Serum Testosterone Associates With Lower High-Density Lipoprotein Cholesterol in Black and White Males, 10 to 15 Years of Age, Through Lowered Apolipoprotein AI and AII Concentrations

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High-density lipoprotein cholesterol (HDL-C) concentrations decrease during adolescence in males in association with increasing pubertal maturation and free testosterone (F-T). To determine whether F-T effects lower HDL-C levels by decreasing the amount of cholesterol associated with the major protein moeities associated with HDL-C (apolipoprotein [apo]AI and AII) or by decreasing the concentrations of these proteins, we studied 251 black and 285 white boys, ages 10 to 15 years. In cross-sectional analysis, advancing puberty associated with decreasing HDL-C, apoAI, and apoAII in boys of each ethnic group. The decreases were greater in white (1.49 to 1.24 mmol/L) than black boys (1.68 to 1.53 mmol/L). Backward stepwise regression analyses indicated that F-T was a significant negative predictor of all 3 lipid parameters—HDL-C, apoAI, and apoAII. Ethnic group was associated with HDL-C (blacks higher) and apoAII (whites higher), but not apoAI. The ratio of HDL-C to apo (AI+AII) varied significantly (and negatively) with body mass index (BMI; kg/m^2), but not with pubertal stage or F-T. Thus, increased F-T appears to explain decreased HDL-C via decreased apoAI and apoAII, not decreases in the amount of cholesterol associated with these proteins.

THE ADOLESCENT decline in high-density lipoprotein cholesterol (HDL-C) in males profoundly alters the risk of coronary heart disease relative to females.[1-3] An earlier report from the Sex Hormones and Lipoproteins in Adolescent Males study showed that changes in sex hormones and body mass play significant roles in the changes in male atherogenic lipid parameters and the low-denisty lipoprotein cholesterol (LDL-C)/HDL-C ratio. Increased free testosterone (F-T) levels associated significantly with lower HDL-C and higher ratios of LDL-C/HDL-C, while increased estradiol (E_2) associated with lower ratios.[4] Studies of pubertal changes in HDL-C and apolipoproteins (apo) AI and AII, the primary protein moeities associated with HDL-C, have not defined definitively the effects of sex hormones on these lipid parameters.[5,6] Nor have they determined whether F-T decreases HDL-C by decreasing concentrations of apoAI and apoAII or by decreasing the amount of cholesterol associated with these proteins. To explicate the effects of F-T and E_2 on HDL-C, apoAI, and apoII, and their interrelationships in males, we studied black and white boys 10 to 15 years of age. We hypothesized that increasing F-T would associate with decreases in both apoAI and AII and the amount of cholesterol per apoAI and AII.

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MATERIALS AND METHODS

Study Population

The Sex Hormones and Lipoproteins in Adolescent Males Study has been described previously.[3] Briefly, black and white boys in grades 5 to 9 at selected Cincinnati area schools were recruited in 1984, targeting ages 10 to 15 years. Data on subject's date of birth and ethnic group were collected from the subject's parents at the time signed informed consent was obtained. The University of Cincinnati College of Medicine Institutional Review Board approved the protocol.

Clinical Assessments

The clinical measurements have been described previously.[3] Briefly, height and weight were measured according to a standard protocol[3] and body mass index (BMI) was calculated as kg/m^2. The stages of puberty were scored by measurement of testicular volume compared to Prader beads and visual assessment of pubic hair following the modification of Tanner by Biro et al.[7] Stage 1 was no pubic hair and testicular volume less than 3 cc; stage 2a was no pubic hair and testicular volume 3 cc or greater; stages 2b, 3, 4, and 5 had pubic hair at Tanner stages 2, 3, 4, and 5, respectively. Stage 1 represents prepuberty, stages 2a and 2b represent early puberty, stages 3 and 4 represent mid-puberty, and stage 5 represents late puberty. A 10-mL quantity of blood was drawn into tubes containing EDTA for determination of lipids and 20 mL into empty tubes for hormone assays. Following blood aspiration, the vacutainers were kept cool and delivered to the laboratory within 2 hours.

