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Original Research

Synthetic astaxanthin is significantly inferior to algal-based astaxanthin as an antioxidant and may not be suitable as a human nutraceutical supplement

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Abstract

Synthetic astaxanthin (S-AX) was tested against natural astaxanthin from *Haematococcus pluvialis* microalgae (N-AX) for antioxidant activity. *In vitro* studies conducted at Creighton University and Brunswick Laboratories showed N-AX to be over 50 times stronger than S-AX in singlet oxygen quenching and approximately 20 times stronger in free radical elimination. N-AX has been widely used over the last 15 years as a human nutraceutical supplement after extensive safety data and several health benefits were established. S-AX, which is synthesised from petrochemicals, has been used as a feed ingredient, primarily to pigment the flesh of salmonids. S-AX has never been demonstrated to be safe for use as a human nutraceutical supplement and has not been tested for health benefits in humans. Due to safety concerns with the use of synthetic forms of other carotenoids such as canthaxanthin and beta-carotene in humans, the authors recommend against the use of S-AX as a human nutraceutical supplement until extensive, long-term safety parameters have been established and human clinical trials have been conducted showing potential health benefits. Additionally, differences in various other properties between S-AX and N-AX such as stereochemistry, esterification and the presence of supporting naturally occurring carotenoids in N-AX are discussed, all of which elicit further questions as to the safety and potential health benefits of S-AX. Ultimately, should S-AX prove safe for direct human consumption, dosage levels roughly 20–30 times greater than N-AX should be used as a result of the extreme difference in antioxidant activity between the two forms.

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Introduction

Astaxanthin is a member of the carotenoid family. Carotenoids are divided into two groups: carotenes such as beta-carotene and lycopene, and xanthophylls such as astaxanthin, lutein and canthaxanthin. The main structural difference between the two groups is that xanthophylls exclusively have hydroxyl groups at the end of the molecules. Astaxanthin is unique in that it has more hydroxyl groups than other xanthophylls, which may account for

its superior antioxidant activity and its more diverse and profound health benefits in humans [1].

Natural astaxanthin (N-AX) occurs in *Haematococcus pluvialis*, a ubiquitous unicellular microalgae, which grows in fresh water throughout the world. Commercially, N-AX is extracted from *H. pluvialis* microalgae grown in closed systems or open pond systems by several different companies. When subjected to environmental stress, these algae hyperaccumulate N-AX as a survival mechanism. N-AX protects the algae cells extremely efficiently; the algae can live for over 40 years with no food or water and in extreme heat or cold due to the protective effects of N-AX. This natural form of astaxanthin was first sold as a human nutraceutical supplement in the late 1990s when it was allowed for sale by the US Food and Drug Administration (FDA) as a new dietary ingredient. An extensive array of human clinical trials from around the world have established health benefits for N-AX in areas such as eye and brain health, UV protection and skin health, anti-inflammatory activity, immune system modulation and cardiovascular health among others [2–10].

Synthetic astaxanthin (S-AX) is produced by a highly involved, multistep process from petrochemicals by a handful of large chemical companies. During the steps in this process, the molecule assumes different forms before finally arriving at its final stage, when it attains the same chemical formula as N-AX. S-AX is then sold in the animal feed market where it is added primarily to fish feeds with the purpose of pigmenting the flesh of certain species of commercially farmed fish, predominantly salmonids such as Atlantic salmon and trout. S-AX has not undergone safety testing for direct human use and has not been documented to have any physiological benefits in humans; it has thus never been registered with regulatory authorities for direct human use in any country [1].

The main differences between N-AX and S-AX are three-fold: Firstly, N-AX is comprised of 95% esterified molecules, both monoesterified and diesterified (meaning they have either one or two fatty acid molecules attached to the ends of the astaxanthin molecule). In Fig. 1, a diester of astaxanthin is shown where R and R′ are 16:0 (palmitic acid), 18:1 (oleic acid) or 18:2 (linolenic acid). Comparatively, S-AX is completely different from N-AX; it is exclusively "free" astaxanthin (meaning that it is non-esterified and has no fatty acids attached to the ends of the molecule).

