Cancer-related fatigue during combined treatment of androgen deprivation therapy and radiotherapy is associated with mitochondrial dysfunction

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Received September 30, 2019; Accepted December 6, 2019

DOI: 10.3892/ijmm.2019.4435

Abstract. Combined androgen deprivation therapy (ADT) and radiation therapy (RT) is the standard of care treatment for non-metastatic prostate cancer (NMPC). Despite the efficacy, treatment-related symptoms including fatigue greatly reduce the quality of life of cancer patients. The goal of the study is to examine the influence of combined ADT/RT on fatigue and understand its underlying mechanisms. A total of 64 participants with NMPC were enrolled. Fatigue was assessed using the Functional Assessment of Cancer Therapy-Fatigue. Mitochondrial function parameters were measured as oxygen consumption from peripheral blood mononuclear cells (PBMCs) extracted from participants' whole blood. An ADT/RT-induced fatigue mouse model was developed, with fatigue measured as a reduction in voluntary wheel-running activity (VWRA) in 54 mice. Mitochondrial function was assessed in the ADT/RT mouse brains using western blot analysis of glucose transporter 4 (GLUT4) and transcription factor A, mitochondrial (TFAM). The results demonstrated that fatigue in the ADT group was exacerbated during RT compared with the non-ADT group. This effect was specific to fatigue, as depressive symptoms were unaffected. PBMCs of fatigued subjects exhibited decreased ATP coupling efficiency compared to non-fatigued subjects, indicative of mitochondrial dysfunction. The ADT/RT mice demonstrated the synergistic effect of ADT and RT in decreasing VWRA. Brain tissues of ADT/RT mice exhibited decreased levels of GLUT4 and TFAM suggesting that impaired neuronal metabolic homeostasis may contribute to fatigue pathogenesis. In conclusion, these findings suggest that fatigue induced by ADT/RT may be attributable to mitochondrial dysfunction both peripherally and in the central nervous system (CNS). The synergistic effect of ADT/RT is behaviorally reproducible in a mouse model and its mechanism may be related to bioenergetics in the CNS.

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Abbreviations: ADT, androgen deprivation therapy; RT, radiation therapy; NMPC, non-metastatic prostate cancer; PBMC, peripheral blood mononuclear cells; VWRA, voluntary wheel-running activity; GLUT4, glucose transporter 4; TFAM, transcription factor A mitochondrial; CNS, central nervous system; EBRT, external beam radiation therapy; Gy, Gray; GnRH, gonadotropin-releasing hormone agonist; FACT-F, Functional Assessment of Cancer Therapy-Fatigue; HAM-D, Hamilton Depression Rating Scale; CTL, control; Irrad, irradiated; OCR, oxygen consumption rate; ANOVA, analysis of variance

Key words: cancer-related fatigue, radiation therapy, prostate cancer, androgen deprivation therapy, mitochondria

Introduction

Androgen-deprivation therapy (ADT) in combination with radiation therapy (RT) is the standard of care for locally advanced prostate cancer (1). ADT, or chemical castration, is a highly effective therapeutic option for patients with prostate cancer and has been shown to prolong survival (2,3). The beneficial effect of ADT relates to the dependence of prostate tumor cell growth on androgen receptor signaling. Upon binding to testosterone, androgen receptors homodimerize, leading to phosphorylation, subsequent nuclear translocation and binding to the androgen response elements, which eventually results in transcription of target genes that facilitate tumor cell growth, thus extending survival (2). RT uses ionizing radiation to induce double-stranded DNA breaks in cancer cells and is well-established as an effective treatment for localized prostate cancer (4). Patients treated with RT were less likely to further develop metastases and exhibited a decreased rate of disease progression compared to an active surveillance (control) group (5).

A number of large, randomized clinical trials in previous years have demonstrated improved long-term survival using combined ADT and RT compared to RT or ADT alone (6-11).

The synergistic effect of ADT and RT is likely related to the role of ADT as a 'radiosensitizer' by downregulating DNA repair genes, thus accelerating DNA damage induced by RT (12-14). Alternatively, an enhanced immune response has been suggested as another possible mechanism of the synergistic effect of ADT and RT (15,16).

Despite these benefits, treatment with ADT can result in a multitude of iatrogenic conditions including increased risk for diabetes, cardiovascular diseases, sexual health dysfunction, depression, cognitive and mood dysfunction (11,17). These treatment-related conditions can greatly reduce health-related quality of life (18). One of the most common and burdensome symptoms experienced during RT with concomitant ADT is fatigue (19,20). A total of ~40% of men experience clinically-significant fatigue while taking ADT and 60% experience clinically-significant fatigue during RT for non-metastatic prostate cancer (19).

Various mechanisms have been hypothesized for the etiology of treatment-related fatigue; however, the exact underlying mechanism especially related to combined therapies remains unknown (20,21). Previous studies suggest a possible role of mitochondrial dysfunction in cancer-related fatigue (22-24). Interestingly, oxidative stress induced by RT as well as androgen deprivation can separately influence mitochondrial function (25). Furthermore, anemia induced by combined treatment of ADT and RT can lead to cerebral hypoxia and neuronal mitochondrial dysfunction (26,27).

Given the prominent use of combined ADT and RT for localized prostate cancer, there is an increasing need to better understand adverse effects of the combined treatment. The goal of the current study is to explore the influence of ADT on fatigue progression and mitochondrial function during RT for localized prostate cancer. To examine mitochondrial function in human blood samples, a method previously published by the present group was used (28). The effect of the ADT and RT on brain mitochondria was examined using a previously developed mouse model of radiation-induced fatigue (29).

Materials and methods

Participants. The present study (NCT00852111) was approved by the Institutional Review Board of the National Institutes of Health (NIH). All participants enrolled in this study were male, ≥18 years of age, diagnosed with non-metastatic prostate cancer with or without prior prostatectomy and scheduled to receive external beam radiation therapy (EBRT). Patient characteristics are presented in Table I. Participants were excluded if they had known progressive diseases causing significant fatigue including mitochondrial diseases, psychiatric disease within the past five years, uncorrected hypothyroidism or anemia, or a second malignancy. Individuals who used sedatives, steroids, or non-steroidal anti-inflammatory agents were also excluded. All participants recruited in the study completed EBRT which lasted 38-44 days with a total dosage of 68.4-75.6 Gray (Gy), depending on the clinical stage of the disease. The majority of the enrolled participants received neoadjuvant ADT 76 days before EBRT and continued to receive ADT during and after EBRT. Participants receiving ADT were on a daily dose of 50 mg Bicalutamide, an androgen receptor antagonist, prior to receiving a gonadotropin-releasing hormone agonist (GnRH) (30). Participants often receive an injection of 22.5 mg leuprolide acetate, the GnRH, two weeks after receiving the androgen receptor antagonist. They continue to receive the leuprolide acetate every three months. This combination stops the release of hormones and prevents any residual hormones from functioning. The Bicalutamide continued throughout RT and often stopped on the last day of radiation. Participants remained on the leuprolide acetate up to 2-3 years after completing RT. Participants were recruited between September 2009 and November 2015 at the Magnuson Clinical Research Center at the NIH. Signed written informed consents were obtained prior to study participation.

Instruments. Clinical and demographic data were obtained from chart review. Fatigue was measured using the frequently-used 13-item Functional Assessment of Cancer Therapy-Fatigue (FACT-F), a validated, reliable, stand-alone measure of fatigue in cancer therapy (questionnaire items and scoring method can be found at www.facit.org) (31). FACT-F had good internal consistency reliability with a Cronbach's α=0.81 when tested in the present study participants. Each item response is rated on a 0-4 scale (0='not at all', 4='very much'). Total FACT-F scores typically range from 16-53, with lower scores reflecting higher fatigue intensity. A FACT-F score of 43 best divides fatigue scores of cancer patients and the general population (32), and is used for cross-sectional comparisons with the US general population (32). Subjects with a FACT-F score <43 were considered fatigued. Subjects were considered to have significantly increased fatigue when there was at least a three-point decrease in FACT-F score relative to the baseline. The 3-point change in FACT-F score satisfies the Clinically Minimally Important Difference threshold, which has been shown to be clinically meaningful (defined by effect size: Cohen's d >0.2) (33,34).

Depressive symptoms were measured using the validated, 24-item Hamilton Depression Rating Scale (HAM-D) (35,36). Scores ranged from 0-54 with high scores reflecting increasing severity of depressive symptoms: A score of 0-7 indicated no depression, 8-16 indicated mild depression and a score of ≥17 indicated moderate to severe depression (37). HAM-D has good internal consistency (standardized Cronbach α=0.67-0.80) and test-retest reliability (Pearson correlation coefficient=0.88, P<0.001) (38).

Blood cell counts were measured using standard procedures adapted by the Department of Laboratory Medicine, NIH. Anemia was defined as hemoglobin level <13 g/dl for men based on the World Health Organization guidelines (39).

