on permethrin absorption via the skin. However, to our knowledge, there are no human occupational exposure studies that have examined possible interactions between concurrent occupational exposures, such as DEET, and permethrin exposure from wearing treated uniforms or clothing.

This study focuses on examining the relationships between body composition (%BF) and physical workload (TEE) and permethrin exposure among US Army National Guard (ARNG) Soldiers with a history of prior exposure to permethrin from wearing treated uniforms. We hypothesized that %BF and TEE would positively affect biomarker concentrations, but we anticipated that number of uniform launderings over time might affect permethrin metabolite concentrations as uniform fabric permethrin levels decrease over successive launderings (personal communication, Ms. Perry, Textile Materials Evaluation Team; (5)). In addition, we examined whether co-application of DEET (as measured by the urinary metabolite 3-(diethylcarbamoyl)benzoic acid, DCBA) and reported fuel exposures were associated with lower urinary permethrin biomarkers.

METHODS

The protocol was reviewed and approved by the US Army Research Institute of Environmental Medicine Institutional Review Board (IRB) and the US Army Medical Research and Materiel Command IRB and was in compliance with human subjects review procedure at the Centers for Disease Control and Prevention (CDC). The involvement of the CDC laboratory did not constitute engagement in human subjects research. The investigators adhered to the policies for protection of human subjects as prescribed in Army Regulation 70–25, and the research was conducted in adherence with the provisions of 32 CFR Part 219. . All participants gave their informed consent prior to the research study. Approval to conduct this research with ARNG Soldiers was also obtained through the National Guard Bureau and State-specific ARNG headquarters.

Study Participants and Design

This study was designed as a repeated measures prospective cohort study. ARNG Soldiers were eligible for inclusion if they owned permethrin-treated uniforms. The study was conducted with three separate groups of ARNG Soldiers in the summer of 2016 and 2017 during their 2-week Annual Training (AT) period. A total of 50 participants from several different unit types (e.g., aviation support, engineering, and infantry) enrolled and consented to participate (June 2016: 15 participants; August 2016: 14 participants; August 2017: 21 participants). Three Soldiers dropped out of the study due to changes in their training schedules, leaving 47 participants who completed the study. Calculations determined *a priori* maintained that a sample size of 50 individuals, with a 20% dropout rate (or n=40 remaining) would be sufficient to test the study's primary hypotheses, based on simulations indicating at least 80% power of showing within subjects associations at the 2-tailed $p<0.05$ level.

The study protocol was conducted with each of the three groups consecutively over a nine day study period, which began on the third or fourth day of the two week AT period. Participants carried out their planned ARNG daily activities during the study period (as study participation did not impose any restrictions on activities). On each Study Day, participants reported to the research team in the morning for approximately 20–30 minutes between the hours of 0600–0800 and again for approximately 5–10 minutes between 1600–1800 (Table 1). No visits occurred in the late afternoon of Day 3 or Day 9.

Measures

The study measures and procedures employed are identical to those followed in an earlier study (17) that examined relationships between %BF and TEE on permethrin biomarkers among US Army trainees wearing treated uniforms for the first time during basic combat training.

The Army Combat Uniform (ACU) is a tightly woven intimate blend of 50/50 Nylon and Cotton in a rip stop weave construction (i.e., plain weave construction with reinforcement yarns in both the warp and fill) and these reinforcement yarns give the uniform fabric the grid-like pattern seen on the material. The ACU material is a wind resistant material with an air permeability requirement of less than 10CFM; the weight requirement for the material is 6–7osy. A wrinkle-free finish applied to the material in the fabric stage prior to uniform manufacture. The permethrin is currently applied to the fully assembled garment at a rate of 0.52% w/w (Wpermethrin/Wfabric) +/− as required by the EPA registration with a +/− 10% variance allowed by FIFRA (Federal Insecticide, Fungicide, Rodenticide Act). The permethrin formulations applied to the uniforms at the factory include binder systems to ensure wash durability. The permethrin formulations and binder systems used by the permethrin applicators do vary and are proprietary to the company, however the treated uniforms undergo strict testing by the Military to ensure that the dose rate and efficacy requirements are being met initially and after 20 and 50 washes.