Laboratory Measurements

Measurements of plasma total cholesterol, triglyceride, and HDL-C were performed in a National Heart, Lung, and Blood Institute–Centers for Disease control (NHLBI-CDC)–standardized Lipid Laboratory on a Hitachi 705 (Boehringer Mannheim, Indianapolis, IN) using enzymatic procedures for cholesterol and triglyceride measurement as well as triglyceride blanking, and the modified Lipid Research Clinics procedure (heparin–2 mol $MnCl_2$) to HDL-C.[8-10] ApoAI was assayed by electroimmunoassay[11] and apoAII by a monoclonal enzyme-linked immunosorbent assay (ELISA).[12] Total testosterone was quantitated following the method of Coyotupa et al[13] with the antibody used in this assay as described by Nolten et al.[14] F-T was assayed with a clinical adsorption assay according to Moll and Rosenfield.[15] E_2 was measured by competitive protein-binding methods .[16,17] The coefficients of vari-

Table 1. Mean ± SD Age, Body Composition Measures, Lipid Parameters, and Sex Steroid Hormones in Black and White Males, Ages 10 to 15 Years, By Ethnicity: The Sex Hormones and Lipoproteins in Adolescent Males Study

Variable	Black Males (n = 251)	White Males (n = 285)	*P* Value	Adjusted *P* Value*
Age	12.9 ± 1.4	12.7 ± 1.5	.06	.05
Body composition				
Height (cm)	160.3 ± 12.0	156.0 ± 12.1	<.001	.41
Weight (kg)	51.3 ± 13.9	46.1 ± 12.8	<.001	.03
BMI	19.7 ± 3.7	18.6 ± 3.1	<.001	.02
Lipids (mmol/L)				
Triglycerides	0.6 ± 0.3	0.8 ± 0.4	<.001	<.001
HDL-C	1.6 ± 0.3	1.4 ± 0.3	<.001	<.001
ApoAI (mg/dL)	149.0 ± 25.2	148.1 ± 22.5	.65	.17
ApoAII (mg/dL)	55.5 ± 9.9	56.5 ± 9.2	.22	.95
HDL-C/apoAI†	0.41 ± 0.09	0.37 ± 0.06	<.001	<.001
HDL-C/(apoAI + apoAII)†	0.30 ± 0.06	0.26 ± 0.04	<.001	<.001
Sex hormones				
E_2 (pmol/L)	111.9 ± 95.2	75.5 ± 69.3	<.001	.01
F-T (nmol/L)	23.7 ± 24.7	17.0 ± 21.3	.001	.79

**P* values reflect adjustment in the models for pubertal maturation stage.

†Calculated with HDL-C in mg/dL.

ation were less than 2% for triglycerides, less than 5% for HDL-C, 8.0% for total testosterone, 6.0 % for F-T, and 3.3 % for E_2.

Statistical Analysis

Black-white comparisons of age were made using Student's *t* test and of the anthropometric, lipid, and sex steroid hormone variables using Student's *t* test and analysis of covariance, adjusting for pubertal stage. For this analysis, the stages of maturation were coded 1 to 4 for the prepubertal through early, mid, and late stages, respectively. Differences in the relation of age to pubertal stage by ethnic group were assessed using a 2-way analysis of variance. The effects tested in this model were ethnic group, maturation stage, and their interaction. Backwards stepwise regression analyses were used to identify predictors of lipid concentrations in multivariate analyses with age, ethnic group, pubertal stage, BMI, F-T, and E_2 as potential explanatory variables. Because only 8 participants had missing values for variables used in this analysis, missing data were not a problem for the backwards stepwise procedure. Unless otherwise specified, statistical testing was conducted at the .05 significance level. Two-tailed tests were used. In the regression models, explanatory variables were kept in the models with a *P* value of 0.10. Ethnic group was coded whites = 0, blacks = 1. All analyses were performed using the SAS statistical analysis system.[18]

RESULTS

This report is based on study results from 285 white and 251 black boys, 10 to 15 years of age, representing 74% and 68% of eligible white and black students, respectively. Black boys were marginally older than white boys ($P = .06$), but the difference amounted to only 2.5 months (Table 1). Few black (n = 17, 6.8%) and white boys (n =16, 5.6%) were 15 years old. The number of boys in the other 1-year age groups ranged from 25 (10.0%, age 10) to 65 (25.9%, age 12) in black boys and from 43 (15.1%, age 10) to 64 (22.5%, age 12) in white boys. Although pubertal stage tended to be more advanced with increased age, as expected, there were white boys in every pubertal stage at age 13 and black boys in every pubertal stage at ages 12 and 13. In addition, as expected, there were stepwise increments in mean age, height, weight, and BMI across pubertal stages (Table 2). There was a significant ethnic group by age interaction in the model for pubertal stage ($P = .005$) such that black boys were more advanced in puberty than white boys at each age.