Mitochondrial function measurement. Mitochondrial function parameters were measured as previously described (28). Briefly, peripheral blood mononuclear cells (PBMCs) were isolated from human peripheral blood samples using the BD Vacutainer CPT Mononuclear Cell Preparation Tubes based on manufacturer's protocol (BD Biosciences; Becton, Dickinson and Company). Each well in the cell plate was coated with freshly prepared Cell-Tak cell and tissue adhesive solution (Corning, Inc.). PBMCs were plated at $1.5x10^5$ cells/well to reach 80-90% confluency. Respiratory inhibitors were reconstituted immediately before the experiment to reach the following working concentrations: Oligomycin, 1 μM; carbonyl

Table I. Demographics and clinical characteristics of sample population.

Characteristics	Total (n=64)	+ADT all time points (n=27)	+ADT during EBRT (n=20)	No ADT (n=17)
Age, years	65.41±7.78	66.37±8.10	65.25±7.06	64.06±8.31
BMI, kg/m^2	30.26±4.91	30.15±4.51	31.20±5.86	29.32±4.34
Ethnicity, %				
Asian	6.25	7.41	0.00	11.76
Black	26.56	25.93	30.00	23.53
Hispanic	3.13	0.00	0.00	11.76
White	64.06	66.67	70.00	11.76
Education, %				
Did not complete high school	6.25	3.70	3.70	0.00
High school grad/GED	10.94	11.11	10.00	11.76
Associate degree/some college	7.81	11.11	5.00	5.88
Bachelor's degree	46.88	48.15	40.00	52.94
Advanced degree	26.56	22.22	30.00	29.41
No answer	1.56	3.70	0.00	0.00
T stage, %				
T1c	29.69	18.52	45.00	29.41
T2	1.56	0.00	0.00	5.88
T2a	29.69	29.63	35.00	23.53
T2b	6.25	7.41	0.00	11.76
T2c	10.94	11.11	5.00	17.65
T3	21.88	33.33	15.00	11.76
Gleason score, %				
3+3=6	6.25	3.70	0.00	17.65
3+4=7	26.56	0.00	50.00	41.18
3+5=8	1.56	3.70	0.00	0.00
4+3=7	14.06	14.81	10.00	17.65
4+4=8	32.81	44.44	25.00	23.53
4+5=9	14.06	29.63	5.000	0.00
5+4=9	4.69	3.70	10.00	0.00
CBC, 1,000/μl				
WBC	6.52±1.74	6.03±1.68	6.77±1.92	7.01±1.47
RBC	4.59±0.42	4.46±0.43	4.54±0.32	4.83±0.42
Neutrophils absolute	3.86±1.42	3.54±1.49	3.96±1.36	4.27±1.32
Lymphocytes absolute	1.91±0.63	1.79±0.60	2.06±0.79	1.92±0.45
Monocytes absolute	0.54±0.23	0.46±0.13	0.61±0.34	0.57±0.16
Eosinophils absolute	0.19±0.15	0.19±0.18	0.19±0.11	0.19±0.16
Basophils absolute	0.03±0.02	0.03±0.02	0.04±0.02	0.03±0.01
PSA baseline, ng/ml	5.99±13.59	4.32±6.64	9.77±22.76	4.19±3.83
PSA completion of EBRT, ng/ml	0.41±0.92	0.07±0.10	0.07±0.07	1.35±1.43

Data are presented as mean ± standard deviation or percentages. BMI, body mass index; EBRT, external beam radiation therapy; ADT, androgen deprivation therapy; CBC, complete blood count; WBC, white blood cell; RBC, red blood cell; PSA, prostate specific antigen.

cyanide-4-(trifluoromethoxy)phenylhydrazone, 1 μM; and antimycin A/rotenone, 0.5 μM. After incubating in a non-CO_2 incubator for 45-60 min in the presence of pH 7.4 assay media [for details on assay media preparation, see (28)], the cell plate was inserted in to a Seahorse XFp extracellular flux instrument (Agilent Technologies, Inc.). The respiratory inhibitors were injected sequentially into the corresponding ports and oxygen consumption rate (OCR) was measured. Live cells were stained with the Cell Proliferation Assay (Thermo Fisher Scientific, Inc.) and quantified using the Cytation 1 instrument (Biotek Instruments, Inc.). OCR measurements from each well were normalized to the number of live cells. ATP coupling

efficiency is calculated as: Coupling Efficiency=100% x (ATP Production-linked OCR)/(Basal Respiration Rate). Basal respiration rate was measured as baseline OCR before the injection of mitochondrial respiration inhibitors and ATP production-linked OCR was measured as the difference between basal respiration rate and the post-oligomycin OCR.

Mouse model of cancer-related fatigue

Ethics. This study was approved by the National Heart Lung and Blood Institute (NHLBI) Animal Care and Use Committee of the NIH. All investigators working with animals were properly trained by the NIH Office of Animal Care and Use and the NHLBI Murine Phenotyping Core. All interactions with animals in this study were in compliance with The Guide for the Care and Use of Laboratory Animals (40).

Animals. A total of 60 male C57Bl/6 mice were ordered from Charles River Laboratories and were 6-9 weeks old and 15-20 g at the beginning of each study. Mice were given *ad libitum* access to food and water and were individually housed on a 12-h light-dark cycle at ~22.2˚C and 50% humidity throughout all studies. Tails were tattooed for identification and mice received three days of gentle handling by experimenters before procedures began. Mice received daily visual health inspection and were removed from study if any health problems were apparent.

Flutamide implants. Mice were randomly split into two groups. Implant surgery took place over a two-day period, with half the animals in each group receiving implants on each of the two days. The 'ADT' group had a flutamide pellet (SA-152 5 mg/pellet, 60 Day Release; Innovative Research of America) surgically implanted subcutaneously on their backs. Mice were anesthetized using isoflurane anesthesia (3-5% isoflurane was used to induce anesthesia and 1-3% was used to maintain anesthesia) and placed atop a heating pad. A stab incision was made at the nape of the neck, the incision site was swabbed with disinfectant and a pellet was inserted subcutaneously after all disinfectant had completely dried. The wound was closed with tissue glue and a skin staple and mice were returned to their cages. The control (CTL) group underwent the same surgery as the ADT group, but no pellet was implanted. Skin staples were removed one week after surgery. When removing the staples, implanted pellets could be felt when touching the animal's back and could often be seen as a small bump underneath the skin; mice were removed from the study if they were in the ADT group but a pellet was not detected in this way.

Irradiation. Mice in each of the ADT and CTL groups were randomly subdivided into irradiated (Irrad) or sham (Sham) groups, resulting in four groups: Irrad-ADT, Irrad-CTL, Sham-ADT, and Sham-CTL. The procedure is described in detail by Wolff *et al* (2017), though in the present study a lower dose of radiation was used. In brief, on each of the three days of irradiation, all mice were anesthetized with an intraperitoneal injection of ketamine (100 mg/kg; VetOne/MWI Animal Health) and xylazine (10 mg/kg; Akorn Animal Health). Mice were placed inside a lead shielding and received 4 Gy of γ radiation at a dose rate of 1 Gy/min targeted to a narrow pelvic region. Mice in the sham group were anesthetized and placed inside the shielding but remained outside the irradiator. All mice recovered from the anesthesia in their cages above heating pads.

Voluntary wheel running activity (VWRA). Mice were housed in cages with a running wheel (Lafayette Instrument Neuroscience), which recorded wheel rotation in one-min intervals. After at least one week acclimating to the animal facility in standard plastic home cages, mice were housed in running wheel cages for at least 2 weeks before surgery, then for ~2 weeks prior to irradiation, then for 10-12 days after irradiation and before euthanasia. Mice were removed from their running wheel cages during two days of surgery and the three days of irradiation. Mice that did not consistently use the running wheels were removed from the study. Data were collected by the Lafayette Running Wheel software (Lafayette Instrument Neuroscience; version 11.16). The VWRA outcome measure was time spent using the wheel.

Tissue harvest and western blotting. Mice were anesthetized with ketamine/xylazine (120/20 mg/kg) and decapitated immediately after exsanguination. The skull was opened and the brain was removed. The whole brain was placed in a petri dish with ice-cold PBS. Cortical tissues were extracted by removing the brain stem, cerebellum, midbrains and were immediately placed into ceramic bead tubes on ice. Modified radioimmunoprecipitation assay buffer (50 mm Tris-HCl pH 7.4, 1% NP-40, 0.25% sodium deoxycholate and 150 mm NaCl) supplemented with protease inhibitor cocktail (Sigma-Aldrich; Merck KGaA) was added to the samples for cell lysis using a bead-mill homogenizer (Thermo Fisher Scientific, Inc.). Lysates were centrifuged at 14,000 x g 15 min at 4˚C. Supernatants were retained as the soluble lysate, boiled at 100˚C for 10 min in the presence of Laemmli Sample Buffer (Bio-Rad Laboratories, Inc.) supplemented with dithiothreitol. All protein samples (30 μg at 1 μg/μl) were subjected to denaturing 10% SDS-polyacrylamide gel electrophoresis followed by transfer to polyvinylidene difluoride membranes using the Trans-Blot Turbo Transfer System (Bio-Rad Laboratories, Inc.). The membranes were blocked at 4˚C for 1 h with a 5% Non-Fat Dry Milk Omniblock (AB10109-00100; AmericanBio, Inc.) or bovine serum albumin (Sigma-Aldrich; Merck KGaA) solution in phosphate-buffered saline with 0.1% Tween and incubated overnight at 4˚C with a rabbit polyclonal antibodies mitochondrial transcription factor A (TFAM; 1:1,000; cat. no: ab131607; Abcam) and glucose transporter 4 (GLUT4; 1:750; cat. no: ab654; Abcam). Membranes were re-probed with a primary antibody against GAPDH (anti-rabbit, 1:1,000, cat. no: ab9485; Abcam) as a loading control. After incubation overnight at 4˚C, the membranes were further probed with a goat anti-rabbit IgG horseradish peroxidase antibody (1:1,000; cat. no: ab6721; Abcam). Immunoreactive complexes were visualized using Super Signal West Pico Chemiluminescent Substrate (Thermo Fisher Scientific), imaged and quantified using the ChemiDoc MP Imaging Systems Image Lab 6.0.1 (Bio-Rad Laboratories, Inc.).