Wearing of undershirts under the uniform is standard Army practice; we assumed that under garments such as underwear were worn during the study but did not confirm by self-report.

Questionnaire—Questions regarding participant demographics, employment history, lifestyle behaviors (current tobacco, alcohol, and caffeine use), and current pesticide exposures were obtained during the first study visit via self-report survey. Also, participants were asked to report on their functional health (Veterans RAND 12-item Health Survey, VR-12 and cognitive functioning (Medical Outcomes Study (MOS) cognitive functioning scale (MOS CF) (23,24)) and health symptoms. The VR-12 evaluates self-perception of physical and emotional impacts on day-today functioning of individuals. VR-12 item responses are calculated to produce physical and mental component summary (PCS and MCS respectively) scores, standardized to U.S. population norms (mean=50, SD=10). The MOS CF scale is scored from 0–100 and evaluates the impact of thinking and attention on day-to-day functioning. Higher scores on each of these scales indicates better performance. The health symptom checklist queried participants on 18 symptoms of interest. This checklist has been utilized in prior studies involving military exposures to neurotoxicants (25,26). Participants were asked how often they experienced each symptom in the past week (Never, Rarely, Sometimes, Often, or Very Often), and each response was scored from 0 (“Never”) to 4 (“Very Often”). The responses were summed to produce a composite score of symptom endorsement (range: 0–72) for each participant to provide an overall indicator of the severity of health symptoms they were experiencing.

Participants’ functional health (VR12, MOS CF) and health symptom reports were evaluated again on the evening of Day 8 to examine whether there were changes in health symptoms or general wellness over the study period.

Body Composition—On the morning of Day 1, measurements of each Soldier’s height (cm) and weight (kg) were obtained and measurements for computation of percent body fat (%BF) were taken. Height was only measured on the first day of the study, while weight was measured 4 additional times (mornings of Day 3, 4, 6, and 9) and %BF measurements were collected 3 more times (mornings of Day 3, 4, and 9) over the duration of the study (Table 1). Height was measured by use of a SECA 2017 stadiometer. Participants were asked to remove their boots and the device was adjusted so that it was level with the top of the participant’s head for height (cm). Soldiers wore t-shirts, shorts, and socks for weight (kg) measurements (using a Doran DS6150 Remote Indicator Scale). Percent BF was calculated using Jackson and Pollock’s 3-site skinfold method with calipers (27). Thigh, abdominal, and chest skinfold measurements were taken for males; tricep, suprailiac, and thigh measurements were determined for females. Each measurement was made in duplicate to the nearest mm; if the measures differed by more than 2 mm then a third measurement was taken.

In addition to the above body composition measurements, body mass index (BMI) was calculated from the collected height and weight data at each relevant point over the study (28).

Biological Samples—Spot urine samples were collected from each participant the each morning and in the evening of Days 1, 2, 4, and 5 to quantify concentrations of permethrin biomarkers (3PBA, *cis*-DCCA, and *trans*-DCCA), DCBA, and creatinine. In total, 13 samples were collected from each individual (Table 1), with 10 urine samples per person analyzed for permethrin biomarkers and DCBA, and 8 urine samples (Days 4–9) analyzed for computation of TEE. Every urine sample was aliquoted into separate vials for permethrin biomarkers and DCBA, creatinine, and doubly-labelled water (DLW) isotope analyses (for latter, see further details below). The urine samples were refrigerated within three hours and frozen within one day of collection, and shipped overnight on dry ice to the analyzing laboratory within 1.5 weeks of collection. At the CDC, samples were analyzed to determine the concentrations of the permethrin metabolites (29) and DCBA (30, 31).

