Metabolic Activity in the Insular Cortex and Hypothalamus Predicts Hot Flashes: An FDG-PET Study

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Context: Hot flashes are a common side effect of adjuvant endocrine therapies (AET; leuprolide, tamoxifen, aromatase inhibitors) that reduce quality of life and treatment adherence in breast cancer patients. Because hot flashes affect only some women, preexisting neurobiological traits might predispose to their development. Previous studies have implicated the insula during the perception of hot flashes and the hypothalamus in thermoregulatory dysfunction.

Objective: The aim of the study was to understand whether neurobiological factors predict hot flashes.

Design: [18F]-Fluorodeoxyglucose (FDG) positron emission tomography (PET) brain scans coregistered with structural magnetic resonance imaging were used to determine whether metabolic activity in the insula and hypothalamic thermoregulatory and estrogen-feedback regions measured before and in response to AET predict hot flashes. Findings were correlated with *CYP2D6* genotype because of *CYP2D6* polymorphism associations with tamoxifen-induced hot flashes.

Outcome Measures: We measured regional cerebral metabolic rate of glucose uptake (rCMRglu) in the insula and hypothalamus on FDG-PET.

Results: Of 18 women without hot flashes who began AET, new-onset hot flashes were reported by 10 (55.6%) and were detected objectively in nine (50%) participants. Prior to the use of all AET, rCMRglu in the insula ($P \leq 0.01$) and hypothalamic thermoregulatory ($P = 0.045$) and estrogen-feedback ($P = 0.007$) regions was lower in women who reported developing hot flashes. In response to AET, rCMRglu was further reduced in the insula in women developing hot flashes ($P \leq 0.02$). Insular and hypothalamic rCMRglu levels were lower in intermediate than extensive *CYP2D6* metabolizers.

Conclusions: Trait neurobiological characteristics predict hot flashes. Genetic variability in *CYP2D6* may underlie the neurobiological predisposition to hot flashes induced by AET. (*J Clin Endocrinol Metab* **97:** 3207–3215, 2012)

Abbreviations: AET, Adjuvant endocrine therapy; AI, aromatase inhibitor; EM, extensive metabolizer; FDG, [18F]-fluorodeoxyglucose; IM, intermediate metabolizer; MRI, magnetic resonance imaging; PET, positron emission tomography; PM, poor metabolizer; rCMRglu, regional cerebral metabolic rate of glucose uptake; ROI, region of interest.

Hot flashes are the most common side effect of adjuvant endocrine therapies (AET) used to treat breast cancer. Hot flashes develop rapidly in half of those treated with AET (1, 2), impairing quality of life and reducing treatment adherence (1, 3). The AET that are used widely clinically are tamoxifen, aromatase inhibitors (AI), and GnRH agonists, all of which result in estrogen deprivation at a cellular level due to decreased estrogen synthesis or blockade of its action. Identifying preexisting traits that predict the development of hot flashes on AET will inform strategies for early symptom management in breast cancer patients.

Thermoregulatory (4, 5) and brain activity changes during individual hot flash episodes (6) observed in women with hot flashes suggest that hot flashes are under central nervous system influence. Interindividual variability in the response to AET suggests that neurobiological traits preceding the onset of hot flashes may predispose to their development. Two brain regions have been linked to hot flashes. The hypothalamus is a region responsible for thermoregulation (7, 8) and estrogen feedback to GnRH neurons (9). The insula is central to perception of bodily sensations (*e.g.* pain, sweating, temperature sensation) (10, 11) and has been shown to activate transiently during the *perception* of hot flash episodes (6). Importantly, alterations in brain regions activated during symptomatic episodes have also been found during the asymptomatic state in other conditions such as anxiety disorders, suggesting that similar relationships may pertain for hot flashes (12).

The cytochrome P450 enzyme CYP2D6 metabolizes tamoxifen to its active metabolites in the liver. It has been suggested that *CYP2D6* polymorphisms influence the occurrence of hot flashes on tamoxifen (13, 14), but the association has not been studied in other AET. The CYP2D6 enzyme is active in the brain in areas presumed to be important in hot flashes (15) and biotransforms serotonin and other neurotransmitters implicated in hot flashes (16, 17). These observations raise the possibility that the association of *CYP2D6* genotype with hot flashes may extend beyond tamoxifen to other AET through CYP2D6 activity in the brain.

Our goal was to determine whether basal levels of metabolic activity in the insula and hypothalamus distinguish women susceptible to developing hot flashes on AET, as measured subjectively and objectively. We hypothesized that differences in metabolic activity in these regions are biomarkers for hot flash risk and are associated with reduced CYP2D6 enzymatic activity.