Statistical analysis. Descriptive analyses were used to describe demographic characteristics of the sample. All data

were expressed as the mean ± standard error of mean. To assess changes in clinical variables over time, a two-way repeated-measures analysis of variance (ANOVA) was employed. The presence or absence of ADT was defined as between-subject factors, while the within-subject factor was defined by study time points. The sphericity assumption was tested with Mauchly's test and fatigue differences at each time point were determined by non-directional Student's t-test with Bonferroni corrections for multiple comparisons. One-way ANOVA was used to determine significant differences in comparisons involving more than 2 groups. Post hoc non-directional Student's t-test with Bonferroni correction was used for between group comparisons. $P<0.05$ was considered to indicate a statistically significant difference. Statistical analyses were performed with SPSS statistics software version 23 (IBM Corps.). Behavioral data analysis was conducted in python using the statsmodels library for ANOVA and the scipy and pandas modules for all other tests. Two-way ANOVAs were used to test for main effects, Shapiro-Wilk tests were used for evaluating normality and post-hoc t-tests were used for pairwise comparisons with Holm-Sidak corrections for multiple comparisons. Pearson correlation coefficient analysis was performed to analyze correlations between variables. All experiments were performed with an $n>8$ and repeated three times. In all plots, error bars represent the standard error of the mean. Threshold α values were 0.05 for all tests.

Results

Clinical characteristics of participants. The clinical sample was predominantly Caucasian (62.50%) with an average age of 65.23±7.41 years and a body mass index (BMI) of 30.3±4.96 (Table I). The majority of the subjects had locally confined prostate cancer with 28.07% at T stage T1, 47.37% at T2 and 15.79% at T3. Of the 64 subjects enrolled in the study, 17 subjects received only EBRT with no ADT at any study time point. A total of 27 subjects received EBRT with concomitant ADT at all study time points (baseline, midpoint, completion and one-year post-EBRT). ADT (both Bicalutamide and leuprolide acetate) treatment was terminated upon EBRT completion in 20 of the subjects. There were no significant differences in clinical characteristics between the two groups including BMI, Gleason scores and T stage (Table I). In addition, there was no significant correlation between FACT-F scores and prostate-specific antigen levels after treatment completion ($r=0.162$, $P=0.255$).

Fatigue worsened during EBRT and ADT treatment. Subjects receiving ADT experienced significantly worse fatigue at the midpoint ($P=0.00005$) as well as completion of EBRT ($P=0.0008$), compared to subjects without ADT (Fig. 1A). Using the previously described definition of fatigue, a FACT-F cutoff score of 43 (21), the present study found that 61% of subjects in the ADT group reported fatigue compared to 13% in the non-ADT group prior to EBRT initiation (Fig. 1B). The percentage of fatigued subjects in the ADT group increased to 72% at midpoint and 68% at EBRT completion. The effect of ADT on fatigue lessened one year after EBRT with 32% of the ADT group experiencing fatigue (Fig. 1B). On the other hand, the percentage of fatigued subjects in the non-ADT group remained stable during EBRT (midpoint: 24%, completion: 21%) and at one-year post-EBRT (28%) (Fig. 1B).

A 3-point longitudinal change in FACT-F score has been found to represent clinically important worsening of fatigue (33). The percentage of subjects receiving ADT with worsened fatigue increased during EBRT (63% at midpoint, 49% at completion of EBRT). Only 19% of non-ADT subjects experienced significantly worsened fatigue during EBRT and 30% after treatment completion (Fig. 1C).

Hemoglobin levels decreased significantly over time in subjects treated with ADT (Fig. 1D; $F_{3,37}=6.12$, $P=0.002$). Prior to EBRT initiation (at baseline), 28% of subjects receiving ADT were anemic with hemoglobin levels lower than 13 g/dl compared to 7% of subjects without ADT (Fig. 1E). The percentage of subjects with anemia increased in the ADT group during EBRT-62% at the midpoint and 80% upon completion of EBRT, compared to 6% at the midpoint and 12% at completion of EBRT in the no ADT group (Fig. 1E). One year after EBRT completion, ADT continued to affect hemoglobin levels, though to a lesser degree, resulting in 48% of anemia in the ADT group, compared to 18% in the no ADT group (Fig. 1E).

Despite the similarity of depressive symptoms with fatigue in terms of the subjective experience and underlying physiological mechanism (41), the effect of ADT during EBRT appeared to be specific to fatigue, as ADT did not affect HAM-D scores at any study time point (Fig. 1F; $F_{3,40}=0.34$, $P=0.80$).

Fatigued subjects exhibit lower mitochondrial coupling efficiency. A schematic illustration of the mitochondrial function assay and coupling efficiency calculation is shown in Fig. 2A. Although ADT treatment during RT did not affect mitochondria coupling efficiency (+ADT vs. -ADT $P=0.633$, Fig. 2B), fatigued subjects during RT exhibited lower ATP coupling efficiency compared to non-fatigued controls (fatigued vs. non-fatigued $P=0.017$; Fig. 2C).

Mouse model of ADT/RT-induced fatigue. Due to limitations of sample availability (assessing brain mitochondrial function was not possible in human subjects), the present study decided to use a previously published mouse model of fatigue (29), to examine the role of brain mitochondria in fatigue behavior. VWRA was used to measure fatigue-like behavior in mice. To model ADT, flutamide pellets were implanted that slowly released 5 mg flutamide over a 60-day period. Over the next two weeks, VWRA was monitored (Fig. 3A) and found there was no significant difference between these ADT mice and control (CTL) mice (Fig. 3B, $t_{48}=0.48$, $P=0.63$).

A total of two weeks after initiating ADT, mice received three days of irradiation targeted to the pelvic region. Previous studies indicate that lower-abdominal irradiation causes fatigue-like behavior that lasts about six days (29,42), so the present study measured average VWRA across six days post-irradiation (Fig. 3C). All mice, including Sham-CTL, showed a reduction in wheel running, with mice in the Irrad-ADT group showing the largest decrease. A two-way ANOVA reported a significant effect of irradiation ($F_{2,50}=8.82$, $P=0.0046$) but not of ADT ($F_{2,50}=0.47$, $P=0.50$) on VWRA (Fig. 3D). Post-hoc pairwise comparisons only