Daily Activity and Workload—At each evening study visit, a one page survey was administered asking about each Soldier's activities, smoking and alcohol use, and uniform wear during the previous 24 hour period. Questions asked included what time of day that participants went to bed and awoke each day and how many hours they slept, the number of hours that they wore their uniform during the day, the number of times the uniform worn that 24-hour period had been washed, and the age of the specific uniform worn that day (i.e., how many months/years they had owned the uniform). Although owning permethrin-treated uniforms was an inclusion criteria for this study, participants were asked whether the uniform worn that day was permethrin-treated, to confirm whether or not older non-treated uniforms were being worn during the study period. In addition, questions were asked about the participants' primary daily activities, any known exposure to chemicals (DEET, jet fuel, and gasoline) or substances (tobacco, alcohol, and caffeine), and whether they showered during the day.

Physical activity levels were tracked for two separate time intervals by an activity monitor (Philips brand Actical (32)) worn at the participant's ankle or above the boot: during the i) first 48 hours of the study (morning of Day 1 to morning of Day 3) and ii) the last 120 hours of the study period (morning of Day 4 to morning of Day 9). The monitor recorded data on the participant's movement and computed the estimated energy expenditure (kcals, in 1 min intervals). After each recording period, raw data were downloaded from the devices and individual-level average daily activity levels were determined to provide assessment of daily energy expenditure.

The technique utilizing DLW is the established gold standard method to calculate the total energy expended over a selected time period (33) and was included in the study design to compute total energy expenditure (TEE). On the morning of Day 4, 90 g of doubly-labelled water (99.9% atom percent excess ^2H_2O and 10% H_2O^{18}) was distributed to each participant for ingestion; urine samples collected on Days 4 – 9 (1 pre-dose and 7 post-dose) were aliquoted and measured for isotope enrichment by the Pennington Biomedical Research

Center via Isotope Ratio Mass Spectrometry. The results of the isotope analysis provide TEE (kcal) averaged over the respective 5 day period.

Statistical Analysis

Descriptive analyses were run to examine study group characteristics at baseline and over the study period, including information on demographics, permethrin metabolites, DCBA and creatinine concentrations, energy expenditure levels, body composition, and uniform age and laundering history. Changes in measured weight, BMI, %BF, and reported health symptoms and functional health over the study period were examined by paired t-tests. Analyses of variance (ANOVA) were conducted to examine permethrin biomarker concentrations among those self-reporting exposure to DEET, jet fuel, and gasoline compared to those with no reported exposure. *Cis*-DCCA and *trans*-DCCA concentrations were summed to yield a total DCAA concentration (∑DCCA) that was utilized for some analyses. For statistical purposes, concentrations that were not detectable (i.e., below the limits of detection (LOD)), were assigned a value of LOD/2 (34).

Urine samples with creatinine concentrations outside the range of 20–350 mg/dL (17) were excluded (n=28, 8%) from the analyses to minimize the effect of samples being too dilute or concentrated and eliminate possible participants with potential kidney dysfunction or hydration issues that might affect results.

A method described (12) and applied in previous studies (14, 15, 17), computed the average daily permethrin dose for each day that the study was conducted (in μg/kg/day).

Linear mixed or regression model analyses were performed to address the primary study hypotheses. There were six model sets: three examining the hypotheses pertaining to 3-PBA (Model A. 1–3) and three examining these same hypotheses with the sum of *trans*-DCCA and *cis*-DCCA (∑DCCA) (Model B. 1–3). Models A.1 and B.1 examined the independent association between repeat %BF measures over the study and each biomarker (3-PBA and ∑DCCA) respectively, using linear mixed modeling with a spatial power correlation structure. Linear regression models examined the final study day (Day 9) associations between measured TEE (Models A.2 and B.2) and both %BF and TEE in the model together (Models A.3 and B.3) and permethrin biomarker concentrations. The permethrin metabolites, creatinine, TEE, and %BF were log-transformed to preserve the assumption of normality for the residuals. All models were adjusted for sex, age, creatinine concentrations, tobacco use, number of times that the participant's uniform worn during the day prior to collection had been washed, and the number of hours that the uniform had been worn during the day prior to collection. Tobacco use was coded as a binary variable with users defined as those who reported smoking cigarettes, chewing tobacco, or using e-cigarettes, and nonusers defined as those who reported no current use of these products. The uniform wash history variable was dichotomized into ≤25 reported washings (reference) and >25 reported washings.

