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Resveratrol inhibits the deleterious effects of diet-induced obesity on thymic function

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Abstract

Obesity is associated with an increased risk of infectious diseases. It has been shown to have deleterious effects on cell-mediated immunity, including reducing thymocyte numbers and altering responses of thymocytes to pathogens. In the current study, we examined the efficacy of the antiobesity phytochemical resveratrol in preventing the deleterious effects of a high-fat diet on thymic anatomy and function. Compared to C57Bl/6 male mice fed a low-fat diet, mice on a high-fat diet had a significant increase in thymic weight and lipid content, and a disrupted anatomy, including a reduction of the medullary compartment and absence of a corticomedullary junction. There were a decrease in thymic cellularity and mature T-cell output, and a disrupted T-cell maturation, as evidenced by increased double-negative and decreased single- and double-positive thymocytes. Mice that had been fed resveratrol along with a high-fat diet had a dose-dependent reversal in all these parameters. Western blots from thymi showed that obese mice had lower levels of the key stimulators of lipid metabolism, phospho-5′ adenosine monophosphate-activated protein kinase and its downstream target, carnitine palmitoyl transferase-1; this was restored to normal levels in resveratrol-fed mice. Resveratrol also reversed an increase in glycerol-3-phosphate acyltransferase-1, the enzyme that catalyzes the first step in triglycerol synthesis. Taken together, these results indicate that resveratrol is a potent inhibitor of the deleterious effects of diet-induced obesity on thymic anatomy and function, and this may hold promise in preventing obesity-related deficits in cell-mediated immunity.

Keywords: Thymus; Mouse; Obesity; Resveratrol; T cell; Diet

1. Introduction

The thymus plays a critical role in cell-mediated immunity by providing an environment in which T cells can differentiate and mature before being released into the blood. The thymus involutes during aging, fat accumulation replaces the active mass of the thymus, and T-cell generation declines, resulting in immunosenescence [1]. Thus, susceptibility to infections increases with age. Obesity, like aging, is associated with increased susceptibility to infections [2–4]. Recent work has demonstrated that diet-induced obesity adversely affects the thymus, causing premature involution and fat accumulation, with a resulting decline in T-cell production [5]. Indeed, it has been hypothesized that the adipocyte is an active driver of thymic involution, not merely a passive participant [5]. Calorie restriction, on the other hand, inhibits thymic adipogenesis and reduces age-related thymic involution [6]. Thus, the increasing prevalence of obesity, particularly in children [7], has considerable implications for adaptive immunity. Strategies to reverse or inhibit obesity-induced thymic fat accumulation and the resulting decline in thymic function may be efficacious in protecting lifelong cell-mediated immunity.

Resveratrol (3,5,4′-trihydroxystilbene) is a naturally occurring polyphenol found abundantly in the skin of grapes, peanuts and berries. Resveratrol has been shown to prevent diet-induced obesity in young mice and reverse the deleterious effects of obesity in older mice [8], including obesity-induced insulin resistance, hyperglycemia and dyslipidemia. Resveratrol extends the life span of obese mice but does not affect the life span of normal-weight mice, suggesting that its effects are mediated by reversal of obesity-induced changes [9]. Resveratrol prevents an increase in liver size and hepatic lipid accumulation in mice on a high-fat diet [9], demonstrating the potential benefits of resveratrol on specific organ pathology. These findings in mice have been corroborated in obese humans in a recent study using low-dose resveratrol supplementation for 30 days [10]. To the best of our knowledge, the effects of resveratrol on obesity-induced changes in thymic structure and function have not been studied. Therefore, we designed the experiments in the current study to confirm the obesity-induced deleterious effects of a high-fat diet on the thymus in mice first reported by Yang et al. [5] and to test the possible efficacious effects of resveratrol in restoring thymic function. We demonstrate, for the first time, that resveratrol can inhibit obesity-induced changes in thymic anatomy and function and restore T-cell production.

Abbreviations: AMPK, 5′ adenosine monophosphate-activated protein kinase; CPT-1, carnitine palmitoyl transferase-1; DN or DP, double negative or double positive; GPAT-1, glycerol-3-phosphate acyltransferase-1; SP, single positive.

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2. Experimental procedure

2.1. Animals and study design

C57Bl/6 mice (4 weeks of age) were purchased from Jackson Laboratory (Bar Harbor, MA, USA). All animal procedures followed current regulations on animal experimentation approved by the University of Texas Animal Use and Care Committee. Mice were singly housed on a 12-h light/dark cycle. After 1 week, mice were randomly assigned to a low-fat diet (10% energy from fat; LF) or a high-fat diet (60% energy from fat; HF). Diets were from Research Diets (New Brunswick, NJ, USA) and were fed *ad libitum* for 10 weeks. Mice received 0, 200 (low resveratrol; LR) or 400 (high resveratrol; HR) mg/kg diet (n=10 mice/group). Fresh food was provided twice weekly. Mice were sacrificed by CO_2 inhalation followed by cervical dislocation. Fresh thymi were harvested, and whole blood was collected by cardiac puncture. Body weight was measured weekly; percent body fat was determined by dual-energy X-ray absorptiometry (DEXA) using a Lunar PIXImus Densitometer (GE Medical Systems, Madison, WI, USA).

2.2. Thymocyte isolation and subset analysis

Thymocytes were isolated as described previously [11]. Cell viability was confirmed by trypan blue exclusion, and cell counts were determined by CellCyn 900 Hematology Analyzer (Sequoia-Turner Corp., Mountainview, CA, USA). T-cell subsets were analyzed by flow cytometry as previously described [12]. Cells were stained with APC-conjugated anti-mouse CD4, PE-conjugated anti-mouse CD8a, APC-conjugated anti-mouse CD44 and PE-conjugated anti-mouse CD25 (BD Biosciences, San Diego, CA, USA). After 30 min on ice, excess antibody was removed by washing three times with phosphate-buffered saline (PBS) with sodium azide, vortexed and centrifuged. The cells were then resuspended in 1 ml of PBS + 5% fetal bovine serum and analyzed using the using an Accuri C6 flow cytometer (Accuri Cytometers Inc. Ann Arbor, MI, USA) (n=10 mice/group).