Ethnic Differences in Body Composition, Lipids, and Sex Hormones

Black boys had significantly higher HDL-C ($P < .001$) and lower triglycerides ($P < .001$) than white boys. Differences in apoAI and AII were not significant; consequently, black boys had more HDL-C per apoAI and AII. Black boys were significantly taller, heavier, and had greater BMI, as well as higher mean E_2 and F-T (both $P \leq .001$). After controlling for pubertal stage, ethnic differences in height were no longer significant, but differences in weight and BMI (both $P < .05$), and HDL-C and triglycerides (both $P < .001$) remained significant (Table 1). As expected, serum concentrations of E_2 and F-T were incrementally higher at more advanced stages of pubertal development in each ethnic group (Table 2). After controlling for pubertal stage, F-T levels did not differ between ethnic groups (Table 1), but E_2 levels were significantly higher in black boys, with the difference appearing in the mid and late stages, (Tables 1 and 2; $P = .01$).

Predictors of Lipid Levels in Adolescent Boys

In backward stepwise regression analyses, pubertal stage was not retained in any of the multivariate models for HDL-C, apoAI, and apoAII, but F-T and BMI, two factors strongly associated with pubertal stage (Table 3), appeared in one or more final models. F-T (negative) and ethnic group (blacks higher), were retained in the apoAI and HDL-C models and explained 7.2% and 15.5% of the variation, respectively. In the apoAII model, ethnic group (blacks lower) and F-T (negative) were retained, and there was a significant E_2 by ethnic group interaction such that higher E_2 associated with higher levels of

Table 2. Mean ± SD Age, Body Composition Measures, Lipid Parameters, and Sex Steroid Hormones in Black and White Males, Ages 10 to 15 Years, By Pubertal Stage: The Sex Hormones and Lipoproteins in Adolescent Males Study

	Prepubertal		Early		Mid		Late	
Variable	Black Males (n = 23)	White Males (n = 58)	Black Males (n = 93)	White Males (n = 130)	Black Males (n = 120)	White Males (n = 87)	Black Males (n = 15)	White Males (n = 10)
Age (yr)	11.5 ± 1.00	11.2 ± 0.90	12.2 ± 1.16	12.4 ± 1.15	13.6 ± 1.19	14.0 ± 0.97	14.3 ± 0.89	14.2 ± 0.71
Height (cm)	146.4 ± 8.73	144.4 ± 7.88	154.1 ± 9.70	152.8 ± 8.62	166.3 ± 9.41	166.9 ± 8.94	171.6 ± 6.42	170.5 ± 4.37
Weight (kg)	39.8 ± 13.90	36.9 ± 9.26	46.3 ± 13.42	42.4 ± 9.81	55.7 ± 11.69	55.1 ± 10.56	64.5 ± 8.66	67.6 ± 9.46
BMI	18.3 ± 4.78	17.5 ± 2.90	19.3 ± 4.08	18.0 ± 2.86	20.0 ± 3.20	19.7 ± 2.84	21.8 ± 1.98	23.3 ± 3.45
Lipids (mmol/L)								
Triglycerides	0.59 ± 0.24	0.74 ± 0.38	0.66 ± 0.36	0.75 ± 0.43	0.60 ± 0.33	0.79 ± 0.44	0.51 ± 0.23	0.71 ± 0.33
HDL-C	1.68 ± 0.32	1.49 ± 0.26	1.64 ± 0.39	1.42 ± 0.27	1.49 ± 0.31	1.28 ± 0.25	1.53 ± 0.18	1.24 ± 0.24
Apo (mg/dL)								
ApoAI	151.6 ± 24.10	156.5 ± 21.42	152.3 ± 24.80	149.7 ± 20.52	146.0 ± 25.91	141.4 ± 24.38	145.8 ± 22.02	135.5 ± 18.09
ApoAII	60.0 ± 10.70	60.0 ± 9.65	57.2 ± 10.32	57.3 ± 8.22	53.8 ± 9.19	53.2 ± 9.53	51.1 ± 7.69	54.5 ± 7.50
Ratios*								
HDL-C/apoAI	0.43 ± 0.09	0.37 ± 0.06	0.42 ± 0.10	0.37 ± 0.06	0.40 ± 0.08	0.36 ± 0.06	0.42 ± 0.09	0.36 ± 0.06
HDL-C/ (apoAI + apoAII)	0.31 ± 0.06	0.27 ± 0.04	0.31 ± 0.07	0.27 ± 0.04	0.29 ± 0.06	0.26 ± 0.04	0.31 ± 0.05	0.25 ± 0.04
Sex steroid hormones								
E_2 (pmol/L)	37.35 ± 26.37	25.06 ± 22.07	57.20 ± 48.50	53.82 ± 49.32	161.00 ± 98.37	134.83 ± 72.20	184.96 ± 91.66	145.37 ± 45.33
F-T (nmol/L)	2.21 ± 3.94	1.78 ± 4.10	9.93 ± 12.51	8.84 ± 13.37	35.27 ± 24.81	36.76 ± 22.09	54.44 ± 23.60	43.74 ± 8.85