Parameter estimates were obtained using maximum likelihood statements. The permethrin metabolites were log transformed for analyses, so that the linear models met their homoscedasticity assumption. The parameter estimates were exponentiated to allow for ease of interpretation of the effect estimated by %BF and TEE.

Interaction terms (%BF*uniform wash history; TEE*uniform wash history) were added to the respective models to examine the effect that a high number of launderings had on the relationship between %BF and TEE on permethrin exposure. One participant who owned multiple uniforms did not wear the permethrin-treated uniform during the study. For the analyses, this person was coded as wearing uniforms that had been washed > 25 times. We also performed post hoc sensitivity analyses, excluding this person completely from the analyses.

In separate post hoc analyses, the role of concurrent exposure to DEET on the relationship between %BF and permethrin biomarkers was explored by adding DCBA concentrations to the linear regression (Models A.3 and B.3) models. All analyses were performed using SAS, version 9.4 (SAS Institute, Inc., Cary, NC).

RESULTS

The average (SD) age of the 47 ARNG participants was 28.27 (7.86) years and ranged from 20.0 to 53.3 years old; the majority of the group was male (n=40; 85%), identified as white, Caucasian (n=38; 81%), and had a college education or higher (n=32; 68%). At baseline on Day 1, current tobacco use was reported by 19 (40%) participants; weekly or more frequent alcohol use was reported by 22 (47%) participants. Eleven (22%) persons reported having regular occupational exposure to jet fuel or gasoline. The average (SD) height and weight of the group on Day 1 was 176.05 (9.08) cm and 83.8 (17.07) kg. On Day 1, the mean (SD) VR-12 PCS and MCS were 52.84 (5.02) and 55.49 (7.71), respectively, the MOS CF score was 88.82 (12.13), and the average health symptom score was 5.11 (5.94).

Over the course of the study period, there was a significant decrease in %BF (Day 1 AM: 17.52 (6.12); Day 9 AM: 16.50 (5.81); paired t=2.62, p=0.012), but there were no significant changes in weight or BMI. On average, participants reported 6.39 (1.41) hours/sleep per night (range: 3.70–8.70). The average (SD) estimated energy expenditure measured by the worn actigraph during the 120 hour period (Day 4-Day 9) was 3,396.35 (829.35) kcals/day, while the average measured through the DLW method was 3,383.61 (558.97) kcals/day for the same period (Spearman's rho=0.75).

The mean, standard error (SE), geometric mean (GM), median, and 95th percentile for all creatinine-adjusted permethrin biomarker and DCBA concentrations, and the estimated daily permethrin dose (μg/g creatinine/day) at each morning collection point during the study period as well as over the study period are presented in Supplementary Tables 1 and 2. Figure 1 displays the mean creatinine-adjusted (in μg/g creatinine) concentration of the three metabolites (3-PBA, *trans*-DCCA, *cis*-DCCA) measured from the morning samples over the study. On Day 1, the mean 3-PBA concentration among the study population was 23.73 μg/g creatinine (SE = 3.58). On the morning of Day 9, the mean 3-PBA concentration was 26.03 μg/g creatinine (SE = 4.79). No significant differences between the mean concentrations of 3-PBA overall during the study (F=0.71 p=0.6631) were observed. Figure 2 presents the mean daily permethrin dose estimates of the participants. Overall, there was a significant difference in estimated permethrin dose between collection points ($F=2.14$, $p=0.039$). There were no significant changes in reported health functioning or health symptoms over the study period.

Uniforms were worn on average 13.5 hours/day throughout the study period (range = 4.7 – 18.0 hours); the average number of times the worn uniforms were reportedly washed over their history was 50.4 ± 111.6 (median: 25) times. The average age of the uniforms was 2.3 years (range = 0–7.0). Further description of uniform characteristics is included in Supplementary Table 3.