2.3. T-cell receptor excision circle (TREC) analysis

TREC analysis was performed essentially as previously described [13]. DNA was isolated from 300 μl whole blood (n=10 mice/group) and purified using a Promega Genomic DNA Purification Kit (Promega Corporation, Madison, WI, USA). DNA was quantified by a NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies, Inc., Wilmington, DE, USA). sjTRECs in the purified DNA were detected by real-time polymerase chain reaction (PCR) based on SYBR Green I fluorescence. The analysis was performed in a 96-well plate with 25-μl reactions containing 12.5 μl of Fast SYBR Green Master Mix, 0.4 μM each of sjTREC forward 5′-CCAAGCTGACGGCAGGTTT-3′ and sjTREC reverse 5′-AGCATGGCAAGCAG-CACC-3′ custom primers, and 10.5-μl mixture of 0.1 ng purified DNA and DEPC-treated water. To compensate for variations in input DNA, GAPDH forward 5′-GTGAGGCCGGTGCTGAGTAT-3′ and reverse 5′-TCATGAGCCCTTCCA-CAATG-3′ primers at a concentration of 0.4 μM were used as internal control or housekeeping gene for normalization of the real-time PCR data. All reagents and custom primers were from Applied Biosystems (Foster City, CA, USA). The plate was briefly centrifuged, and the PCR was run using the Eppendorf Mastercycler ep gradient S Thermocycler (Eppendorf North America, Inc. Hauppauge, NY, USA). The thermocycling conditions used were 95°C for 20 s for enzyme activation followed by 40 cycles of denaturing at 95°C for 1 s and annealing/extension at 60°C for 20 s. Each of the reactions was run in triplicate, and a minimum of three separate experiments were carried out for each sample. Relative quantification (RQ) of PCR for different treatments was performed using the cycle threshold (Ct) values. First, the ΔCt value for each sample was obtained by subtracting the Ct of the gene of interest (sjTREC) from that of the housekeeping gene (GAPDH). The ΔΔCt values were finally calculated as the difference between the ΔCt values of treatment and control groups.

2.4. Thymic architecture analysis by hematoxylin and eosin (H&E) and immunohistochemical staining

Thymi (n=5 mice/group) were frozen or formalin fixed and sent to MD Anderson Science Park (Smithville, TX, USA) Histology Core Facility for processing. Briefly, formalin-fixed tissue was embedded in paraffin and cut into 4-μm-thick sections for H&E and immunohistochemical staining. The slides were deparaffinized in xylene and hydrated in ethanol. Endogenous peroxidase activity was then blocked, followed by exposing of antigenic sites. The slides were then blocked with casein in buffer for 10 min, drained and stained with primary antibodies for differences in thymic cortex and medulla (keratins 8 and 5), proliferation (Ki67) and apoptosis (TUNEL) for 30 min. The slides were then washed with buffer and incubated anti-rabbit/immunoglobulin F (IgG) horseradish peroxidase polymer for 30 min. The slides were then washed, counterstained and dehydrated.

2.5. Western blot analysis

Lysates were prepared (n=10 mice/group), protein was quantified using BCA assay kit (Thermo Scientific, Rockford, IL, USA), and Western immunoblot was performed as we previously described [14]. Briefly, lysates were diluted to 3 μg/μl with distilled water and sample buffer, and 50 μg of protein was loaded per well and subjected to electrophoresis in 0.1 M Tris–0.1 M HEPES–3 mM sodium dodecyl sulfate. Proteins were transferred to a PolyScreen PVDF membrane (NEN Life Sciences, Boston, MA, USA), and the membrane was blocked using 5% Blotto. The membrane was probed overnight with a diluted primary antibody [glycerol-3-phosphate acyltransferase-1 (GPAT-1) (1:1000), carnitine palmitoyl transferase-1 (CPT-1) (1:500), p-5′ adenosine monophosphate-activated protein kinase (AMPK) (1:500) or β-actin (1:1000)] at 4°C. Following the overnight incubation, the membrane was washed thrice with a solution of 1× PBS–0.1% Tween and probed with diluted goat polyclonal secondary antibody to rabbit IgG (Abcam, Cambridge, MA, USA) (1:5000) for 90 min. The blot was then washed as mentioned above and incubated in 1-Step NBT/BCIP solution for approximately 15 to 20 min until desired color had developed. The membrane was finally rinsed with ddH2O and imaged using Syngene. All above reagents and supplies were purchased from Thermo Scientific (Rockford, IL, USA) unless otherwise specified.

2.6. Statistical analyses

Data were analyzed using one-way analysis of variance. Statistical significance was determined using Tukey's multiple comparison test with GraphPad Prism software (San Diego, CA, USA). The level of significance was set at $P \leq .05$ in all analyses.

3. Results

3.1. Effect of resveratrol on body weight and percent body fat

Mice on an HF diet for 10 weeks were significantly heavier than their LF counterparts (Fig. 1A and B). Supplementation of the HF diet with resveratrol reduced body weight gain (Fig. 1A and B). Mice fed an HF diet had significantly increased percent body fat compared to LF-fed mice, and percent body fat was reduced in a dose-dependent manner by resveratrol supplementation (Fig. 1C and D). Resveratrol