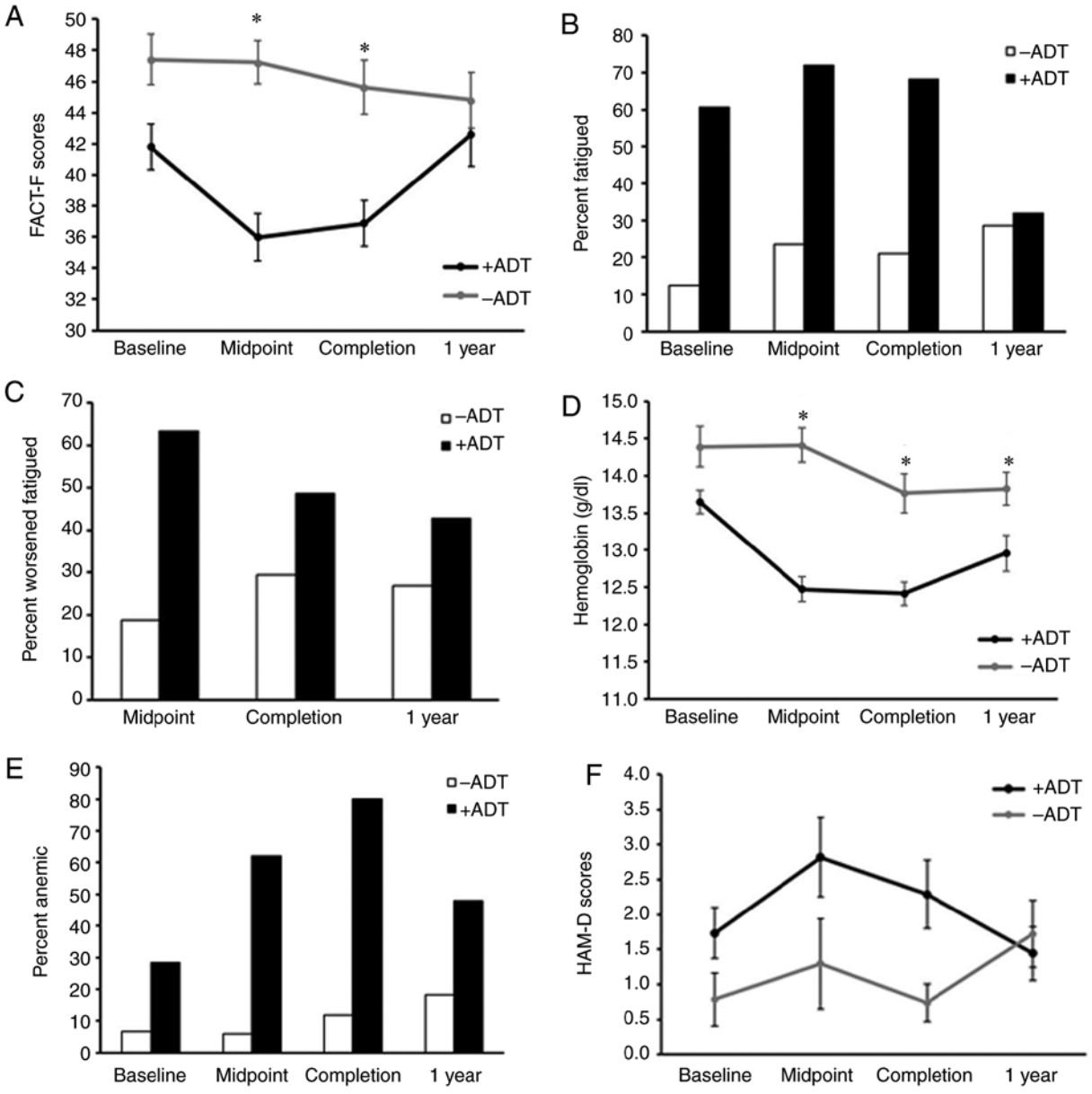

Figure 1. ADT contributes to anemia during RT in men with non-metastatic prostate cancer. (A) ADT significantly affected fatigue development over time ($F_{3,42}$=3.80, P=0.02) with the most significant difference occurring at midpoint (P=0.00005) and completion of RT (P=0.0008). (B) Percentage of subjects with fatigue, or a FACT-F score less than 43. (C) Percentage of subjects with worsened fatigue, or a decrease in FACT-F scores ≥3 from baseline. (D) ADT treatment significantly affects changes in hemoglobin levels over time ($F_{3,37}$=6.12, P=0.002). (E) Percentage of subjects with anemia at each time point. (F) Percentage of subjects with anemia at each time point. The presence of absence of ADT did not affect levels of depressive symptoms ($F_{3,40}$=0.34, P=0.80). *P<0.05 vs. -ADT. ADT, androgen deprivation therapy; FACT-F, Functional Assessment of Cancer Therapy-Fatigue; RT, radiation therapy.

reported a significant reduction in VWRA from the combination of ADT and irradiation (t_{28}=2.91, P=0.036) compared to Sham-CTL.

Hemoglobin levels were measured 9 days after irradiation to test whether the mice showed anemia-like conditions (Fig. 3E). As with VWRA, a two-way ANOVA showed a significant effect of irradiation ($F_{2,44}$=9.84, P=0.0029) but not ADT ($F_{2,44}$=1.68, P=0.20) on blood hemoglobin levels. Again, similar to VWRA, post-hoc pairwise comparisons of hemoglobin levels only showed a significant reduction in the group that received both irradiation and ADT (t_{16}=3.36, P=0.016) compared to Sham-CTL. In addition, hemoglobin levels across all mice were found to be significantly positively correlated with VWRA totals (r=0.36, P=0.012; Fig. 3F).

Fatigue in the mouse model appears to be related to mitochondrial function in the cerebral cortex. To investigate whether brain mitochondria are affected by the ADT and peripheral irradiation procedures, mice were euthanized 10-12 days after irradiation and whole brain protein lysates were collected and immunoblotted for glucose transporter GLUT4 and mitochondrial transcription factor TFAM. Representative bands from each group are shown in Fig. 4A. Densitometry data were quantified (n=8 mouse samples per group for a total of 32 samples) and shown as bar graphs (Fig. 4B). Levels of GLUT4 were found to be lower in the brains of irradiated mice relative to sham (Fig. 4B) and a two-way ANOVA showed a significant effect of irradiation ($F_{2,27}$=4.87, P=0.036) but not ADT ($F_{2,27}$=1.00, P=0.33). The Sham-ADT group showed a lower

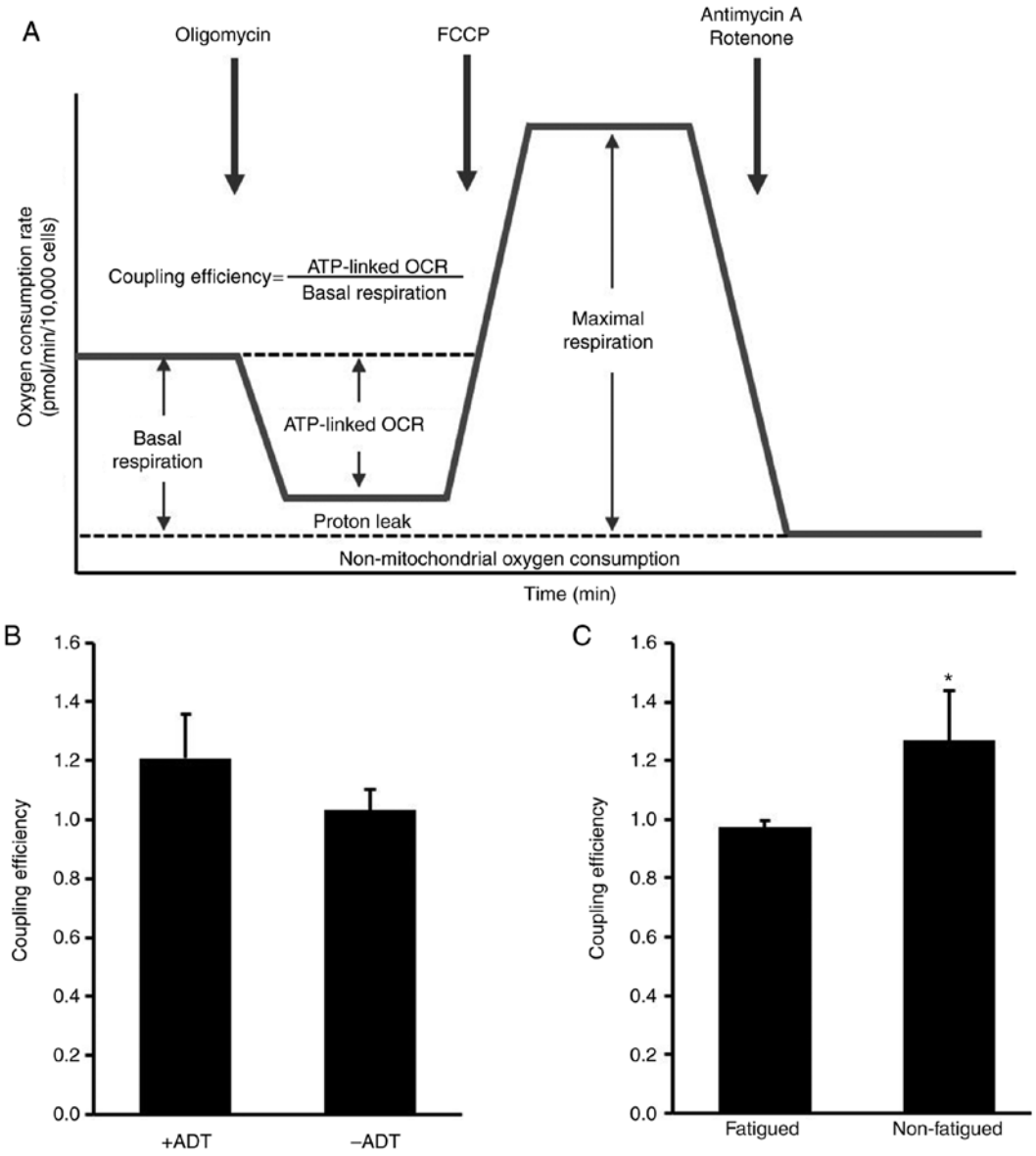

Figure 2. Mitochondrial dysfunction contributes to self-reported fatigue in men with non-metastatic prostate cancer. (A) Schematic diagram of OCR profile of the extracellular flux mito stress test. (B) ADT treatment during radiation therapy did not affect mitochondrial coupling efficiency (+ADT vs. -ADT P=0.633). (C) Non-fatigued subjects exhibited higher mitochondrial coupling efficiency compared to fatigued subjects (P=0.017). *P<0.05 vs. fatigued. OCR, oxygen consumption rate; ADT, androgen deprivation therapy.

mean level of GLUT4 than Sham-CTL, which could suggest that floor effects were masking an effect of ADT, but the interaction term of the ANOVA was just shy of significance ($F_{2,27}$=3.03, P=0.052). On brain levels of TFAM (Fig. 4B), a significant effect of irradiation was found ($F_{2,25}$=5.54, P=0.027) but not ADT ($F_{2,25}$=0.18, P=0.68) and no interaction ($F_{2,25}$=0.0010, P=0.97). Across all GLUT4 and TFAM post-hoc pairwise comparisons, only one comparison was significant: GLUT4, Irrad-CTL vs. Sham-CTL (t_{14}=-3.31, P=0.0342).