On examination of the study hypotheses, neither %BF nor TEE were significant predictors of permethrin exposure (assessed from 3-PBA or ∑DCCA concentrations) either separately (Models 1 and 2 respectively) or when included together in the model (Model 3) (Table 2). Along with creatinine (data not shown), the number of times the uniform had been laundered was the only significant predictor of permethrin biomarker concentrations, and significant interaction effects on permethrin exposure between %BF and laundering history were observed (Tables 2 and 3). In the combined model (Model 3A) with both %BF and TEE present, a 10% increase in %BF is associated with a 25% increase in urinary 3-PBA concentrations among participants who wore uniforms that were laundered ≤25 times. Exclusion of the one person not wearing permethrin-treated uniforms during the study from the model analyses did not affect the significance of the pattern of results described above.

A total of 24 individuals reported being exposed to either gasoline, jet fuel, or DEET at least one time during the study period, with several reporting concurrent exposures (18 reported occupational exposure to jet fuel, 11 to gasoline, and 9 to DEET). We observed no significant differences in permethrin biomarkers measured on Day 9 between the groups reporting exposure to DEET, jet fuel, or gasoline compared to those reporting no exposure.

Post hoc analyses exploring the role of concurrent DEET exposure, by adding DCBA to the models in Table 3, did not impact the study findings. DCBA did not significantly affect permethrin biomarker concentrations nor did it change the relationships between %BF, TEE, or wash histories and permethrin exposure (results not shown).

DISCUSSION

Findings suggest that among ARNG Soldiers wearing permethrin treated clothing over longer intervals (years), uniform laundering history plays a significant role in the extent of permethrin exposure. Overall, there was no significant association between body composition (%BF) or physical activity (TEE) on permethrin exposure from wearing treated uniforms. However, %BF was a significant factor in permethrin exposure among those wearing uniforms washed ≤25 times. This finding is supported in previous research (15) where higher %BF was significantly associated with permethrin biomarkers among new Army trainees wearing newly acquired permethrin treated uniforms, with minimal laundering histories. Concurrent exposure to DEET did not significantly affect permethrin exposure directly nor did it affect the relationships between %BF and permethrin exposure.

The uniform wear histories among our ARNG study population varied significantly from the majority of other military studies that have been conducted prior to this time as most other military studies have examined acute permethrin exposure, using newly treated clothing and measuring permethrin uptake over the first few months of exposure. In comparison, the average uniform age worn during this study was over 2 years old and median number of times washed was 25 times. As such, these results provide an estimate of the permethrin dose experienced from wearing permethrin-treated clothing over long-time ownership, i.e., extended age and related wider range of laundering histories (Supplementary Tables 2 and 3). Compared to estimated dose levels from those other studies (i.e., 5–6 ug/kg body weight/day; (10, 12, 15), on average, exposure to permethrin from wearing treated clothing in this study is lower (i.e., 1–2 ug/kg body weight/day) and in general, comparable to the lower median baseline (Day 1) biomarker concentrations observed under multiple week-long, steady-state wear conditions (13). In an earlier study (15) with new Army trainees, higher physical workload measured by TEE was significantly associated with permethrin biomarkers. However, TEE was not significantly associated among the ARNG participants in this study. One possible explanation may be the difference in activity levels between the studies as the ARNG group, compared to the Army trainees in the middle of their training period, reported higher average hours of sleep (6.4 versus 5.7 hours/day, respectively) and less energy expenditure levels (3,384 versus 4,004 kcals/day, respectively).

There are a number of strengths to this study. This is the first study to our knowledge that examines permethrin metabolite concentrations among cohorts who have worn treated clothing over several years, although intermittently. As such, these results provide information about the expected permethrin dose levels for people wearing treated clothing for more periodic recreational outdoor use. Furthermore, this population is representative of US Army National Guard personnel as a whole as it includes both sexes, a broad spectrum of ages, and Soldiers with a wide range of time in military service and occupational specialties. We did rely on self-report from the participants for age of their uniform worn and laundering history. However, there is no reason to believe an individual’s ability to recall these factors varied with their permethrin exposure since participants would not have knowledge of their individual permethrin levels. Therefore, any error from self-report is likely to be random with respect to the outcome and would potentially bias the results towards a null finding. Also, because of the disparate reports of environmental exposures and wide differences in uniform age, however, we did not have the statistical power to determine effects of multiple interactive occupational factors on exposure (such as potential interactions between exposure to DEET and fuel on permethrin biomarkers).