Subjects and Methods

Subjects

Of 25 women who consented to be screened, 18 women were eligible for the study. The remaining seven women were excluded because they had hot flashes before starting AET. Participants included pre- and postmenopausal women without hot flashes who were scheduled to start taking an AET in a breast cancer clinic (n = 12) or in a healthy volunteer protocol involving leuprolide administration (n = 6). Enrollment was open to all consecutive women identified through systematic screening of breast cancer patients who were starting AET and willing and eligible to participate in the study, as well as all healthy volunteers initiating leuprolide during the same time period. Study-eligible participants were premenopausal women starting treatment with the GnRH agonist leuprolide 3.75 mg depot (n = 7) and postmenopausal women starting tamoxifen 20 mg/d (n = 6) or an AI (n = 5; letrozole 2.5 mg/d, anastrozole 1 mg/d). Participants were not pregnant, lactating, or using centrally active medications that alter hot flashes (*e.g.* hormones, antidepressants). The absence of hot flashes was confirmed subjectively using a hot flash diary and objectively with a sternal skin-conductance monitor (Biolog; UFI, Morro Bay, CA). The ambulatory skin-conductance monitor has been validated as a physiological measure of hot flashes (18, 19). We used conventional criteria to establish the presence of each hot flash (increase of 2 μmho or greater within 30 sec).

Women with clinical depression were excluded using standardized psychiatric assessments [Patient Health Questionnaire (20) and a Beck Depression Inventory score $\geq$15 (21)]. Subjects provided written informed consent. Study procedures were approved by Partners Health Care and Dana-Farber/Harvard Cancer Center Institutional Review Boards.

Procedures

Before AET, participants completed resting-state [18F]-fluorodeoxyglucose (FDG) positron emission tomography (PET) brain scans according to routine FDG-PET procedures. Glucose uptake measured with FDG-PET is a marker of neuronal metabolic activity. The level of activity measured at rest at the time of the FDG injection in each anatomically defined brain region is quantified by the regional cerebral metabolic rate of glucose uptake (rCMRglu). Each subject completed a structural brain magnetic resonance imaging (MRI) scan to coregister hypothalamic structures.

Hot flashes have been measured by both subjective and objective methods using validated methodologies. Although objective hot flashes have been proposed as the "gold standard" for studies examining hot flash mechanisms (18), objective monitors are known to measure events that are perceived as hot flashes as well as other physiological events that are not perceived as hot flashes (22–24). Because perceived symptoms are more likely to influence a woman's willingness to continue taking AET, we first examined our data according to subjective hot flash status and then by objective hot flash status. Subjective hot flashes were assessed using a hot flash diary that was completed daily for 4 wk and a brief interview after 8 wk on therapy. During the fourth treatment week, objective hot flashes were assessed with a skin-conductance monitor worn for a 24-h period. Subjects were classified as having subjective hot flashes if they perceived hot flashes at any point during the follow-up period and as having objective

hot flashes if at least one hot flash was measured on the monitor during the 24-h period of observation.

A second FDG-PET scan was completed after changes in reproductive hormones for each AET were expected to have already occurred. Specifically, follow-up scans were obtained after 2–3 wk on AET to determine whether changes in metabolic activity in the insula and hypothalamus occurring in response to AET between the pretreatment and posttreatment scans differed between women who did or did not develop hot flashes. Blood was drawn for estradiol, estrone, FSH, and LH measurement before AET and two to three additional times on AET. Estradiol and estrone were measured using liquid chromatography-tandem mass spectrometry (Mayo Clinic, Rochester, NY) (25) to achieve accuracy and precision in this low range (interassay coefficients of variation, 8.6 and 8.7%, respectively) (25).

FDG-PET scans

After fasting for 6 h or more, subjects received 5 mCi FDG in a dimly lit room with their eyes closed. Subjects were positioned supine in a Siemens HR+, 32-ring, 63-slice body tomograph (Siemens Medical Solutions USA, Inc., Malvern, PA) 45 min later so that transverse section slices were parallel to the canthomeatal line. A single 20-min emission measurement was acquired. Head movement was minimized, and projection data were corrected as previously described (9).

MRI scans

T1-Weighted three-dimensional structural scans were acquired on a General Electric 1.5-T Signa scanner (General Electric Healthcare, Hatfield, United Kingdom), using a contiguous axial three-dimensional T1-weighted spoiled gradient echo pulse sequence (repetition time, 30 msec; echo time, 9 msec; flip angle, 25°; band width, 15.63; number of scan locs, 124; field of view, 22; matrix, 256 × 192).