*Ratios have been calculated with HDL-C in units of mg/dL.

apoAII in black boys than in white boys. The model explained 7.8% of the variance in apoAII (Table 3). Multivariate analyses of the associations of ethnic group, BMI, F-T, and E_2 with the amount of cholesterol associated with apoAI (HDL-C/apoAI) indicated that only BMI (negative) and ethnic group (blacks higher) were significant factors. The ratio of lipid to (apoAI and AII) was a factor of ethnicity (blacks higher) BMI (negative) and E_2, with E_2 having a marginally significant and negative effect that was greater in black boys than white boys. Pubertal stage, F-T, and E_2 were not retained in these models, which explained 14.9% and 18.2% of the variation in the ratios of lipid to apoAI and (AI + AII).

Next, apoAI and AII were introduced into the HDL-C models as potential explanatory variables. As might be expected both apoAI and AII were retained, and the model R^2 increased markedly to 31.69% (Table 4). Finally, triglycerides were introduced as an explanatory variable for HDL-C. In this model, ethnic group (blacks higher), triglycerides (negative), and apoAI and AII (both positive) were retained in the model. The proportion of the variability in HDL-C explained in this final

Table 3. Final Models Predicting Protective Lipid Parameters in Black and White Adolescent Boys: The Sex Hormone and Lipoproteins in Adolescent Males Study

Dependent Variable	Parameter	β-Coefficient	SE β	$P > \|T\|$	R^2	Model *P* Value
HDL-C (mmol/L)	Intercept	1.685	0.025	<.0001	0.155	<.0001
	Ethnicity	0.210	0.026	<.0001		
	Ln F-T	−0.052	0.007	<.0001		
ApoAI	Intercept	157.456	1.967	<.0001	0.072	<.0001
	Ethnicity	3.383	2.036	.097		
	Ln F-T	−3.654	0.576	<.0001		
ApoAII	Intercept	57.417	3.210	<.0001	0.078	<.0001
	Ethnicity	−6.924	3.607	.055		
	Ln E_2	0.114	0.864	.895		
	Ln E_2 · ethnicity	1.680	0.851	.049		
	Ln F-T	−1.050	0.414	.012		
HDL-C/apoAI	Intercept	0.768	0.056	<.0001	0.149	<.0001
	Ethnicity	0.053	0.007	<.0001		
	Ln BMI	−0.120	0.019	<.0001		
HDL-C/(apoAI + apoAII)	Intercept	0.588	0.040	<.0001	0.182	<.0001
	Ethnicity	0.073	0.020	.0003		
	Ln BMI	−0.092	0.013	<.0001		
	Ln E_2	−0.004	0.003	.216		
	Ln E_2 · ethnicity	−0.008	0.005	.088		

Abbreviations: |T|, probability of a greater absolute T statistic; LN, natural logarithm of the variable.