To conclude, within this ARNG population overall, we found no significant association between either %BF or TEE and permethrin metabolite concentrations (3-PBA and/or *cis*-DCCA, *trans*-DCCA, and ∑DCCA) in urine over a 9-day ARNG Annual Training period. A statistically significant positive association between %BF and 3-PBA concentrations was found among those wearing uniforms washed ≤25 times, suggesting a threshold effect. It is important to note that the estimated mean permethrin dose in this study was lower than doses observed in other studies where treated clothing was never washed or washed fewer times. These study results provide some guidance for the permethrin biomarker concentrations expected from longer-term (at times intermittent) exposure among U.S. Army populations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Clinical Significance

This study demonstrates significant impacts of percent body fat on occupational exposure to permethrin (measured by urinary biomarkers) while wearing treated clothing among Army National Guard members with newer, less laundered uniforms (less than 25 times). Neither increased energy expenditure (physical workload) nor concurrent DEET exposure affected permethrin biomarker concentrations.

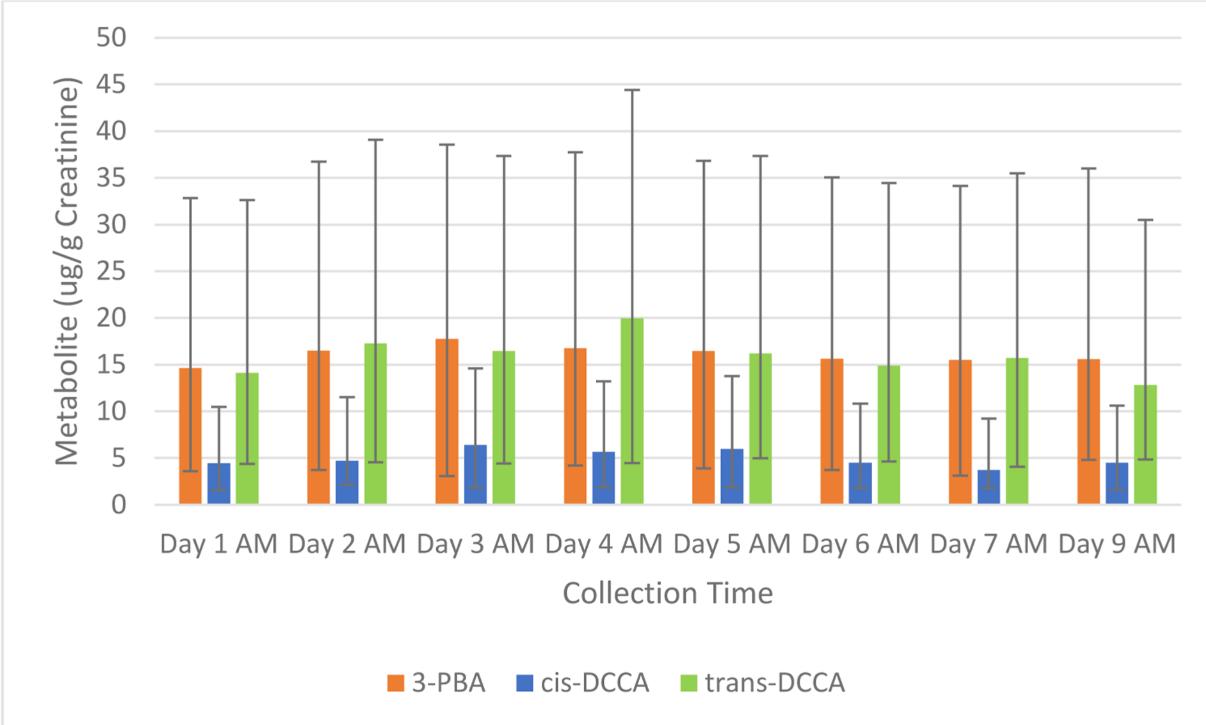

Figure 1: Creatinine-adjusted Geometric Mean Permethrin Metabolite Concentrations (with Standard Error Bars)