Whether these levels of brain mitochondrial proteins correlated with either the anemia measure (hemoglobin) or the fatigue measure (VWRA) was looked at next. The present study found that hemoglobin levels were positively correlated with both GLUT4 (r=0.38, P=0.050; Fig. 4C) and the GLUT4/TFAM ratio (r=0.46, P=0.017; Fig. 4E), but not with TFAM (r=0.03, P=0.904; Fig. 4D). VWRA did not correlate with any of these measures (all P>0.35; Fig. 4F-H).

Discussion

The current study describes a novel finding that RT exacerbated the effects of ADT on fatigue in patients with non-metastatic localized prostate cancer. The present study showed that the combination of ADT and RT caused worsened fatigue that was associated with anemia and mitochondrial dysfunction. This combined effect of ADT and radiotherapy appeared to be specific to fatigue, as depressive symptoms were unaffected. As anemia can lead to hypoxia in brain tissues (43), the mouse model was used to examine markers of brain bioenergetics, TFAM and GLUT4 (44,45). The mouse model did not use tumor-bearing mice but showed that the combination of ADT and RT were sufficient to induce a fatigue-like behavior that mimicked the clinical behaviors that were observed: ADT alone did not cause fatigue; instead, ADT exacerbated the effect of RT on fatigue. The present findings suggest a possible

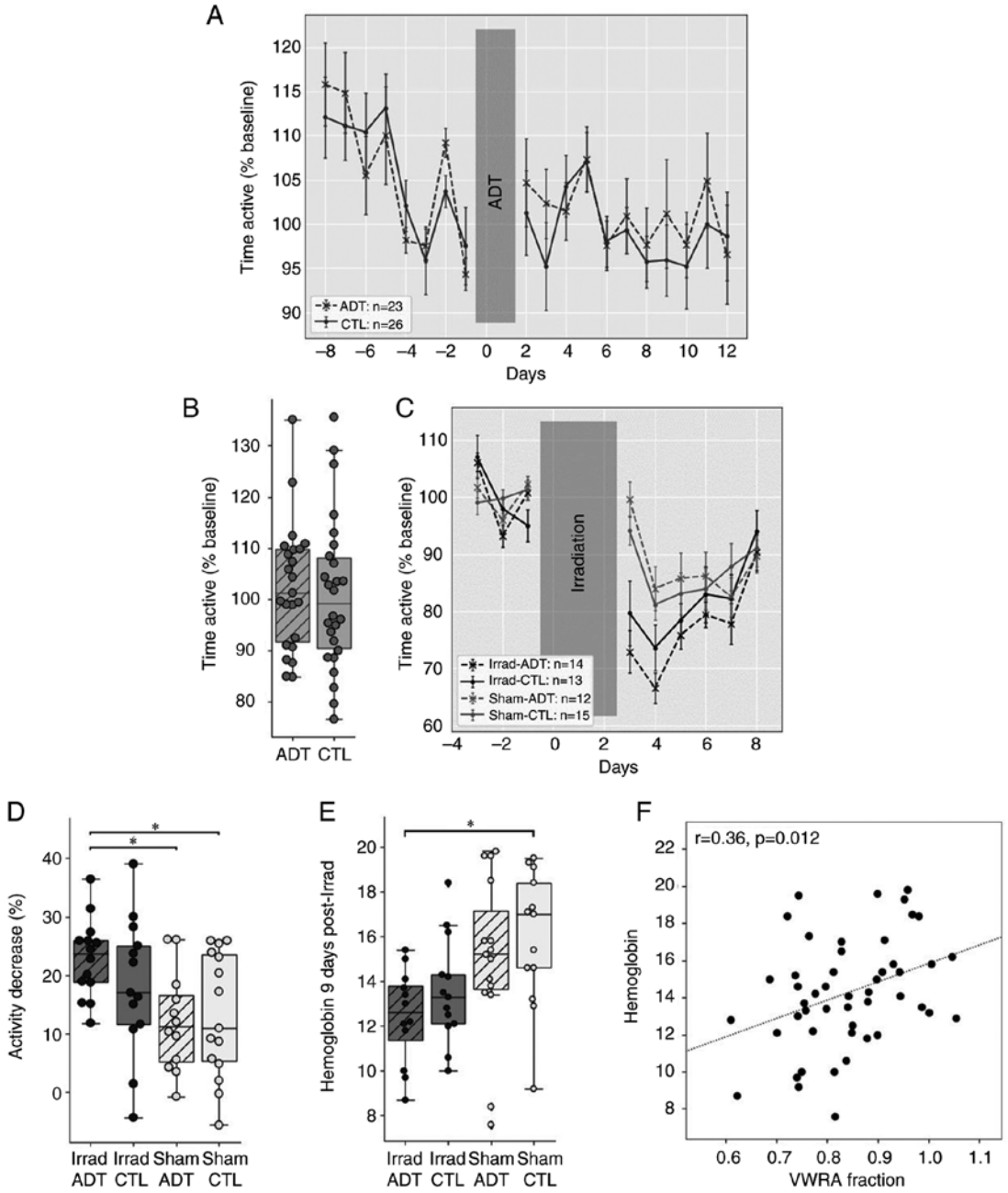

Figure 3. ADT/radiation therapy-induced fatigue in a mouse model. (A) VWRA before and after implanting flutamide pellets on days 0 and 1. Data are normalized to the mean daily VWRA over the four days before surgery (n=12-15 mice per group). (B) There was no difference between groups in total activity over the 12 days post-surgery (t_{48}=0.48, P=0.63). (C) VWRA before and after three days of irradiation on days 0-2. Data are normalized to the mean VWRA over the four days before irradiation. (D) There was a significant effect of irradiation ($F_{2,50}$=8.82, P=0.0046) but not ADT ($F_{2,50}$=0.47, P=0.50) on VWRA totals over the six days after irradiation. Post-hoc comparisons showed a significant difference only when comparing the Irrad-ADT group to Sham-ADT (t_{25}=3.62, P=0.0084) or Sham-CTL (t_{28}=2.91, P=0.036). (E) There was a significant effect of irradiation ($F_{2,44}$=4.62, P=0.037) but not ADT ($F_{2,44}$=0.66, P=0.42) on hemoglobin levels. Post-hoc comparisons showed a significant effect only when comparing Irrad-ADT to Sham-CTL (t_{16}=3.36, P=0.016). (F) Hemoglobin levels showed a significant correlation with VWRA (r=0.33, P=0.022). *P<0.05. ADT, androgen deprivation therapy; Irrad, irradiated; CTL, control; VWRA, voluntary wheel-running activity.

role of brain bioenergetics in decreased wheel running activity and fatigue. It is possible that ADT combined with RT not only results in anemia in patients, but also mitochondrial dysfunction in the CNS leading to cognitive fatigue.

Although it has been shown that ADT and RT may independently cause anemia and fatigue, adverse effects of combined therapy have been less explored (46). In the current study, ADT or RT alone did not significantly affect anemia or fatigue. Instead, ADT and RT acted synergistically to worsen anemia and fatigue during EBRT, and the trajectory of hemoglobin level changes mimicked that of fatigue. Interestingly, a subset of subjects who did not receive ADT still experienced anemia one year after RT. This may be due to the fact that aging contributes to anemia in elderly patients-for example, both the Framingham cohort and the Third National Health and Nutrition Examination Survey found that the prevalence of anemia in men over 65 years of age is 6.1-11% (47), comparable to what was observed in