Table 4. Predictors of HDL-C in Black and White Adolescent Boys, Allowing ApoAI, ApoAII, and Triglycerides to Enter the Models: The Sex Hormones and Lipoproteins in Adolescent Males Study

Dependent Variable	Parameter	β-Coefficient	SE β	$P > \|T\|$	R^2	Model P Value
HDL-C (mmol/L)	Intercept	0.705	0.096	<.0001	0.316	<.0001
	Ethnicity	0.198	0.024	<.0001		
	Ln F-T	−0.031	0.007	<.0001		
	ApoAI	0.005	0.001	<.0001		
	ApoAII	0.005	0.001	0.001		
HDL-C (mmol/L)	Intercept	0.430	0.092	<.0001	0.430	<.0001
	Ethnicity	0.154	0.022	<.0001		
	Ln F-T	−0.030	0.006	<.0001		
	ApoAI	0.005	0.0005	<.0001		
	ApoAII	0.007	0.001	<.0001		
	Triglycerides	−0.219	0.021	<.0001		
HDL-C/apoAI	Intercept	0.634	0.056	0.0001	0.231	<.0001
	Ethnicity	0.041	0.006	0.0001		
	Ln BMI	−0.085	0.019	0.0001		
	Triglycerides	−0.047	0.006	0.0001		
HDL-C/(apoAI + apoAII)	Intercept	0.477	0.039	<.0001	0.297	<.0001
	Ethnicity	0.067	0.018	0.0003		
	Ln BMI	−0.057	0.013	<.0001		
	Ln E_2	−0.008	0.003	0.015		
	Ln E_2 · ethnicity	−0.009	0.004	0.046		
	Triglycerides	−0.039	0.004	<.0001		

Abbreviations: |T|, probability of a greater absolute T statistic; LN, natural logarithm of the variable.

model was 0.43. When triglycerides were allowed to enter the models for the amount of cholesterol associated with AII and AII, it had a significantly negative effect in each model and increased the proportion of the variance in these ratios explained to 23.1 and 29.7% respectively. Ethnic group and BMI were both retained in these models (Table 4).

DISCUSSION

Previous studies of the adolescent changes in male HDL-C have differed among themselves in the lipid parameters evaluated, the ethnic composition of the study population, and in the sex hormones measured. Some studies have reported decreases in HDL-C, apoAI, and apoAII in boys with advancing age and pubertal stage.[19,20] Other investigations reported significant associations between HDL-C and both BMI and pubertal stage in black and white boys, separately by ethnic group, but without sex hormones,[21,22] correlations between sex hormones and lipids,[23] and BMI–hormone–HDL-C associations in boys, without ethnic group comparisons.[24,25] The current report shows that F-T is inversely associated with HDL-C in both black and white boys in multivariate analysis, even after controlling for BMI. Kirkland et al[26] examined testosterone-HDL-C associations in 3 groups of adolescent males: (1) 10- to 17-year-old boys with normal puberty, (2) boys with delayed puberty who were treated with testosterone enthanate, and (3) boys with delayed puberty who spontaneously entered puberty. In each group, increasing testosterone was associated with marked decreases in HDL-C. Similarly, data from the Bogalusa Heart Study showed negative correlations between testosterone and HDL-C in white boys 13 to 14 years of age, and in black boys 15 to 17 years of age.[23] In contrast to these findings in adolescents, adult studies have reported positive,[27-29] as well as negative associations between F-T and HDL-C.[30] Although the strongly negative correlations between BMI and both F-T[27] and HDL-C[31] could partially account for these discrepant findings, multivariate analyses controlled for BMI in the above studies.[27-29] Goldberg et al[32] chemically suppressed testosterone levels in 8 adult men and produced concomitant increases in both HDL-C and apoAI.

The current report extends the understanding of the adolescent decline in male HDL-C, showing that the primary pubertal effect of F-T on HDL-C may be the reduction of the major apolipoproteins associated with HDL-C and not the amount of cholesterol associated with these proteins. That is, while the amount of HDL-C per apoAI and AII does differ between black and white males in this cohort, it does not vary within ethnic group across pubertal stages, nor with F-T. Moreover, when apoAI and AII were introduced in the models, they were both significant predictors of HDL-C, and the beta coefficient associated with F-T decreased. Finally, F-T was not kept in the models for the ratios of lipid to (apoAI and apoAII). This suggests that the primary effect of increasing F-T levels is to lower concentrations of apoAI and AII, and lower apoAI and AII, in turn, associate with lower HDL-C.