3-PBA = 3-phenoxybenzoic acid (3-PBA)

cis-DCCA = *cis*-3-(2,2-dichlorovinyl)-2,2-dimethyl-cyclopropane-1-carboxylic acid

trans-DCCA = *trans*-3-(2,2-dichlorovinyl)-2,2-dimethyl-cyclopropane-1-carboxylic acid

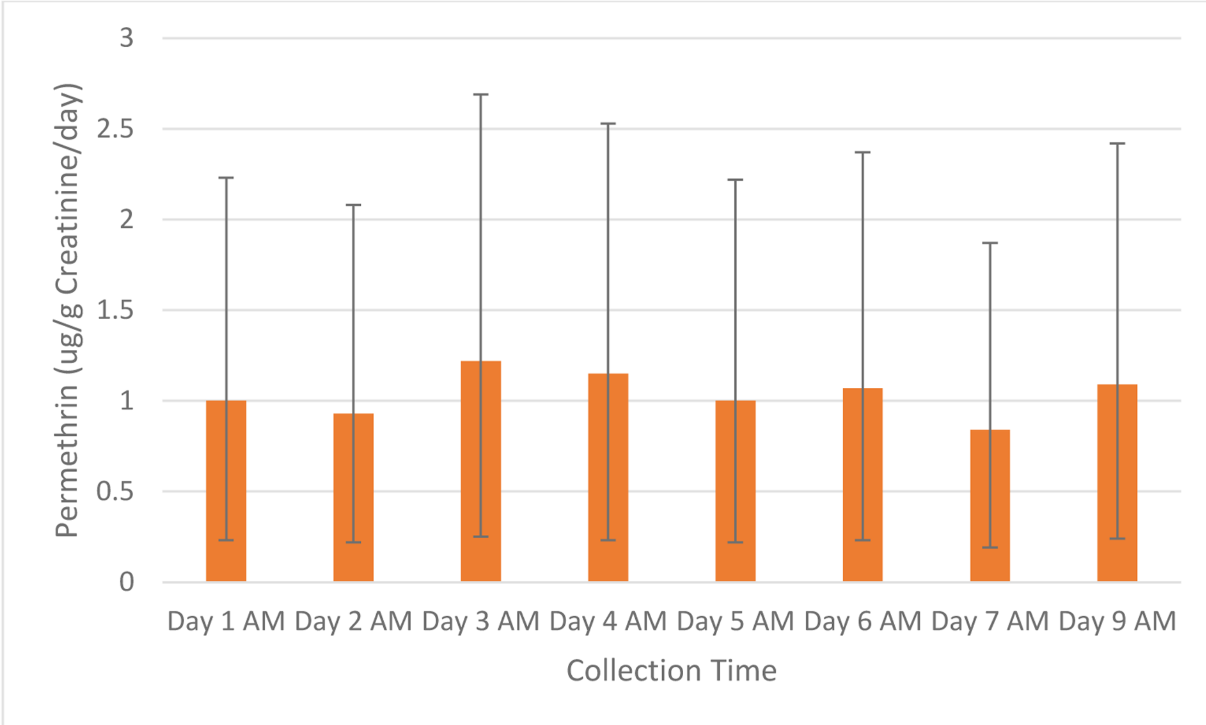

Figure 2:
Geometric Mean Estimated Daily Permethrin Dose by Study Day (with Standard Error Bars)

Table 1:

Data Collection Timeline of Study

Day	1	2	3	4	5	6	7	8	9	
Urine										
AM	X[p]	X[p]	X[p]	X[p,a]	X[p,a]	X[p,a]	X[pa]	[X][a]	X[p,a]	
PM	X[p]	X[p]		[X][a]	[X][a]					
%BF	M		M	M					M	
Height	M									
Weight	M		M	M		M			M	

X urine sample collected

[X] urine sample collected but not analyzed for permethrin metabolites (analyzed for DLW isotopes only)