Image reconstruction

For insula analyses, PET images were corrected for head movement and spatially normalized to the Montreal Neurological Institute brain template using Statistical Parametric Mapping (SPM2) software for neuroimaging data (Welcome Cognitive Neurology Department, London, UK) and smoothed with a three-dimensional Gaussian filter of 12-mm full width half maximum. For the hypothalamus analysis, PET images were movement-corrected and coregistered with structural MRI but not smoothed due to the small structure being examined. rCMRglu from regions of interest (ROI) in both the insula and hypothalamus were extracted using the MARSeille Boîte À Région d'Intérêt (MARSBAR) tool for SPM. The Wake Forest University PickAtlas was used to mask the insula to restrict the analysis to this ROI.

Morphometric analysis of hypothalamus

FDG-PET rCMRglu was coregistered with hypothalamic subregions on structural MRI to investigate thermoregulatory (anterior preoptic and superior tuberal) and GnRH (inferior tuberal or medial basal) areas. The hypothalamus was functionally segmented for each participant based on her MRI anatomic landmarks. The hypothalamus was subdivided into three ROI bordered laterally by the internal capsule. The anterior preoptic ROI extended from the anterior-most tip of the anterior commissure to the anterior-most tip of the infundibulum. The superior and inferior tuberal ROI extended from the anterior-most section containing the infundibulum to the coronal section anterior to the mammillary body. The border between the superior and inferior tuberal ROI was set at the superior-most level of the floor of the basal forebrain.

Genotyping

CYP2D6 genotyping was performed using allelic discrimination Taqman assays (Applied Biosystems, Inc., Foster City, CA): *4 (C_27102431_D0, rs3892097), *10 (C_11484460_40; rs1065852), *6 (C_32407243_20; rs5030655), *3 (C_32407232_50; rs35742686), *41 (C_34816116_20; rs28371725), *2 (C_27102414_10; rs1135840). Participants were defined as extensive metabolizers (EM; two fully functional or one full and one reduced function allele), intermediate metabolizers (IM; one fully functional and one null or two reduced function alleles), or poor metabolizers (PM; two nonfunctional alleles).

Statistical analysis

rCMRglu was extracted from each ROI. Because subjective and objective hot flash measurements were discordant in some women, analyses were conducted separately by subjective and objective hot flash status.

For the insula, SPM2 was used to conduct an ANOVA with hot flash status as the group characteristic. Extracted voxels were selected using an uncorrected $P < 0.05$ threshold because this was a ROI analysis restricted to the insula. Nonparametric Wilcoxon rank-sum tests were used to determine whether the extracted values of rCMRglu in the insula before initiation of AET differed between those developing and not developing hot flashes. The same approach was used to study the secondary endpoints of the change in rCMRglu in the insula in response to AET and in the hypothalamic subregions both before and in response to AET. For each of the hypothalamic ROI, rCMRglu from the left and right were averaged because there were no lateral differences in rCMRglu. Connectivity analyses were conducted using Spearman's correlation coefficients.

Fisher's exact tests were used to determine the association between hot flashes and CYP2D6 metabolizer status (IM vs. EM), and Wilcoxon rank-sum tests were used to determine the association between CYP2D6 metabolizer status and rCMRglu levels.

Logistic regression models were built to examine potential confounding of the association of extracted values in each ROI with hot flash status by menopause status and the type of AET used. Analyses were conducted using STATA software (Stata-Corp, College Station, TX). Statistical significance was established using two-sided $\alpha = 0.05$, and statistical trend was defined as $0.05 \geq P < 0.10$.

Results

Subjects

Ten women (55.6%) had subjective hot flashes, and nine (50%) had objective hot flashes. Concordance between subjective and objective measurements was 61%, with six subjects developing hot flashes that were both reported subjectively and measured objectively (Table 1). Among those developing hot flashes, symptoms began before the posttreatment FDG-PET scan, but none were re-