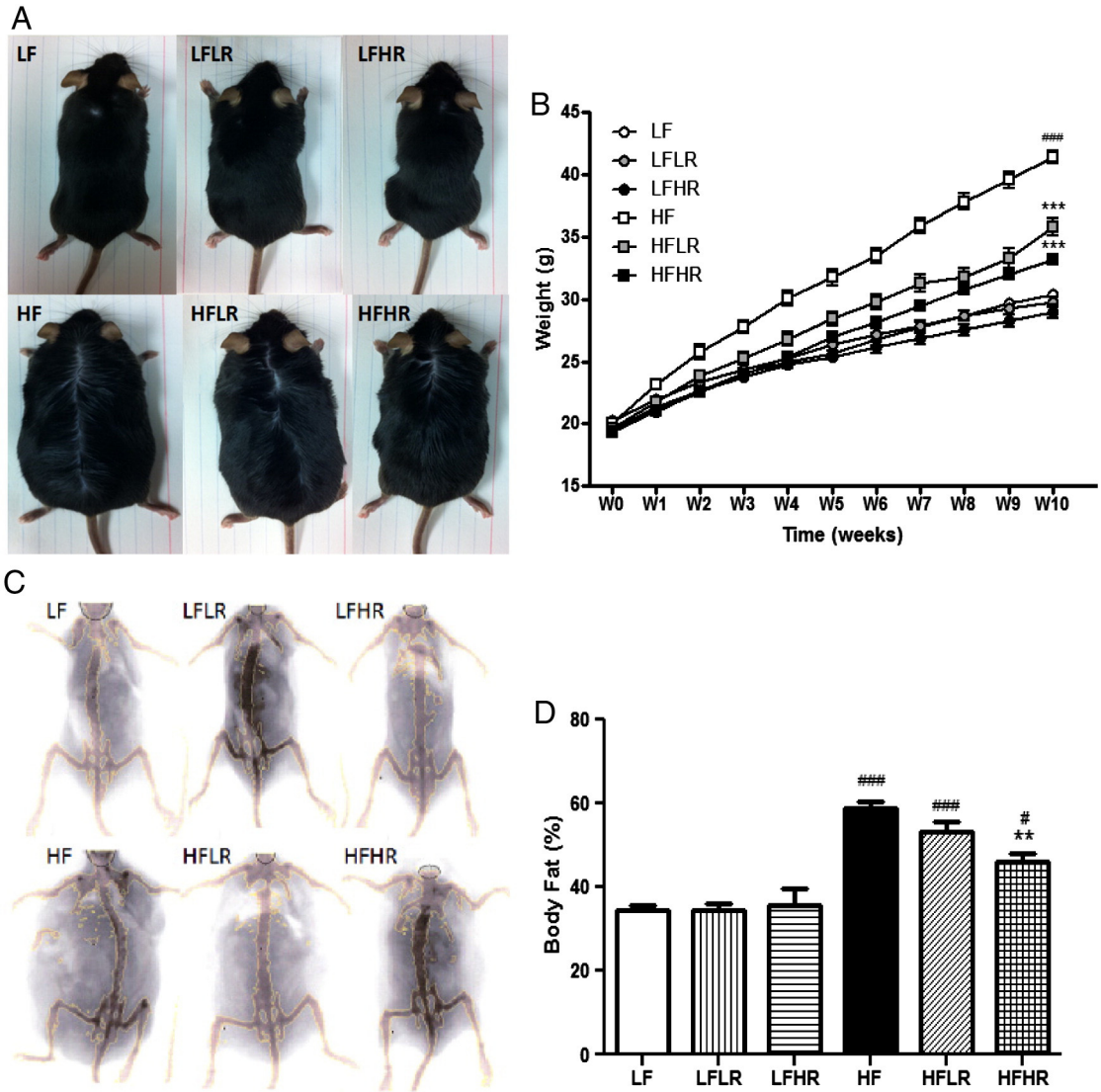

Fig. 1. Resveratrol inhibited the increase in body weight and percent body fat of mice on an HF diet. C57BL/6 mice were fed LF or HF diet in the presence or absence of low resveratrol (LR; 200 mg/kg diet) or high resveratrol (HR; 400 mg/kg). Body weights were recorded weekly (A), and the percent body fat was measured by DEXA at the end of the diet treatment period. Each bar represents the mean ± S.E.M. of 10 individual mice. *P<.05; **P<.01; ***P<.001. $^{@}$, $^{\#}$ and * indicate differences within LF groups, between LF and HF groups, and within HF groups, respectively.

had no effect on body weight or percent body fat in mice fed an LF diet (Fig. 1A–D).

3.2. Effect of resveratrol on thymic weight, lipid accumulation and T-cell number

The weight of fresh thymi isolated from mice on the HF diet was significantly heavier than that of mice from the LF group (Fig. 2A). Resveratrol reduced thymus weight in the HF group in a dose-dependent manner but did not affect the thymus weight in the LF group (Fig. 2A). Total thymic T-cell numbers were significantly reduced in the HF group compared to the LF mice, but T-cell numbers were enhanced in the HF group fed the higher dose of resveratrol (Fig. 2B). There was no change in T-cell numbers due to resveratrol in the LF group (Fig. 2B). There was a profound increase in lipid content of the thymi from mice fed an HF diet compared to their LF counterparts (Fig. 3). Resveratrol feeding markedly inhibited lipid accumulation in a dose-dependent manner (Fig. 3) but had no effect on LF-fed mice.

3.3. Effect of resveratrol on thymic anatomy

The thymi from mice fed an HF diet evinced a breakdown of normal thymic anatomy. The corticomedullary junction was absent, and the medullary compartment was much reduced compared to LF mice (Fig. 4). Resveratrol at both doses tested prevented these HF-induced changes in thymic anatomy (Fig. 4). Immunohistochemical staining of keratin-8, a marker of cortical epithelial cells, was greatly reduced in HF-fed mice compared to LF mice, indicating a loss of cortical epithelial cells (Fig. 5A). This was prevented in HF mice fed resveratrol. Similarly, medullary epithelial cells, stained by keratin-5, were much reduced in HF mice, but there was no difference from LF mice in mice fed HF with resveratrol (Fig. 5B).