[a]Urine sample analyzed for isotopes to determine total energy expenditure (TEE)

[p]Urine sample analyzed for permethrin and DEET metabolites

M body composition measurements made

Table 2:

Model Results for 3-PBA (A) and ΣDCCA (B), using dichotomous washing categories (>25 washings as the reference)

	A			B		
	LN 3-PBA			LN ∑DCCA		
Model*	**Beta**	**SE**	**F statistic (p)**	**Beta**	**SE**	**F statistic (p)**
Model 1						
%BF	0.3253	0.353	0.85 (0.3586)	−0.1945	0.469	0.17 (0.6789)
Sex	0.3076	0.396	0.60 (0.4390)	0.7414	0.506	2.14 (0.1460)
Tobacco Use	0.1808	0.288	0.39 (0.5313)	0.1803	0.368	0.24 (0.6250)
# hrs worn	0.0405	0.029	1.93 (0.1673)	0.0619	0.042	2.18 (0.1425)
# times washed	0.3004	0.156	3.69 (0.0574)	0.5791	0.233	**6.17 (0.0145)**
Model 2						
TEE, kcal/day	0.3769	1.440	0.07 (0.7952)	−0.5114	2.198	0.05 (0.8176)
Sex	0.5995	0.656	0.83 (0.3679)	0.8901	1.001	0.79 (0.3805)
Tobacco Use	0.3661	0.361	1.03 (0.3178)	0.4584	0.558	0.67 (0.4178)
# hrs worn	0.1153	0.085	1.86 (0.1820)	0.1576	0.129	1.22 (0.2300)
# times washed	0.7932	0.370	**4.60 (0.0396)**	1.0634	0.574	3.43 (0.0737)
Model 3						
%BF	0.4770	0.640	0.55 (0.4622)	−0.2787	0.998	0.08 (0.7821)
TEE, kcal/day	0.2852	1.516	0.04 (0.8520)	−0.0647	2.362	0.00 (0.9783)
Sex	0.4576	0.751	0.37 (0.5469)	1.1746	1.169	1.01 (0.3234)
Tobacco Use	0.3305	0.395	0.70 (0.4089)	0.6152	0.626	0.97 (0.3339)
# hrs worn	0.1178	0.084	1.95 (0.1724)	0.1632	0.131	1.55 (0.2237)
# times washed	0.7458	0.371	4.04 (0.0534)	0.9893	0.590	2.81 (0.1046)

*Models adjusted for sex, age, creatinine, tobacco use (1= current use; 0=no current use), number of hours uniform worn and number of wash times of uniform (1= ≤25 times; 0= >25 times) worn the day prior to urine collection

3-PBA=3-phenoxybenzoic acid (3-PBA); ∑DCCA = sum of *cis*- and *trans*-3-(2,2-dichlorovinyl)-2,2-dimethyl-cyclopropane-1-carboxylic acid

Table 3:

Interaction Effect of %BF and Uniform Wash Frequency on Permethrin Biomarkers

Model*	**LN 3-PBA**	**LN ∑DCCA**
Model 1	**F-statistic (p)**	**F-statistic (p)**
%BF*Washings	**5.69 (0.019)**	**9.25 (0.003)**
Effect of %BF by # of washings		
0–25	**0.86 (0.041)**	0.69 (0.190)
>25	−0.15 (0.694)	**−1.02 (0.043)**
Model 3		
%BF*Washings	**9.56 (0.004)**	**5.00 (0.034)**
Effect of %BF by # of washings		
0–25	**2.35 (0.008)**	1.87 (0.161)
>25	−0.77 (0.278)	−1.66 (0.135)

*Models adjusted for sex, age, creatinine, tobacco use (1= current use; 0=no current use), number of hours uniform worn, number of wash times of uniform (1= ≤25 times; 0= >25 times) worn the day prior to urine collection, and interaction term (%BF*Washings)

3-PBA=3-phenoxybenzoic acid (3-PBA); ∑DCCA = sum of *cis*- and *trans*-3-(2,2-dichlorovinyl)-2,2-dimethyl-cyclopropane-1-carboxylic acid