TABLE 1. Study population characteristics

		Subjective classification		Objective classification	
	All	**Hot flashes reported**	**No hot flashes reported**	**Hot flashes detected**	**No hot flashes detected**
n	18	10	8	9	9
Objective hot flashes, n (%)	9 (50)	6 (66.7)	3 (33.3)	N/A	N/A
Subjective hot flashes, n (%)	10 (55.6)	N/A	N/A	6 (60.0)	4 (40.0)
Age (yr)	46.9 ± 13.3	44.1 ± 11.7	50.4 ± 15.0	42.6 ± 13.0	51.2 ± 12.7
BMI (kg/m^2)[a]	26.7 ± 4.4	24.3 ± 3.0	29.8 ± 4.8	27.2 ± 5.8	26.2 ± 3.6
Non-Hispanic Caucasian, n (%)	15 (83.3)	9 (60.0)	6 (40.0)	7 (46.7)	8 (53.3)
Breast cancer history, n (%)	12 (66.7)	6 (60.0)	6 (75.0)	4 (44.4)	8 (88.9)
Menopause status, n (%)[b]					
Premenopausal	7 (38.9)	5 (71.4)	2 (28.6)	6 (85.7)	1 (14.3)
Postmenopausal	11 (61.1)	5 (45.5)	6 (54.5)	3 (27.3)	8 (72.7)
Antiestrogen therapy used, n (%)[c]					
Tamoxifen	6 (33.3)	3 (50.0)	3 (50.0)	2 (33.3)	4 (66.7)
AI	5 (27.8)	2 (40.0)	3 (60.0)	1 (20.0)	4 (80.0)
Leuprolide	7 (38.9)	5 (71.4)	2 (28.6)	6 (85.7)	1 (14.3)
Prior chemotherapy, n (%)					
Yes	7 (38.9)	4 (57.1)	3 (42.9)	3 (42.9)	4 (57.1)
No	11 (61.1)	6 (54.5)	5 (45.5)	6 (54.5)	5 (45.5)
Depressive symptoms on BDI	3.9 ± 3.7	3.0 ± 2.7	5.0 ± 4.6	2.2 ± 2.5	5.6 ± 4.0

Normally distributed data are presented as mean ± SD, and categorical data are presented as number (% of row total). BMI, Body mass index; BDI, Beck Depression Inventory. N/A, Not applicable.

[a] $P = 0.008$, group differences using subjective hot flash classification.

[b] $P = 0.05$; [c] $P = 0.08$, group differences using objective hot flash classification.

ported or detected during FDG-PET scans. Changes in estradiol, estrone, FSH, and LH did not differ between hot flash groups. All participants on leuprolide (n = 7) had estradiol below 10 pg/ml (36.71 pmol/liter) with a gonadotropin flare that resolved before the posttreatment FDG-

PET scan. All of those on AI (n = 5) had estradiol below 10 pg/ml (36.71 pmol/liter) and FSH above 70 mIU/ml (70 IU/liter). As evidence of successful estrogen blockade, FSH levels were above 40 mIU/ml (70 IU/liter) in all women on tamoxifen (n = 6).

TABLE 2. Insula cortex coordinates and rCMRglu levels for FDG-PET studies conducted before initiation of and in response to endocrine therapy

		Coordinates[a]						FDG-PET rCMRglu levels[b]		
Hot flash classification	**Region**	**K$_E$**	**MNI-coordinates[c]**			**Z-score**	**P value**	**No hot flashes (median, IQR)**	**Hot flashes (median, IQR)**	**P value**
			x	**y**	**z**					
Prior to initiation of endocrine therapy										
Subjective	1	773	30	10	−16	3.40	<0.001	79.0 (75.8 to 80.1)	72.4 (70.2 to 74.2)	0.004
	2	109	−24	20	−14	2.49	0.006	80.9 (76.4 to 87.0)	74.6 (70.2 to 78.5)	0.02
	3	180	−40	−18	−4	2.47	0.007	81.5 (78.6 to 82.8)	76.7 (67.4 to 78.7)	0.01
Objective	1	48	−36	−14	22	2.32	0.01	54.6 (53.7 to 55.8)	52.0 (50.4 to 52.5)	0.02
In response to endocrine therapy										
Subjective	1	26	30	−18	18	2.20	0.014	1.1 (−0.1 to 2.2)	− 1.5 (− 2.2 to − 0.7)	0.03
Objective	1	352	−32	10	−14	3.04	0.001	1.0 (0 to 3.4)	− 2.6 (− 5.9 to − 1.2)	0.003
	2	63	−30	12	14	2.05	0.020	2.2 (−1.3 to 2.4)	−3.1 (−3.9 to 0.6)	0.047

Non-normal data are presented as median (interquartile range). IQR, Interquartile range; MNI, Montreal Neurological Institute.

[a] Coordinates for insula regions with significant differences in rCMRglu.

[b] rCMRglu levels for insula regions of maximum significant differences using subjective (diary) and objective (skin-conductance monitor) hot flash classification.

[c] x indicates right (+) or left (−); y indicates anterior (+) or posterior (−); z indicates superior (+) or inferior (−) to the anterior commissure; by convention, the (+) direction is indicated by the absence of a (−); K$_E$ = number of voxels in a cluster; $P < 0.05$ statistical threshold.