3.4. Effects of resveratrol on T-cell maturation and output

T-cell maturation is a complex, multistep process in the thymus. The four basic stages of thymic T-cell maturation are identified by the expression, or the lack of it, of the CD4 and CD8 plasma membrane

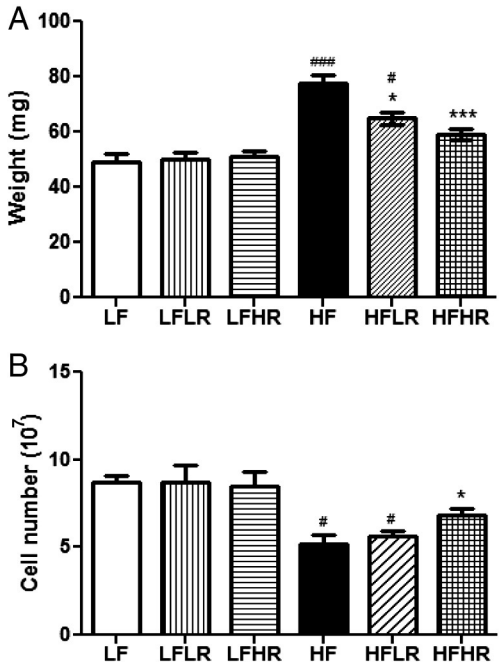

Fig. 2. Resveratrol prevented the increase in thymic weight and decrease in thymic cellularity caused by HF diet. Thymus weights were recorded at the end of the study (A), and the cellularity was obtained by counting isolated thymic T cells on a hemocytometer using trypan blue exclusion. Each bar represents the mean ± S.E.M. of 10 individual mice. *P<.05; **P<.01; ***P<.001. @, # and * indicate differences within LF groups, between LF and HF groups, and within HF groups, respectively.

receptors. Progenitor cells enter the thymus as double-negative cells (DN; $CD4^-$,$CD8^-$) then become double positive (DP; $CD4^+$,$CD8^+$) followed by development into either single-positive (SP) $CD4^+CD8^-$ helper T cells or SP $CD4^-CD8^+$ cytotoxic T cells. The steps within the DP stage can be further subdivided based on the expression of the CD44 and CD25 plasma membrane receptors. The DN1 stage is $CD44^+$,$CD25^-$; DN2 is $CD44^+$,$CD25^+$; DN3 is $CD44^-$,$CD25^+$; DN4 is $CD44^-$,$CD25^-$. Flow cytometric analysis of the various stages of T-cell maturation revealed that mice on an HF diet had a significantly increased double-negative subset of T cells compared to LF mice (Fig. 6A). The double-negative-4 subset was the only significantly increased subset in HF mice (Fig. 6B). There were a decrease in double-positive and SP-8 T cells in HF mice and no change in SP-4 T cells (Fig. 6A). Resveratrol decreased the DN subset in both LF and HF mice, restored the DP subset in HF mice and reduced the numbers of DN4 cells in a dose-dependent manner (Fig. 6A and B). The output of recently generated T cells into the blood stream was measured by peripheral lymphocytes bearing T-cell receptor excision circles [9]. As shown in Fig. 7, there was a significant decrease in output of newly generated T cells in mice on an HF diet; resveratrol restored this output, and the higher tested dose also increased T-cell output in LF-fed mice. An HF diet did not alter the percent of T cells undergoing apoptosis, as measured by TUNEL assay, and resveratrol also had no effect on the apoptotic index (data not shown).

3.5. Effects of resveratrol on key lipid-regulating enzymes

As determined by Western blot, there was a significant reduction in the amount of p-AMPK in the thymus of HF-fed mice (Fig. 8A and B). Resveratrol treatment was associated with an increase in p-AMPK levels in HF- but not LF-fed mice (Fig. 8A and B). Thymic CPT-1 was

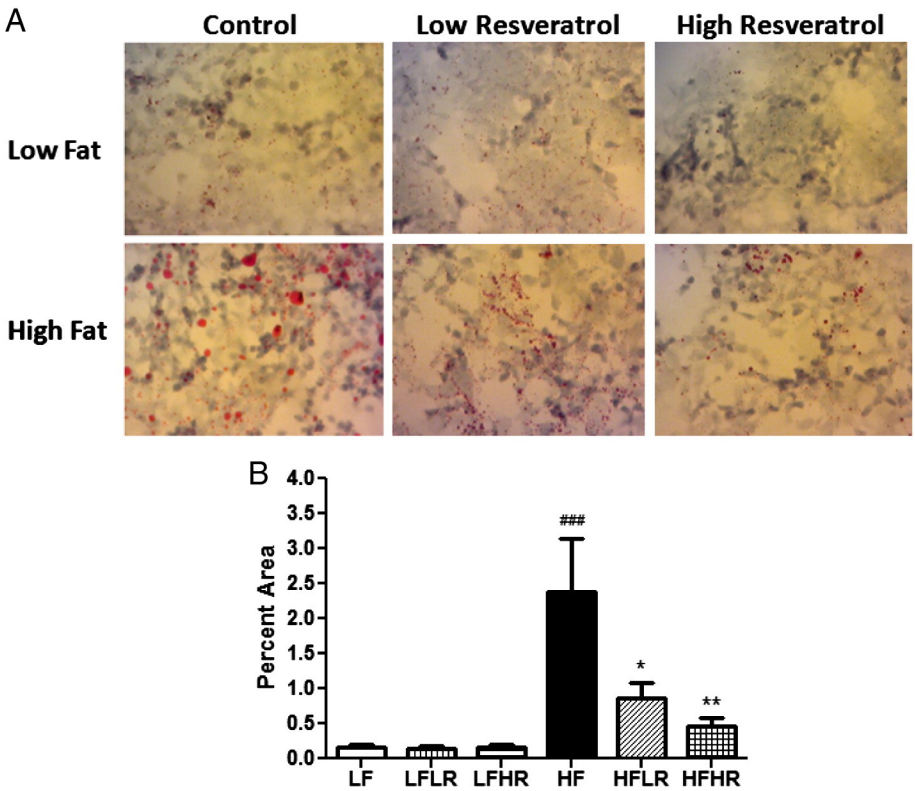

Fig. 3. Resveratrol inhibited the accumulation of lipid droplets in HF mice. Frozen sections of thymi were stained with Oil Red O to measure the amount of fat accumulation within the active mass of the thymus. The density of the red-stained lipid droplets was measured using Image Pro Plus. Each bar represents the mean ± S.E.M. of five individual mice. *P<.05; **P<.01. @, # and * indicate differences within LF groups, between LF and HF groups, and within HF groups, respectively.