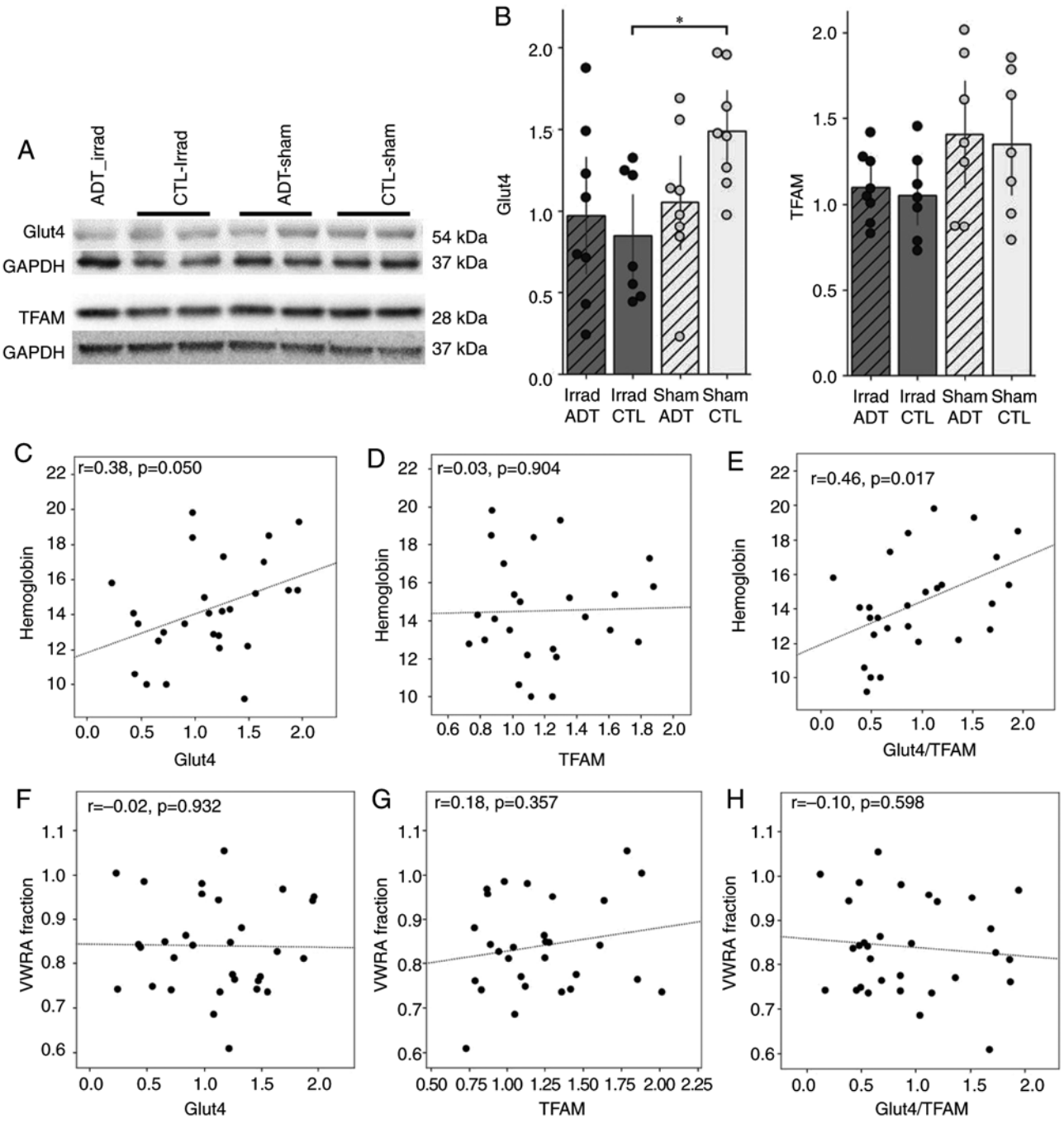

Figure 4. Brain mitochondrial effects in the mouse model of ADT/radiation therapy-induced fatigue. (A) Western blot analysis of GLUT4 and TFAM. Blots indicate representative bands. (B) Densitometry data were quantified (n=8 mouse samples per group for a total of 32 samples) and shown as bar graphs. Irradiation had a significant effect on GLUT4 levels in the mouse brain ($F_{2,27}$=4.87, P=0.036) but ADT did not ($F_{2,27}$=1.00, P=0.33); the interaction of irradiation and ADT was on the edge of significance ($F_{2,27}$=3.03, P=0.052). Irradiation had a significant effect on TFAM levels in the mouse brain ($F_{2,25}$=5.54, P=0.027) but ADT did not (ADT: $F_{2,25}$=0.18, P=0.68). Hemoglobin levels of (C) GLUT4, (D) TFAM and (E) GLUT4/TFAM showed a borderline significant correlation with GLUT4 levels in the mouse brain (C: r=0.38, P=0.050), no significant correlation with TFAM (D: r=0.03, P=0.90) and a strong correlation with GLUT4/TFAM ratio (r=-0.56, P=0.003). VWRA did not significantly correlate with (F) GLUT4, (G) TFAM, nor (H) TFAM/GLUT4 ratio (P>0.35 for all). *P<0.05. ADT, androgen deprivation therapy; Irrad, irradiated; CTL, control; VWRA, voluntary wheel-running activity; GLUT4, glucose transporter 4; TFAM, transcription factor A mitochondrial.

the non-ADT group. Depressive symptoms were included in the present analysis due to the significant overlap between fatigue and depression both in regard to the subjective experience and the underlying mechanism (48,49). No difference in HAM-D was observed at any study time point. This suggests that the effect of combined ADT and radiotherapy appeared to be specific to fatigue, as depressive symptoms did not follow the same longitudinal trend of anemia or fatigue.

Mitochondria coupling efficiency represents the proportion of O_2 consumed to drive ATP synthesis and is calculated as the fraction of basal mitochondrial respiration rate used for ATP production (50). The present study found that self-reported subjective fatigue appeared to be associated

with decreased ATP coupling efficiency, indicative of less efficient mitochondrial ATP production. A limitation with the clinical study is that it was only able to obtain blood samples. Even though there is evidence in the literature to support the validity of measuring mitochondrial function in PBMCs as a proxy marker for systemic bioenergetics (51), the similarity in bioenergetics between PBMCs and neurons remains to be established. In order to examine whether brain bioenergetics is influenced by ADT/RT, a mouse model was developed using a low irradiation dosage and subcutaneous flutamide implants, which was not sufficient to induce fatigue on their own. Similar to the present clinical findings, ADT and irradiation induced fatigue only when administered together. Also supporting current clinical findings, hemoglobin levels after irradiation correlated with changes in wheel running.

The present study showed that irradiation in the ADT/RT-induced fatigue mouse model resulted in the downregulation of GLUT4, a glucose transporter that is preferentially expressed in brain regions involved in the control of motor activity and is essential for maintaining neuronal metabolic homeostasis (52,53). In addition to its role in glycolysis, GLUT4 levels have also been shown to decrease in the presence of mitochondrial dysfunction and serves as an indirect marker of mitochondrial health (52,54). ADT with the sham irradiation procedure also decreased GLUT4 expression, though the effect did not reach statistical significance (P=0.052). It is possible that irradiation caused floor effects masking any effect of ADT in the downregulation of GLUT4, even at a dosage that did not cause a behavioral change. Similarly, irradiation resulted in a downregulation of TFAM, a mitochondrial transcription factor that is important for maintaining normal cellular respiratory function and serves as a marker for mitochondrial function (55). Interestingly, altered TFAM levels has been shown to contribute to mitochondrial dysfunction in various neurodegenerative diseases (54,56). The addition of ADT did not appear to further contribute to GLUT4 or TFAM downregulation. This suggests that the synergistic effect of ADT and irradiation in behavior may extend beyond brain bioenergetics. Future studies will examine the effects of different irradiation and ADT dosages, as well as the contribution of mitochondrial dysfunction in skeletal muscle tissues.

A limitation in the present study is the small clinical sample size, particularly in the non-ADT group. Future studies could validate findings from this study in a larger sample size. The current study did not detect any significant difference between fatigue scores and anemia status between subjects with or without ADT at baseline, at which point subjects had already received ADT but not RT. Future studies will examine the fatigue status in subjects with metastatic cancer that receive ADT monotherapy. PBMCs functional tests were used to assess the role of mitochondrial function in cancer-related fatigue. Even though as a heterogenous population, PBMCs serve as a useful tool for assessing global mitochondrial dysfunction. Future studies will further examine bioenergetics in each cell subpopulation. Additional efforts will also be made to establish baseline mitochondrial function test measurements in healthy individuals. In the current clinical study, the sample type available was a limitation. Blood collection is well tolerated by patients even at multiple time points. Future studies will examine markers in cerebral-spinal fluid to get a better picture of the CNS aspect of fatigue.

The ADT/RT mouse model mimicked clinical symptoms of human subjects going through the combined therapy. However, fatigue measured by VWRA lasted for days in mice, but lasts for months up to years in human subjects. Continued efforts are made to develop a mouse model with the more physiologically relevant 'persistent' fatigue. One limitation in the ADT model is that patients received ADT for on average 76 days prior to EBRT initiation, whereas mice in the animal model of fatigue received 12 days of ADT prior to irradiation. While it is difficult to determine what the 'equivalent' ADT treatment time would be for a mouse (57), the mouse model is consistent with the clinical observation that ADT without radiation did not cause fatigue. Furthermore, a small pilot study (data not shown) treating with flutamide for 38 days was conducted and no difference in VWRA in the flutamide-treated animals relative to controls was seen; therefore on a shorter experiment design was decided upon.