Subject characteristics by hot flash group status are listed in Table 1. There were no differences in menopause status or type of AET used when hot flashes were categorized subjectively, but there was a statistical trend for women with objective hot flashes to be premenopausal ($P = 0.05$) and to be treated with leuprolide ($P = 0.08$). Body mass index was lower in those with than those without subjective hot flashes ($P = 0.008$), but it was not different between objective hot flash groups. The hot flash groups did not differ in age, race, breast cancer history, prior exposure to chemotherapy, or level of depressive symptoms.

Insula

Before AET, rCMRglu in the insula differed between those developing and not developing hot flashes (Table 2). SPM analysis (Table 2 and Fig. 1A) revealed bilateral differences in activity levels in the anterior and posterior insula between subjective hot flash groups ($P \leq 0.007$) and in the left posterior insula between objective hot flash groups ($P = 0.01$), with lower rCMRglu levels observed in both subjective ($P \leq 0.02$) and objective ($P = 0.02$) hot flash groups (Table 2 and Fig. 1B). Within-woman differences in rCMRglu between the FDG-PET scans obtained before and on AET were calculated to indicate changes in metabolic activity in the insula in response to AET. rCMRglu did not change in response to AET for the group as a whole. However, women developing hot flashes had greater decreases in rCMRglu than those without hot flashes (Table 2). Furthermore, changes in metabolic activity differed between subjective hot flash groups in the right posterior insula ($P = 0.01$; Table 2 and Fig. 2A) and between objective hot flash groups in the left anterior insula ($P \leq 0.02$). rCMRglu levels in regions of peak between-group differences declined after initiation of AET among those developing hot flashes (Table 2), whereas activity levels increased in those without subjective ($P = 0.03$) or objective ($P \leq 0.047$; Fig. 2B) hot flashes.
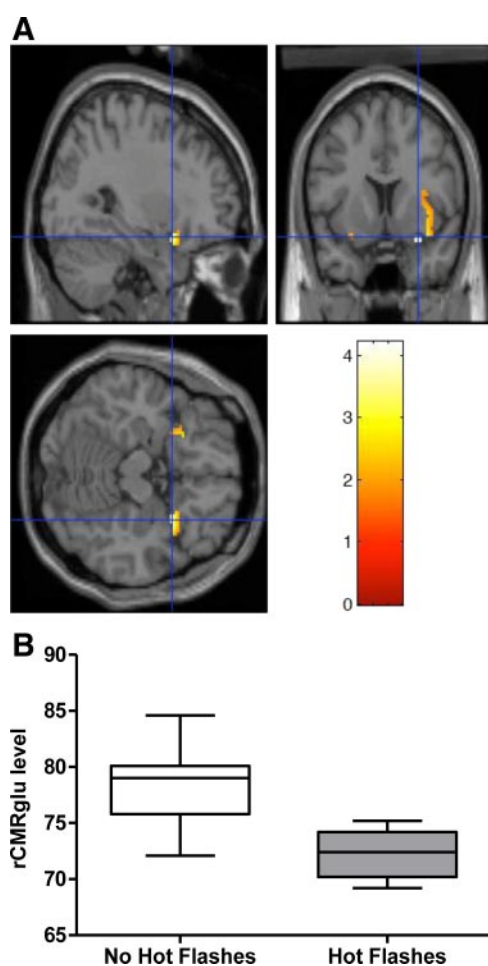

FIG. 1. Difference in rCMRglu levels in the insula before initiation of AET between the groups of women who did and did not develop subjective hot flashes. A, Region of peak difference in rCMRglu in the insula before AET between subjective hot flash groups ($x = 30$, $y = 10$, $z = -16$; $P < 0.001$). B, Box plots (median, interquartile range, and extreme values) for rCMRglu levels from corresponding region of peak difference between subjective hot flash groups before AET ($P = 0.004$).
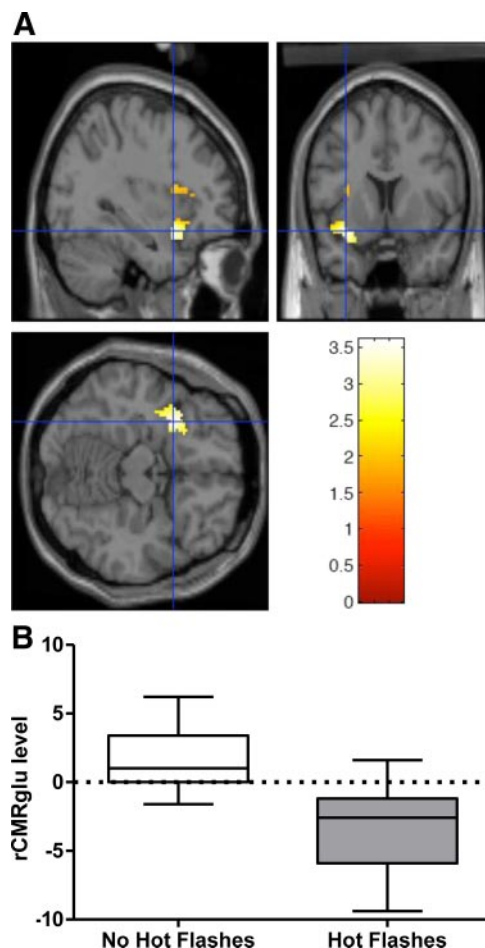
FIG. 2. Difference in rCMRglu levels in the insula in response to initiation of AET between the groups of women who did and did not develop objective hot flashes. A, Region of peak difference in rCMRglu in the insula in response to AET between objective hot flash groups ($x = -32$, $y = 10$, $z = -14$; $P = 0.001$). B, Box plots (median, interquartile range, and extreme values) for rCMRglu levels from corresponding region of peak difference between objective hot flash groups in response to AET ($P = 0.003$).