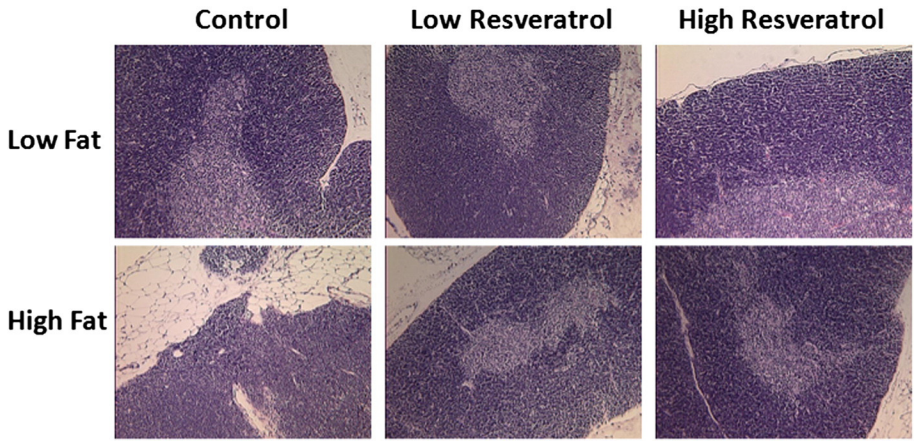

Fig. 4. Resveratrol inhibited the changes in thymic anatomy caused by an HF diet. Paraffin-embedded sections of thymi were deparaffinized, stained with H&E and analyzed for changes in the architecture of the thymus. The sections are representative of five individual mice.

reduced by an HF diet; resveratrol cofeeding caused a nonsignificant trend restoring CPT-1 levels (Fig. 8A and C). GPAT-1 was induced in HF mice, which was inhibited in resveratrol-fed mice (Fig. 8A and D).

4. Discussion

Thymic aging, or involution, is characterized by fat accumulation, a loss of normal thymic anatomy and reduced T-cell production. This leads to immunosenescence in later life. In humans, the thymus is most active in childhood and undergoes involution starting as early as 1–2 years of age [1]. Since fat accumulation is a normal part, and perhaps a driver of, thymic aging, childhood obesity, a growing problem in developed countries, may compromise lifelong immunocompetence by causing premature involution. Although it is known that obesity increases the risk of infections and some cancers, until recently, it was not known whether obesity exerts deleterious effects on the thymus. However, a seminal paper by Yang et al. [5] clearly demonstrated that obesity causes a premature involution of the thymus in a mouse model and a decrease in the thymic output of T cells in humans. Strategies to counteract the effects of obesity on the thymus may ameliorate its effects on immunity. Therefore, we tested the hypothesis that resveratrol, which has been shown to inhibit other obesity-related maladies [8,9,15], prevents obesity-induced changes in thymic aging and function. The doses of resveratrol used in our study, 200 or 400 mg/kg diet, are similar to those of several large mouse studies that examined its effects on age- and diet-associated deficits [8,9,15]. Recently, it was demonstrated that resveratrol is bioactive in obese humans and triggers many beneficial changes similar to those seen in mice [10]; in fact, higher plasma levels of resveratrol were achieved at much lower dietary doses in humans than in mice, alleviating fears that resveratrol is not bioavailable in humans.

We fed a standard LF or HF diet to male mice for a period of 10 weeks, to an age of 15 weeks, at the end of which there was a significant difference in body weight and percent body fat between the groups. Our study's endpoint was much earlier than that of Yang et al., who kept mice on similar diets for 13 months [5]. The endpoint in our study did not correspond with human childhood, as male mice are sexually mature at 6–8 weeks, but it is fair to say that these were young mice, compared to the "middle aged" mice in the Yang et al. study [5]. However, in terms of "thymic age," 15-week-old mice do correspond to human children when one considers that the murine thymic involution begins at approximately 6–10 weeks of age in mice, which correspond to approximately 1–2 years of age in humans [1,16]. Thus, our study was designed, as closely as experimentally practicable, to model the effects of childhood obesity on thymic function. It is interesting to note that many of the deleterious effects of an HF diet on the thymus first reported in the Yang et al. paper were reproduced at the much younger age and far fewer weeks of feeding in our study. After only 10 weeks on an HF diet, thymic anatomy was severely disrupted, lipid accumulation was pronounced, the thymus was enlarged, cellularity was reduced, and thymocyte maturation was affected. Therefore, obesity in mice at a young age had a profound effect on the thymus, raising the possibility that childhood obesity may have similar profound implications. Resveratrol was remarkably effective in preventing these deleterious effects. In every parameter examined, resveratrol significantly and substantially inhibited the deleterious effects of an HF diet on the thymus. It decreased HF-induced thymic weight, increased T cellularity, reduced lipid accumulation, prevented the disruption of normal thymic anatomy and protected cortical and medullary epithelial cell numbers. Resveratrol restored the normal maturation of T cells, as demonstrated in the subset analysis, and protected the release of new T cells into the blood.

The question arises as to whether the beneficial effects of resveratrol are secondary to the decreased body weight and percent body fat demonstrated in Fig. 1. However, the data do not support that conclusion as the sole mechanism of action. There was no significant difference in body weight between the low- and high-resveratrol groups, yet in most assays, resveratrol exerted a dose-dependent protective effect. Furthermore, there was no difference in percent body fat between the LF and HF + low resveratrol, yet low resveratrol exerted significant protective effects. These data would suggest that resveratrol exerts its protective effect independent of its effect on body weight, although it is possible that the prevention of total weight gain contributes to the protective effect on the thymus.