Another limitation in the ADT model is that patients received both an anti-androgen (Bicalutamide) and an LH-RH agonist, whereas mice received only an anti-androgen (flutamide). The flutamide-ADT mouse model was developed based on other studies showing that flutamide treatment reduced tumor incidence compared to a placebo in animal models (58-60), but future studies would benefit from combining LH-RH agonists with an anti-androgen to model ADT and fatigue-like behavior. Lastly, measurements of mitochondrial function in the mouse model were limited to indirect markers including TFAM and GLUT4. Due to the limited blood volume that can be collected from a mouse (<0.5 ml), it was not possible to extract enough PBMCs from a mouse to perform the same mitochondrial function assay that was done using human PBMC samples. Future studies will explore mitochondrial function assays that utilize isolated mitochondria from brain tissues of the mouse model.

In conclusion, the present study demonstrated that ADT exacerbated fatigue in subjects receiving RT. Anemia appeared to be a significant contributor of fatigue during EBRT, but not prior to treatment initiation, suggesting worsened hematologic toxicity with a combination of ADT and RT. Furthermore, self-reported fatigue was associated with mitochondrial dysfunction suggesting a physiological basis for the subjective phenomenon. Additionally, ADT/RT induced fatigue-like behaviors in a mouse model and mimicked clinical observations of human subjects receiving concomitant ADT and RT. Using the mouse model, alterations in markers of mitochondrial dysfunction and brain bioenergetics were found in mice receiving irradiation, suggesting the contribution of non-mitochondrial factors in the synergistic effect on fatigue exerted by combined ADT and RT. Results from this study will inform patients and clinicians of mechanisms related to the combined treatment and thus manage these symptoms more effectively.

Acknowledgements

The authors would like to thank Ms. Diane Cooper (National Institutes of Health Library Writing Center, Bethesda, MD, USA) for assistance with manuscript editing.

Funding

The present study is fully supported by the Division of Intramural Research of the National Institute of Nursing Research of the National Institutes of Health, Bethesda, MD, USA (grant no. ZIA NR000020-06).

Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

LRF, JMR, JL and LNS designed, collected, analyzed and interpreted patient data regarding the clinical aspect and mitochondrial function. BSW, SA and SR performed the mouse model experiments and analyzed the data. All authors contributed to writing the manuscript and approved the final version. All authors were involved in drafting the manuscript. All authors have agreed to be accountable for all aspects of the work.

Ethics approval and consent to participate

The study was approved by the Institutional Review Board of the National Institutes of Health (approved protocol no. NCT00852111). Written informed consent was obtained prior to study participation. This study was approved by the National Heart Lung and Blood Institute Animal Care and Use Committee of the NIH.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Spina CS: Androgen deprivation therapy and radiation therapy for prostate cancer: The mechanism underlying therapeutic synergy. Transl Cancer Res 7 (Suppl 6): S695-S703, 2018.
2. Harris WP, Mostaghel EA, Nelson PS and Montgomery B: Androgen deprivation therapy: Progress in understanding mechanisms of resistance and optimizing androgen depletion. Nat Clin Pract Urol 6: 76-85, 2009.
3. Duchesne GM, Woo HH, Bassett JK, Bowe SJ, D'Este C, Frydenberg M, King M, Ledwich L, Loblaw A, Malone S, *et al*: Timing of androgen-deprivation therapy in patients with prostate cancer with a rising PSA [TROG 03.06 and VCOG PR 01-03 (TOAD)]: A randomised, multicentre, non-blinded, phase 3 trial. Lancet Oncol 17: 727-737, 2016.
4. Barker HE, Paget JT, Khan AA and Harrington KJ: The tumour microenvironment after radiotherapy: Mechanisms of resistance and recurrence. Nat Rev Cancer 15: 409-425, 2015.
5. Hamdy FC, Donovan JL, Lane JA, Mason M, Metcalfe C, Holding P, Davis M, Peters TJ, Turner EL, Martin RM, *et al*: 10-year outcomes after monitoring, surgery, or radiotherapy for localized prostate cancer. N Engl J Med 375: 1415-1424, 2016.
6. Rusthoven CG, Jones BL, Flaig TW, Crawford ED, Koshy M, Sher DJ, Mahmood U, Chen RC, Chapin BF, Kavanagh BD and Pugh TJ: Improved survival with prostate radiation in addition to androgen deprivation therapy for men with newly diagnosed metastatic prostate cancer. J Clin Oncol 34: 2835-2842, 2016.
7. Horwitz EM, Bae K, Hanks GE, Porter A, Grignon DJ, Brereton HD, Venkatesan V, Lawton CA, Rosenthal SA, Sandler HM and Shipley WU: Ten-year follow-up of radiation therapy oncology group protocol 92-02: A phase III trial of the duration of elective androgen deprivation in locally advanced prostate cancer. J Clin Oncol 26: 2497-2504, 2008.
8. Roach M III, Bae K, Speight J, Wolkov HB, Rubin P, Lee RJ, Lawton C, Valicenti R, Grignon D and Pilepich MV: Short-term neoadjuvant androgen deprivation therapy and external-beam radiotherapy for locally advanced prostate cancer: Long-term results of RTOG 8610. J Clin Oncol 26: 585-591, 2008.
9. D'Amico AV, Chen MH, Renshaw AA, Loffredo M and Kantoff PW: Androgen suppression and radiation vs. radiation alone for prostate cancer: A randomized trial. JAMA 299: 289-295, 2008.
10. Shipley WU, Seiferheld W, Lukka HR, Major PP, Heney NM, Grignon DJ, Sartor O, Patel MP, Bahary JP, Zietman AL, *et al*: Radiation with or without antiandrogen therapy in recurrent prostate cancer. N Engl J Med 376: 417-428, 2017.
11. Siddiqui ZA and Krauss DJ: Adjuvant androgen deprivation therapy for prostate cancer treated with radiation therapy. Transl Androl Urol 7: 378-389, 2018.
12. Milosevic M, Chung P, Parker C, Bristow R, Toi A, Panzarella T, Warde P, Catton C, Menard C, Bayley A, *et al*: Androgen withdrawal in patients reduces prostate cancer hypoxia: Implications for disease progression and radiation response. Cancer Res 67: 6022-6025, 2007.
13. Yao M, Rogers L, Suchowerska N, Choe D, Al-Dabbas MA, Narula RS, Lyons JG, Sved P, Li Z and Dong Q: Sensitization of prostate cancer to radiation therapy: Molecules and pathways to target. Radiother Oncol 128: 283-300, 2018.
14. Polkinghorn WR, Parker JS, Lee MX, Kass EM, Spratt DE, Iaquinta PJ, Arora VK, Yen WF, Cai L, Zheng D, *et al*: Androgen receptor signaling regulates DNA repair in prostate cancers. Cancer Discov 3: 1245-1253, 2013.
15. Kissick HT, Sanda MG, Dunn LK, Pellegrini KL, On ST, Noel JK and Arredouani MS: Androgens alter T-cell immunity by inhibiting T-helper 1 differentiation. Proc Natl Acad Sci USA 111: 9887-9892, 2014.
16. Kalina JL, Neilson DS, Comber AP, Rauw JM, Alexander AS, Vergidis J and Lum JJ: Immune modulation by androgen deprivation and radiation therapy: Implications for prostate cancer immunotherapy. Cancers (Basel) 9: pii: E13, 2017.
17. Nead KT, Gaskin G, Chester C, Swisher-McClure S, Dudley JT, Leeper NJ and Shah NH: Androgen deprivation therapy and future Alzheimer's disease risk. J Clin Oncol 34: 566-571, 2016.
18. Evans JR, Zhao S, Daignault S, Sanda MG, Michalski J, Sandler HM, Kuban DA, Ciezki J, Kaplan ID, Zietman AL, *et al*: Patient-reported quality of life after stereotactic body radiotherapy (SBRT), intensity modulated radiotherapy (IMRT), and brachytherapy. Radiother Oncol 116: 179-184, 2015.
19. Nelson AM, Gonzalez BD, Jim HS, Cessna JM, Sutton SK, Small BJ, Fishman MN, Zachariah B and Jacobsen PB: Characteristics and predictors of fatigue among men receiving androgen deprivation therapy for prostate cancer: A controlled comparison. Support Care Cancer 24: 4159-4166, 2016.
20. Holliday EB, Dieckmann NF, McDonald TL, Hung AY, Thomas CR Jr and Wood LJ: Relationship between fatigue, sleep quality and inflammatory cytokines during external beam radiation therapy for prostate cancer: A prospective study. Radiother Oncol 118: 105-111, 2016.
21. Feng LR, Dickinson K, Kline N and Saligan LN: Different phenotyping approaches lead to dissimilar biologic profiles in men with chronic fatigue following radiation therapy. J Pain Symptom Manage 52: 832-840, 2016.
22. Vichaya EG, Molkentine JM, Vermeer DW, Walker AK, Feng R, Holder G, Luu K, Mason RM, Saligan L, Heijnen CJ, *et al*: Sickness behavior induced by cisplatin chemotherapy and radiotherapy in a murine head and neck cancer model is associated with altered mitochondrial gene expression. Behav Brain Res 297: 241-250, 2016.
23. Hsiao CP and Hoppel C: Analyzing mitochondrial function in human peripheral blood mononuclear cells. Anal Biochem 549: 12-20, 2018.
24. Hsiao CP, Daly B and Hoppel C: Mitochondrial bioenergetics and cancer-related fatigue in patients with prostate cancer undergoing localized radiation therapy. Oncol Nurs Forum 42: E213, 2015.
25. Toro-Urrego N, Garcia-Segura LM, Echeverria V and Barreto GE: Testosterone protects mitochondrial function and regulates neuroglobin expression in astrocytic cells exposed to glucose deprivation. Front Aging Neurosci 8: 152, 2016.