Hypothalamus

Hypothalamic data were analyzed in 15 women, nine (60%) of whom developed hot flashes, after excluding one women on leuprolide who did not complete the MRI and two on tamoxifen because of MRI technical problems. Women with subjective hot flashes had lower rCMRglu before AET in the anterior preoptic thermoregulatory subregion ($P = 0.045$; Fig. 3A) and the GnRH subregion (inferior tuberal, $P = 0.007$; Fig. 3B), but not the superior tuberal thermoregulatory subregion ($P = 0.08$; Fig. 3C). Changes in rCMRglu in response to AET did not differ between groups. Interestingly, analyses using objective hot flashes revealed no between-group differences in rCMRglu either before treatment or in response to AET.
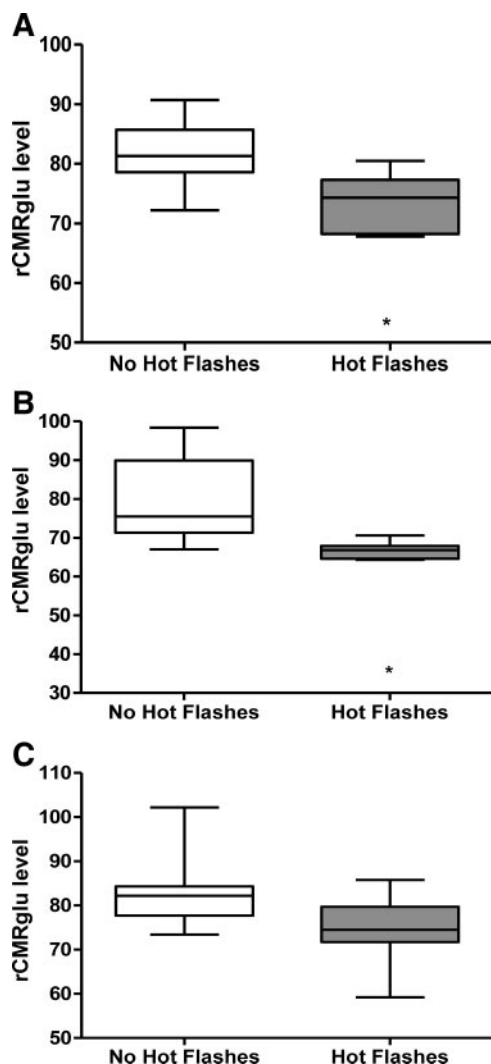
FIG. 3. Box plots (median, interquartile range, and extreme values) showing rCMRglu levels before initiation of endocrine therapies in functional hypothalamic regions in women who did and did not report developing subjective hot flashes. A, Anterior preoptic thermoregulatory region ($P = 0.045$); B, inferior tuberal (medial basal) GnRH region ($P = 0.007$); and C, superior tuberal thermoregulatory region ($P = 0.08$). *, Outlier.

Adjustment for covariates

Adjusting for menopause status and type of AET used did not alter the association of pretreatment rCMRglu levels in the insula with subjective ($P < 0.04$) or objective hot flash status ($P < 0.04$), but the associations were no longer significant for the secondary endpoints of changes in rCMRglu levels in response to AET and in the functional subregions of the hypothalamus.

Connectivity analysis

Pretreatment rCMRglu levels in the hypothalamus correlated with pretreatment rCMRglu levels in the insula for women reporting hot flashes (anterior preoptic $r_s = 0.67-0.72$, $P \leq 0.049$; inferior tuberal $r_s = 0.70-0.82$, $P \leq 0.04$), but not for those without hot flashes. Using objective hot flashes, there were no significant correlations for either hot flash group.