It has been established that resveratrol and other phytochemicals exert pleiotropic effects on organisms and cells [17]. Resveratrol has been shown to be a potent antioxidant and to induce antioxidant enzymes [18], to inhibit various signal transduction pathways relevant to carcinogenesis [19], to mimic the effect of calorie restriction on gene transcription [15] and to induce fatty acid oxidation by elevating p-AMPK levels [20]. As a preliminary attempt to identify the possible mechanism of action, we examined the effect of resveratrol on the protein levels of key enzymes involved in energy regulation and lipid metabolism. p-AMPK is an energy sensor that is thought to be a central mechanism by which calorie restriction extends life span. An HF diet profoundly inhibited p-AMPK in the thymus; resveratrol restored p-AMPK levels in a dose-dependent manner. CPT-1 is the enzyme that transports fatty acids into the mitochondria for

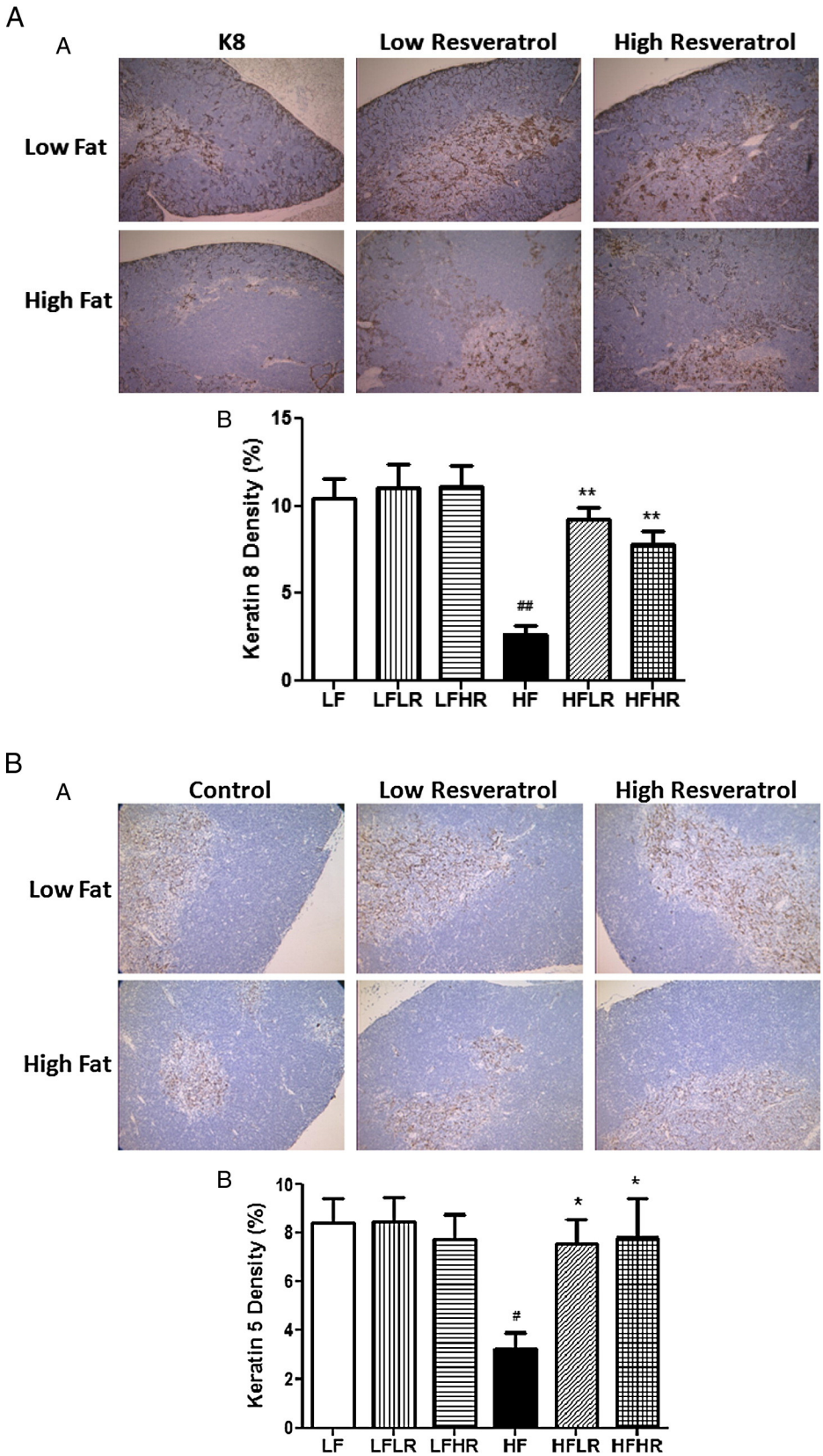

Fig. 5. Resveratrol protected cortical and medullary epithelial cells. Paraffin-embedded sections of thymi were deparaffinized and stained with antibody to keratin 8 to assess changes in the cortical epithelial cells (A) or stained with keratin 5 antibody for medullary epithelial cells (B). The density of the brown keratin-stained cytoplasms was measured using Image Pro Plus. Each bar represents the mean ± S.E.M. of five individual mice. $^{**}P<.01$. $^{@}$, $^{\#}$ and * indicate differences within LF groups, between LF and HF groups, and within HF groups, respectively.