26. Feng LR, Chen MK, Lukkahatai N, Hsiao CP, Kaushal A, Sechrest L and Saligan LN: Clinical predictors of fatigue in men with non-metastatic prostate cancer receiving external beam radiation therapy. Clin J Oncol Nurs 19: 744-750, 2015.
27. Li M, Bertout JA, Ratcliffe SJ, Eckenhoff MF, Simon MC and Floyd TF: Acute anemia elicits cognitive dysfunction and evidence of cerebral cellular hypoxia in older rats with systemic hypertension. Anesthesiology 113: 845-858, 2010.
28. Feng LR, Nguyen Q, Ross A and Saligan LN: Evaluating the role of mitochondrial function in cancer-related fatigue. J Vis Exp, 2018.
29. Wolff BS, Renner MA, Springer DA and Saligan LN: A mouse model of fatigue induced by peripheral irradiation. J Vis Exp, 2017.
30. Merseburger AS, Hammerer P, Rozet F, Roumeguère T, Caffo O, da Silva FC and Alcaraz A: Androgen deprivation therapy in castrate-resistant prostate cancer: How important is GnRH agonist backbone therapy? World J Urol 33: 1079-1085, 2015.
31. Yellen SB, Cella DF, Webster K, Blendowski C and Kaplan E: Measuring fatigue and other anemia-related symptoms with the functional assessment of cancer therapy (FACT) measurement system. J Pain Symptom Manage 13: 63-74, 1997.
32. Cella D, Eton DT, Lai JS, Peterman AH and Merkel DE: Combining anchor and distribution-based methods to derive minimal clinically important differences on the functional assessment of cancer therapy (FACT) anemia and fatigue scales. J Pain Symptom Manage 24: 547-561, 2002.
33. Yost KJ, Eton DT, Garcia SF and Cella D: Minimally important differences were estimated for six patient-reported outcomes measurement information system-cancer scales in advanced-stage cancer patients. J Clin Epidemiol 64: 507-516, 2011.
34. Cella D, Yount S, Sorensen M, Chartash E, Sengupta N and Grober J: Validation of the functional assessment of chronic illness therapy fatigue scale relative to other instrumentation in patients with rheumatoid arthritis. J Rheumatol 32: 811-819, 2005.
35. Lydiatt WM, Denman D, McNeilly DP, Puumula SE and Burke WJ: A randomized, placebo-controlled trial of citalopram for the prevention of major depression during treatment for head and neck cancer. Arch Otolaryngol Head Neck Surg 134: 528-535, 2008.
36. Milam JE, Meeske K, Slaughter RI, Sherman-Bien S, Ritt-Olson A, Kuperberg A, Freyer DR and Hamilton AS: Cancer-related follow-up care among Hispanic and non-Hispanic childhood cancer survivors: The project forward study. Cancer 121: 605-613, 2015.
37. Zimmerman M, Martinez JH, Young D, Chelminski I and Dalrymple K: Severity classification on the Hamilton depression rating scale. J Affect Disord 150: 384-388, 2013.
38. González-Pinto A, Mosquera F, Reed C, Novick D, Barbeito S, Vega P, Bertsch J, Alberich S and Haro JM: Validity and reliability of the Hamilton depression rating scale (5 items) for manic and mixed bipolar disorders. J Nerv Ment Dis 197: 682-686, 2009.
39. Ferrucci L, Maggio M, Bandinelli S, Basaria S, Lauretani F, Ble A, Valenti G, Ershler WB, Guralnik JM and Longo DL: Low testosterone levels and the risk of anemia in older men and women. Arch Intern Med 166: 1380-1388, 2006.
40. National Research Council (US). Committee for the Update of the Guide for the Care and Use of Laboratory Animals. Institute for Laboratory Animal Research (US) and National Academies Press (US): Guide for the care and use of laboratory animals 8th edition. National Academies Press, Washington (DC), 2011.
41. van Dam A: Subgroup analysis in burnout: Relations between fatigue, anxiety, and depression. Front Psychol 7: 90, 2016.
42. Wolff BS, Raheem SA and Saligan LN: Comparing passive measures of fatigue-like behavior in mice. Sci Rep 8: 14238, 2018.
43. Kurtz P, Schmidt M, Claassen J, Carrera E, Fernandez L, Badjatia N, Mayer S and Lee K: Anemia is associated with brain tissue hypoxia and metabolic crisis after severe brain injury. Crit Care 13 (Suppl 1): P92, 2009.
44. Gan L, Ma D, Li M, Yang FC, Rogers RS, Wheatley JL, Koch LG, Britton SL, Thyfault JP, Geiger PC and Stanford JA: Region-specific differences in bioenergetic proteins and protein response to acute high fat diet in brains of low and high capacity runner rats. Neurosci Lett 674: 49-53, 2018.
45. Ding F, Yao J, Rettberg JR, Chen S and Brinton RD: Early decline in glucose transport and metabolism precedes shift to ketogenic system in female aging and Alzheimer's mouse brain: Implication for bioenergetic intervention. PLoS One 8: e79977, 2013.
46. Price EA, Mehra R, Holmes TH and Schrier SL: Anemia in older persons: Etiology and evaluation. Blood Cells Mol Dis 46: 159-165, 2011.
47. Andrès E, Serraj K, Federici L, Vogel T and Kaltenbach G: Anemia in elderly patients: New insight into an old disorder. Geriatr Gerontol Int 13: 519-527, 2013.
48. Bower JE, Ganz PA, Irwin MR, Kwan L, Breen EC and Cole SW: Inflammation and behavioral symptoms after breast cancer treatment: Do fatigue, depression, and sleep disturbance share a common underlying mechanism? J Clin Oncol 29: 3517-3522, 2011.
49. Dantzer R: Cytokine, sickness behavior, and depression. Immunol Allergy Clin North Am 29: 247-264, 2009.
50. Dott W, Mistry P, Wright J, Cain K and Herbert KE: Modulation of mitochondrial bioenergetics in a skeletal muscle cell line model of mitochondrial toxicity. Redox Biol 2: 224-233, 2014.
51. Karamercan MA, Weiss SL, Villarroel JP, Guan Y, Werlin E, Figueredo R, Becker LB and Sims C: Can peripheral blood mononuclear cells be used as a proxy for mitochondrial dysfunction in vital organs during hemorrhagic shock and resuscitation? Shock 40: 476-484, 2013.
52. Ashrafi G, Wu Z, Farrell RJ and Ryan TA: GLUT4 mobilization supports energetic demands of active synapses. Neuron 93: 606-615.e3, 2017.
53. Alquier T, Leloup C, Lorsignol A and Pénicaud L: Translocable glucose transporters in the brain. Diabetes 55 (Suppl 2): S131-S138, 2006.
54. Kang I, Chu CT and Kaufman BA: The mitochondrial transcription factor TFAM in neurodegeneration: Emerging evidence and mechanisms. FEBS Lett 592: 793-811, 2018.
55. Chandrasekaran K, Anjaneyulu M, Inoue T, Choi J, Sagi AR, Chen C, Ide T and Russell JW: Mitochondrial transcription factor A regulation of mitochondrial degeneration in experimental diabetic neuropathy. Am J Physiol Endocrinol Metab 309: E132-E141, 2015.
56. Oka S, Leon J, Sakumi K, Ide T, Kang D, LaFerla FM and Nakabeppu Y: Human mitochondrial transcriptional factor A breaks the mitochondria-mediated vicious cycle in Alzheimer's disease. Sci Rep 6: 37889, 2016.
57. Dutta S and Sengupta P: Men and mice: Relating their ages. Life Sci 152: 244-248, 2016.
58. Ragnow S, Hooshdaran MZ, Sanjay K and Steiner MS: Toremifene prevents prostate cancer in the transgenic adenocarcinoma of mouse prostate model. Cancer Res 62: 1370-1376, 2002.
59. Wu CT, Chen WC and Chen MF: The response of prostate cancer to androgen deprivation and irradiation due to immune modulation. Cancers (Basel) 11: pii: E20, 2018.
60. Lin TH, Lee SO, Niu Y, Xu D, Liang L, Li L, Yeh SD, Fujimoto N, Yeh S and Chang C: Differential androgen deprivation therapies with anti-androgens casodex/bicalutamide or MDV3100/Enzalutamide versus anti-androgen receptor ASC-J9(R) lead to promotion versus suppression of prostate cancer metastasis. J Biol Chem 288: 19359-19369, 2013.