Pretreatment rCMRglu levels were correlated between GnRH and the anterior preoptic thermoregulatory region in the hypothalamus for those developing subjective ($r_s = 0.66$; statistical trend $P = 0.08$) and objective ($r_s = 0.86$; $P = 0.01$) hot flashes, but not for those without.

CYP2D6 genotyping studies

Six (35.3%) women were IM and 11 (64.7%) were EM; none were PM (Table 3). Tamoxifen users comprised 50% of IM and 18% of EM, but *CYP2D6* metabolizer status did not differ between type of AET used ($P = 0.55$). Before AET, IM had lower rCMRglu levels in the insula ($P = 0.03$) relative to EM. Although the overwhelming majority (83.3%) of IM developed subjective hot flashes, the association between *CYP2D6* metabolizer status and AET-induced hot flashes did not reach statistical significance.

Discussion

Results of this functional neuroimaging study indicate a neurobiological predisposition to developing hot flashes on endocrine therapies. Our findings suggest that metabolic activity in the insula and hypothalamus is a biomarker for susceptibility to hot flashes, representing a preexisting trait that precedes and is independent of the perception of hot flashes. Women developing hot flashes had lower rCMRglu before AET in the insula as well as in thermoregulatory and GnRH subregions of the hypothalamus. In response to AET, women reporting hot flashes also had a further decrease in rCMRglu levels in the insula, but not the hypothalamus. Pretreatment rCMRglu levels in the insula and hypothalamus were correlated. Finally, intermediate, relative to extensive, *CYP2D6* metabolizers had lower pretreatment rCMRglu levels, suggesting that genetic variability in CYP2D6

TABLE 3.　Association of cytochrome P450 2D6 metabolizer status with rCMRglu levels before AET and with subjective (diary) and objective (skin-conductance monitor) hot flash group status

| Metabolizer status | rCMRglu level before endocrine therapy | | All (n = 17) | Subjective hot flash classification | | Objective hot flash classification | |
	Insula	Anterior preoptic hypothalamus		Hot flashes reported (n = 10)	No hot flashes reported (n = 7)[a]	Hot flashes detected (n = 9)	No hot flashes detected (n = 8)[a]
PM			0				
IM (n = 6)	71.1 (69.3–74.0)[b]	68.2 (65.3–78.6)	6 (35.3)	5 (83.3%)	1 (16.7%)	3 (50.0%)	3 (50.0%)
EM (n = 11)	75.2 (74.2–79.1)	78.7 (74.3–82.6)	11 (64.7)	5 (45.5%)	6 (54.5%)	6 (54.5%)	5 (55.6%)

Continuous data are non-normal and are presented as median (interquartile range). Categorical data are presented as number (% of row total).

[a] Data not available for one participant on tamoxifen.

[b] $P = 0.03$ for rCMRglu levels before initiation of antiestrogen therapy in IM vs. EM.

enzyme activity may underlie the predisposition to hot flashes among women taking tamoxifen as well as other AET.

The role of the insula in interpreting bodily sensations is divided between the posterior insula, which is primarily responsible for the perception of bodily sensations, and the anterior insula, which integrates emotional awareness of and response to bodily sensations (26–28). We observed resting-state pretreatment differences in rCMRglu in both the anterior and posterior insula, whereas others (6) have found that only the anterior insula is activated on functional MRI during the perception of hot flashes. These results suggest that susceptibility to hot flashes is predicted by decreased metabolic activity in the parts of the insula responsible for both perception and emotional response, whereas the dynamic response of the anterior insula during the experience of hot flashes reflects emotional integration of the bodily sensation.

Insula activity differs between depressed (29) and anxious (30) patients and healthy controls. Because depression and anxiety can precede and increase the risk for subsequent development of hot flashes during the menopause transition and in breast cancer patients (31–33), we hypothesize that lower rCMRglu underlies a propensity to both psychological symptoms and heightened awareness of bodily sensations, such as hot flashes. We are unable to test this hypothesis in the current study because, by design, our study was restricted to women who did not have depression, and anxiety symptom levels were not measured.

Our results link resting-state differences in hypothalamic activity to the perception of subjective hot flashes in humans, as previously hypothesized (34). These findings are consistent with animal studies showing that nuclei in anterior preoptic and midhypothalamic regions control thermoregulation (8, 35, 36) and with studies showing alterations in peripheral markers of thermoregulation in women with hot flashes (37). Because prior functional MRI studies report no activation of the hypothalamus during hot flashes (6), hypothalamic activity may predis-

pose to hot flashes but may not be perturbed during the hot flash experience. Results of connectivity analysis between hypothalamic functional subregions and between the insula and hypothalamus raise the possibility that these regions are involved in the generation of hot flashes independently or through a shared neural circuit.