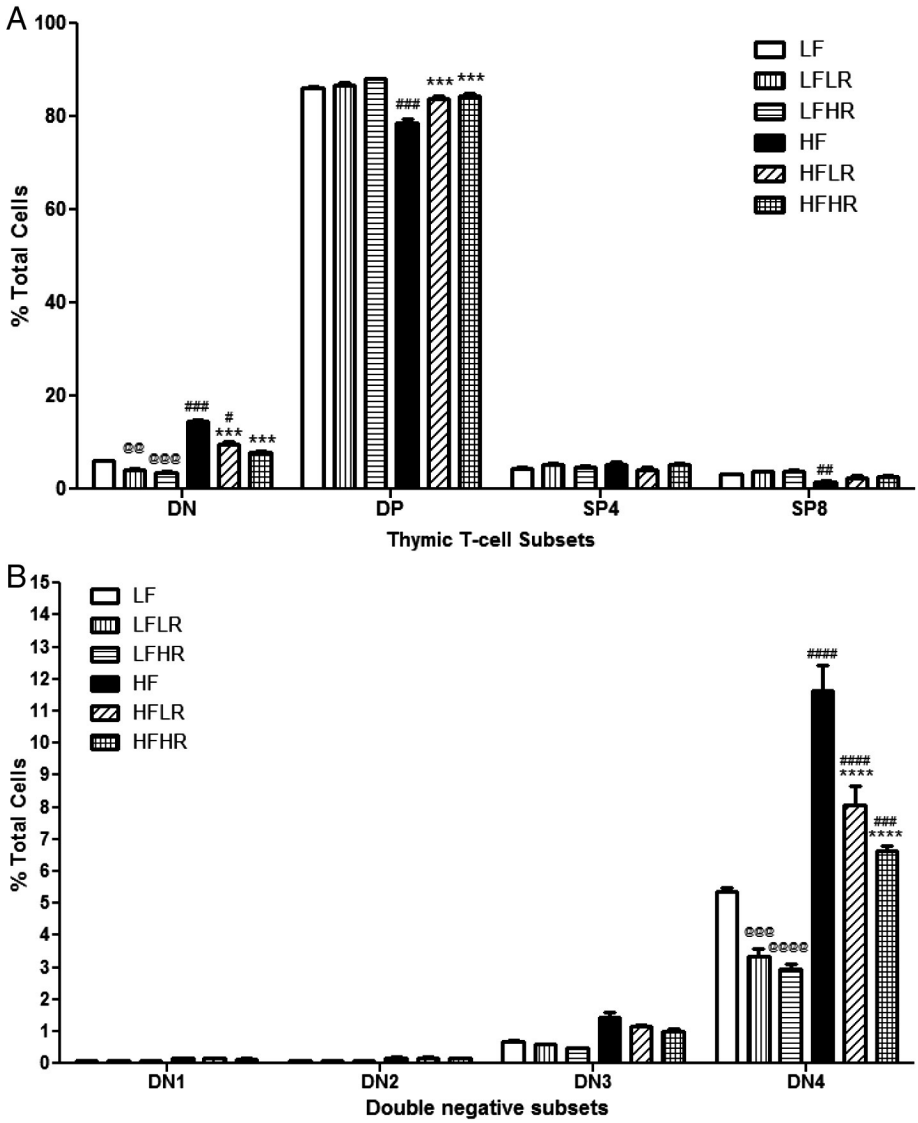

Fig. 6. Resveratrol prevented HF-induced changes in T-cell maturation. Isolated thymocytes were stained with anti-CD4 and anti-CD8 to differentiate between the major subsets (A), and anti-CD44 and anti-CD25 to identify changes in the double-negative stages (B). The T cells were analyzed using a flow cytometer. Each bar represents the mean ± S.E.M. of 10 individual mice. *P<.05; **P<.01; ***P<.001; ****P<.0001. @, # and * indicate differences within LF groups, between LF and HF groups, and within HF groups, respectively.

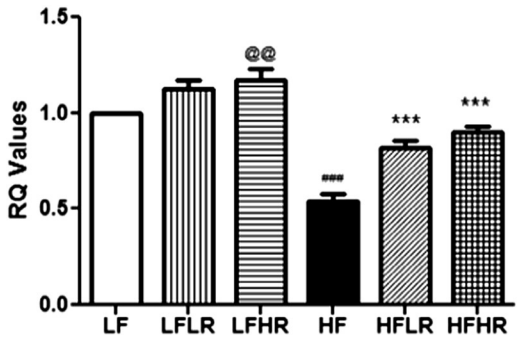

Fig. 7. Resveratrol feeding increased thymic output of new T cells. DNA was purified from whole blood and analyzed for recent thymic emigrants by measuring the signal join T-cell receptor excision circles (SjTRECs) present were detected by real-time PCR based on SYBR Green I fluorescence. The data are expressed as RQ of the SjTRECs gene relative to the GAPDH housekeeping gene calculated as described in the Experimental Procedures. Each bar represents the mean ± S.E.M. of 10 individual mice. **P<.01; ***P<.001. @, # and * indicate differences within LF groups, between LF and HF groups, and within HF groups, respectively.

oxidation. An HF diet dramatically reduced CPT-1 levels, while resveratrol restored CPT-1 levels in a dose-dependent manner. GPAT-1 is the first and rate-limiting step in *de novo* phospholipid and triglyceride biosynthesis. In contrast to CPT-1 and p-AMPK, HF feeding increased GPAT-1 levels, and resveratrol lowered GAPT-1 in a dose-dependent manner. This is the first time that resveratrol has been shown to shift lipid metabolism in a component of the immune system away from biosynthesis to one favoring fatty acid breakdown (oxidation) [9]. Therefore, this provides a molecular mechanism by which resveratrol may prevent fat accumulation in the thymus and subsequent reduction in T-cell production. This is important because obesity has been shown to dysregulate T-cell generation, function and maintenance, thereby impairing the ability to promote a peripheral T-cell-mediated protective immune response, as seen in the case of influenza virus, impaired wound healing and infection [4,21,22]. Resveratrol has been shown to promote the immune response by improve splenic T-cell function [23]. Resveratrol also suppresses tumor-derived regulatory T cells, which are negative regulators of the immune system [24]. In addition, resveratrol has also been shown to maintain T-cell tolerance in mice by regulating the