The mechanisms through which changing F-T leads to lower apoAI and AII, and consequently lower HDL-C, are unknown. A number of candidate mechanisms are possible. Lipoprotein lipase (LpL), an enzyme that influences the maturation of HDL, has been shown to differ markedly between black and white men,[33] and leads to a change in the cholesterol to apo ratio in HDL, which could relate to cholesterol ester transfer protein and the lipid transfer inhibitor protein (LITP).[34] The latter modifies the degree of transfer between very–low-density lipoprotein (VLDL) and HDL, in preference to LDL. This sequence, however, would alter the lipid to protein ratio in HDL-C, and such an alteration was not observed. Alternatively,

the enhanced triglyceride concentration in HDL from such transfers, particularly in Lp(a1) particles, increases HDL and apoAI catabolism[35,36] over that of Lp(AI/AII). Since F-T was not retained in the model for HDL-C/(apoAI and AII) ratio, it is doubtful that F-T is responsible for HDL/apo compositional remodeling. Similarly, the influence of F-T on the HDL scavenger receptor B1 (SRB1), which would preferentially incorporate cholesterol ester into the cell rather than AI, would also dichotomize the HDL-C and apoA1 relation. Therefore, the most likely candidates for the F-T interaction involve the synthetic enzymes, eg, adenosine triphosphate (ATP)-binding casette protein (ABC1). If this were the case, F-T would progressively decrease ABC1 activity during puberty, decreasing A1 and AII secretion rates. This in turn would reduce the level in the plasma of HDL-C. AI and AII are influenced by substantial processing, which also may contribute to HDL-C values.

In our current study, mean concentrations of apoAI and AII did not differ by ethnic group, in contrast to the findings of Srinivasan et al,[5] where blacks had higher AI. However, like the present report, Srinivasan found that black boys had higher HDL-C/AI ratios than white boys.[5] Finally, Srinivasan reported an inverse relationship between the HDL-C/AI ratio and pubertal stage, while neither pubertal stage nor sex hormones were retained in the HDL-C/AI (or AII) models in this study. These differences could partially reflect the wider age range covered in the Bogalusa report (5 to 17 years) than in the present report (10 to 15 years). In addition, environmental factors, including dietary differences between small town Louisiana (Bogalusa) and a participants from a Midwest, metropolitan area could contribute to observed differences. Geographic differences in cardivascular disease–associated mortality have been reported for adults.[37] More importantly, both the Bogalusa and current analyses were cross-sectional in design; consequently, the variance estimates for the dependent and independent variables do not control for intersubject variability as would longitudinal analyses.

Although increased E_2 has been associated with both increased risk of coronary heart disease and low HDL-C in adult men, E_2 did not enter any of the HDL-C or apoAI models examined. This may reflect the high correlation ($r = 0.84$ in black boys and 0.82 in white boys) between E_2 and F-T during the ages covered, as reported previously.[4] When both sex hormones increase markedly as during puberty, increases in one serve to some extent as a marker for the other, especially given the role of F-T as a primary source for male E_2.[38] In adults, the 2 hormones are not correlated.[39] Longitudinal studies are needed to explain these relationships.

In summary, the effects of puberty on HDL-C in boys in this study appear to be mediated through the effects of F-T and BMI on apoAI and AII. Increased BMI and F-T associate with decreased apoAI and AII, which in turn, associate with decreased HDL-C. When TG was introduced into the model, it was significantly and inversely associated with HDL-C, and the proportion of explained variance increased to 43%. Brinton et al[40] have shown that triglyceride levels regulate HDL particle size, which in turn regulates apoAI fractional catabolic rate (FCR), and FCR is a major determinant of HDL-C levels. In our previous analysis of the effects of sex hormones on atherogenic lipids (apoB, LDL-C, and triglycerides), E_2 was significantly and inversely associated with all 3 lipids.[4] Thus, the role of triglycerides in HDL-C metabolism may reflect to some degree the role of E_2 in triglyceride levels, but E_2 effects on HDL-C were not seen in this cross-sectional analysis. Longitudinal analyses, controlling for intersubject variability, should shed more light on these relationships.

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