The link between metabolic activity levels in the insula before AET and the development of hot flashes on AET is not explained by menopause status or by the type of AET that was used. Menopause status and type of AET confounded the association of AET-induced hot flashes with changes in metabolic activity levels in the insula in response to AET and in the hypothalamus before AET was started. Subgroup analyses for pre- and postmenopausal women and for each specific AET used are not feasible due to sample size limitation. Further investigation in larger samples is warranted to examine the mechanism through which menopause status and the specific AET used may explain these associations.

We observed important associations between resting-state metabolic activity, CYP2D6 metabolizer status, and hot flashes. IM had lower metabolic activity than EM, which in turn predicts hot flashes. Our findings are consistent with tamoxifen studies reporting more hot flashes in IM than EM (14). Comparisons with studies reporting fewer hot flashes in PM cannot be made because we had no PM in our study (13). Because PM comprise a small proportion (5–15%) of the overall population (38), studies enrolling a much larger number of women would be required to obtain a large enough sample of PM to address questions applicable to this maximally reduced CYP2D6 metabolizer group. In addition to CYP2D6 metabolizer status, the occurrence of hot flashes on tamoxifen may relate to levels of the tamoxifen metabolite endoxifen and the functionality of the P-glycoprotein membrane transporter, which controls the amount of endoxifen that penetrates across the blood-brain barrier into the brain (39), neither of which was measured in this study.

Our results suggest that CYP2D6 enzyme activity may play a role in hot flashes on tamoxifen as well as AI and leuprolide. Although *CYP2D6* associations with hot flashes have been hypothesized to occur secondary to hepatic metabolism of tamoxifen to endoxifen (13, 14), CYP2D6 enzyme activity in the liver and brain has not been differentiated, nor have *CYP2D6* polymorphisms previously been examined in women on AI or leuprolide. Alterations in CYP2D6 activity in serotonin-rich brain regions (40) suggest that the CYP2D6 enzyme may play a role in serotonin metabolism and that serotonergic tone may be decreased when CYP2D6 function is reduced. The link between *CYP2D6* polymorphisms and susceptibility to hot flashes may therefore involve reduced serotonergic tone, consistent with evidence that selective serotonin reuptake inhibitors alleviate hot flashes (41).

Our findings in the insula by subjective hot flash status are consistent with our findings for the objective hot flash groups. In the hypothalamus, we found differences in metabolic activity before AET in the anterior preoptic thermoregulatory and inferior tuberal GnRH subregions when data were analyzed using subjective hot flash classifications but not using objective hot flash classifications. Any explanation for this discrepancy is speculative.

Analyses were conducted in parallel using subjective and objective hot flash groupings because of discordance between hot flash classifications, which is expected in ambulatory populations (22–24). Hot flashes are more likely to be measured objectively and not subjectively if they are mild or if a woman is distracted by other activities at the time of the flash, whereas hot flashes are more likely to be reported subjectively and not measured objectively if a woman misattributes sweating to a hot flash or if she has negative mood symptoms preceding a hot flash (23, 24). Additional analyses focused specifically on women whose hot flashes were both reported subjectively and measured objectively or who did not have subjectively or objectively measured hot flashes are not feasible because of the small number of women in such subgroups.

Because we studied hot flashes induced by AET, the generalizability of our results to menopause-related hot flashes is unknown. Other limitations relate to inclusion of women taking different types of AET. Although the mechanism through which each AET blocks estradiol synthesis or action differs, women using different types of AET were included because we hypothesized a common pathway through which estrogen-deprivation therapies result in hot flashes. Because of the limited number of women taking each specific AET, we are unable to conduct analyses within each AET subgroup. However, our analyses confirmed that the prediction of hot flashes by pretreatment rCMRglu levels was not restricted to a specific AET.

In conclusion, our results show that specific neurobiological characteristics confer susceptibility to hot flashes and that genetic variability in *CYP2D6* may underlie this predisposition. Hot flashes are an important adverse consequence of AET. Understanding the neural basis of hot flashes will help to identify pretreatment clinical characteristics that correlate with neurobiological biomarkers that are associated with the development of hot flashes on AET. Such approaches will permit the development of early-intervention strategies for hot flashes, with the goal of limiting their adverse impact on quality of life and improving adherence to endocrine therapies.

Acknowledgments

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