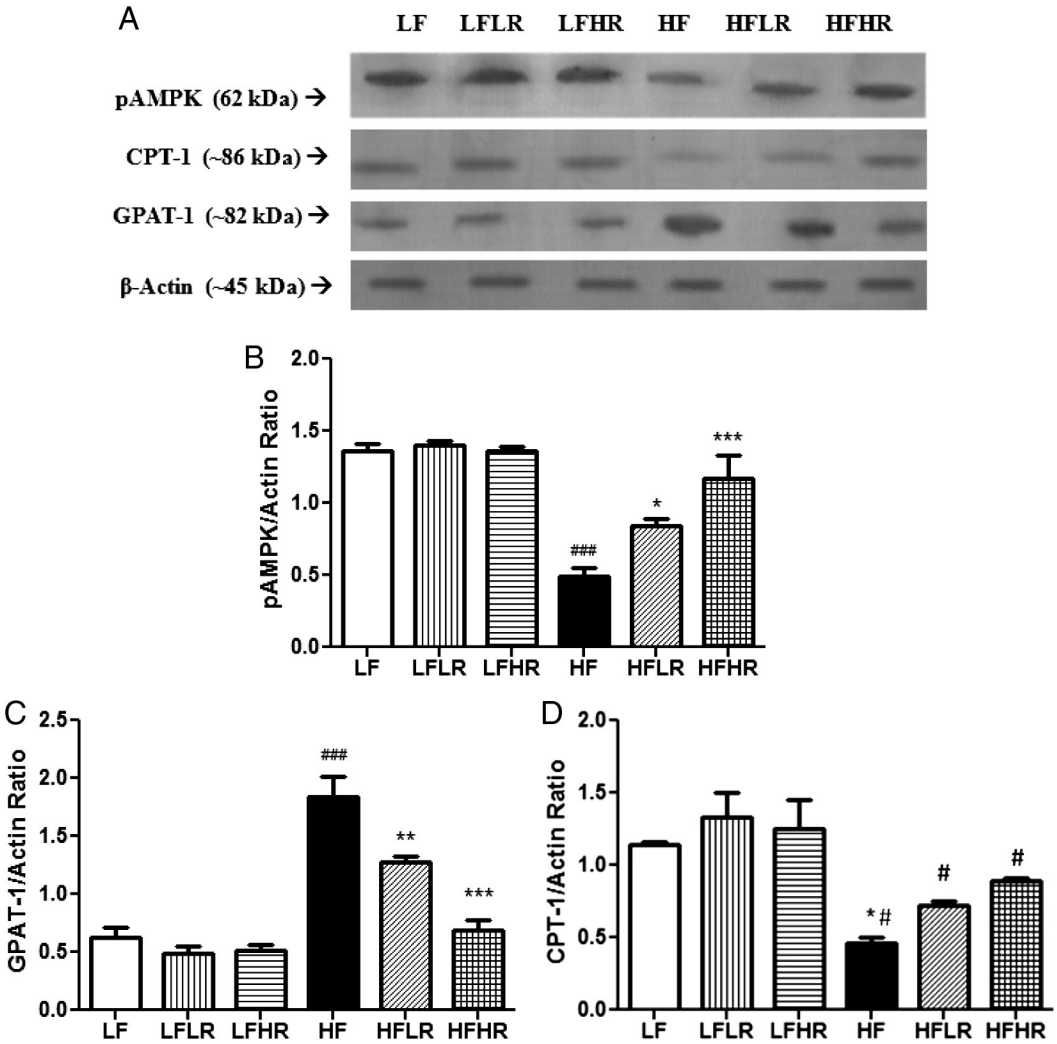

Fig. 8. Effects of resveratrol on protein levels of key lipid metabolic enzymes. Changes in lipid metabolism were analyzed by measuring the activity of p-AMPK, GPAT-1 and CPT-1 in protein fractions purified from the thymus of mice that were fed LF and HF diet or an LF/HF diet with a low (200 mg/kg; LR) or high (400 mg/kg; HR) dose of resveratrol. β-Actin was used as a loading control. Differences in band density were measured using Syngene. Each bar represents the mean ± S.E.M. of 10 individual mice. *P<.05; **P<.01; ***P<.001. $^{\Phi}$, $^{\#}$, and * indicate differences within LF groups, between LF and HF groups, and within HF groups, respectively.

function of sirtuin 1, a type III histone deacetylase, which inhibits activation of self-reactive T cells that escape negative selection in the thymus [25]. Therefore, resveratrol has been shown to enhance peripheral T-cell-mediated immunity in normal-weight mice, but it is not known whether resveratrol can reverse the deleterious effects of obesity on peripheral T-cell function.

Of particular note in our results is the protective effect of resveratrol in preserving cortical and medullary epithelial cell numbers. Interactions of these epithelial cells with developing thymocytes are crucial in thymocyte maturation, and the loss of epithelial cell numbers in mice on an HF diet may be one reason that thymocyte numbers decline in obese mice. Preservation of epithelial numbers by resveratrol, as demonstrated in Fig. 5, in combination with inducing lipid catabolism over anabolism, may in part be the reason that resveratrol maintains T-cell numbers and restores proper maturation. Resveratrol has been shown to protect some cells, such as neurons [20], by inducing mitochondrial biogenesis, although we did not examine this in our study.

The lifelong consequences of premature thymic involution caused by diet-induced obesity in childhood have profound implications for human health. Our study demonstrates, for the first time, that resveratrol is a potent inhibitor of the deleterious effects of an HF diet on thymic anatomy and function and may help preserve cell-mediated immunity in later life. A translational benefit to our study is that the antiobesity effects of resveratrol were observed while the mice consumed a high-fat diet, meaning that an obese human could still receive at least some of the beneficial effects without other dietary modifications. This is especially important as the scientific and clinical communities have begun testing multiple compounds to delay thymic involution (i.e., aging) in an attempt to preserve or improve immune function; however, each compound may have significant negative side effects or may not be as efficacious as hoped [26]. The doses of resveratrol used in our study are equivalent to an effective human dose, in the average 60-kg adult, of 970 mg (low dose) and 1945 mg (high dose) resveratrol per day and 480 mg (low dose) and 960 mg (high dose) per day in a 20-kg child. This calculation corrects for differences in body surface area in order to get a more accurate extrapolation to a human equivalent dose as suggested by Reagan-Shaw et al. [27]. These doses of resveratrol would be feasible through supplementation. However, it is noteworthy that a considerably smaller dose of 150 mg resveratrol per day in humans, as compared to 200–400 mg/kg/day in ours and other murine studies [8,9], supplemented for 30 days has been shown to activate AMPK and improve mitochondrial respiration from a fatty acid substrate.

In addition, this 133- to 266-fold lower dosage in humans also lowered intrahepatic lipid content [10], suggesting a higher potency or bioavailability in humans compared to mice. A recent report in obese humans showing beneficial effects of resveratrol, with no apparent side effects [10], highlights the potential of resveratrol as a viable option for assisting in maintaining optimum immune function as well as inhibiting other obesity-associated maladies.

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