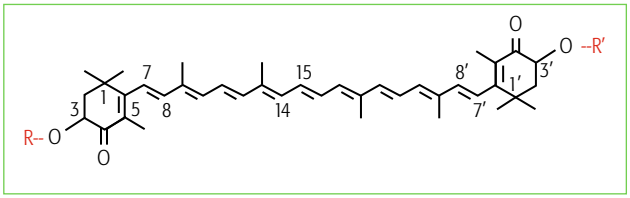

Figure 1 Diester of astaxanthin

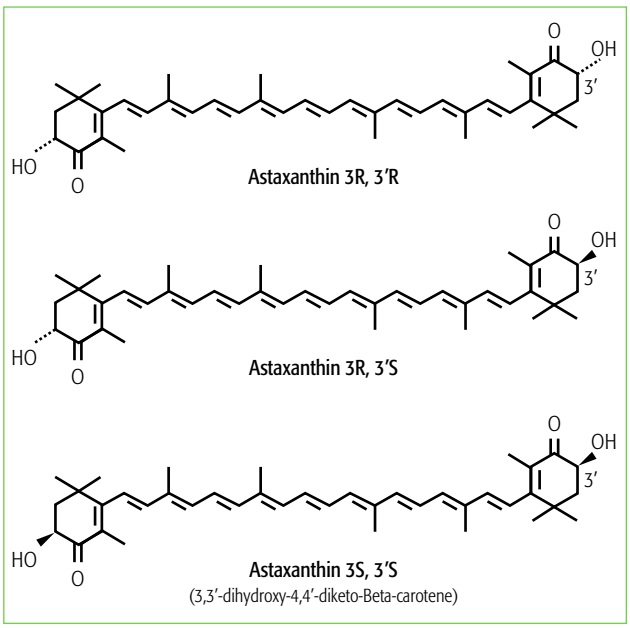

Figure 2 Three different enantiomers of astaxanthin

Secondly, the N-AX and S-AX molecules are shaped differently. This difference in stereochemistry is evidenced by the existence of three distinct enantiomers as seen in Fig. 2: enantiomer 1 is 3S,3′S, enantiomer 2 is 3R,3′R and enantiomer 3 is 3R,3′S (known as "meso"). So, while natural and synthetic astaxanthin share the same molecular formula, 75% of the molecules are shaped differently. The differences between N-AX and S-AX are also quite profound:

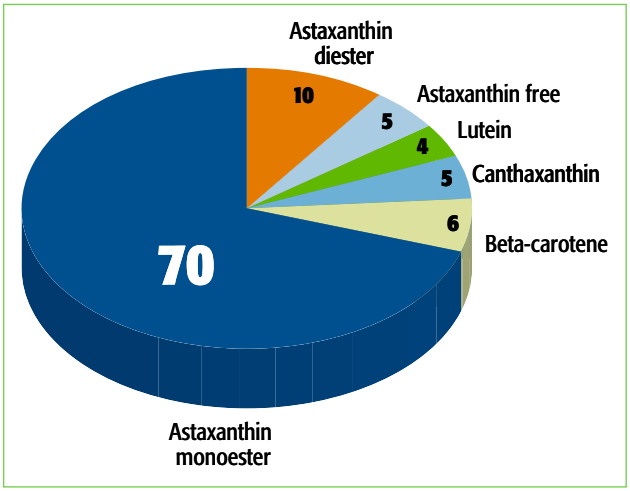

Figure 3 Carotenoid breakdown of N-AX

- N-AX contains 100% 3S,3′S enantiomer.
- S-AX contains a combination of three different enantiomers: It has 25% 3S,3′S (the same shaped molecules as N-AX), but it contains primarily molecules shaped differently from N-AX: 50% is meso-astaxanthin comprised of the 3R,3′S enantiomer. Lastly, 25% is pure "R" enantiomer 3R,3′R.

Thirdly, S-AX is exclusively synthetic astaxanthin and contains no supporting carotenoids, while N-AX is naturally complexed in *Haematococcus* microalgae with other carotenoids, as seen in Fig. 3. When lipids are extracted from the algae, the resulting extract contains primarily N-AX, but it also contains three other naturally occurring carotenoids. The resulting "natural carotenoid complex" contains approximately:

- 70% monoesterified astaxanthin
- 10% diesterified astaxanthin
- 5% free astaxanthin
- 6% beta-carotene
- 5% canthaxanthin
- 4% lutein

Due to three clear differences between these two forms of astaxanthin, N-AX and S-AX cannot be considered the same molecule. While they share the same chemical formula, there are vast differences between N-AX and S-AX in:

- Esterification
- Stereochemistry
- The presence of three additional, naturally occurring carotenoids in N-AX [1]

For these reasons, we suggest that the synthetically produced form must be considered unique from other forms and should not be introduced for direct human use until long-range safety parameters are established and human clinical trials showing potential benefits have been conducted.

Another commercial source of astaxanthin is *Xanthophyllomyces dendrorhous*. This is a species of yeast formerly known as *Phaffia rhodozyma*. While the yeast in nature produces small amounts of astaxanthin, commercial manufacturers use a genetically mutated form to produce higher amounts of astaxanthin. The astaxanthin present in this yeast is extremely different from the astaxanthin found in the marine food chain. For example, similar to S-AX, it has a completely different stereochemistry from N-AX. Another key difference is that it is 100% non-esterified. This astaxanthin product from mutated yeast is allowed for human consumption in some countries; however, due to insufficient safety data, use is only permitted with restrictions. For example, it is allowed by the US FDA, but with restrictions against long-term use, against the use in children and, perhaps most significantly, at dosage levels of only 2 mg/day. Generally, a 2 mg dosage of N-AX has only been shown to be sufficient in human clinical research in the area of immunomodulation [9], one of many potential physiological benefits of astaxanthin. The literature does not contain human clinical research on this yeast form of astaxanthin. For this reason and due to safety concerns, discussion of this form of astaxanthin remains outside the scope of this paper [1].

Materials and methods

The free radicals superoxide anion and hydroxyl radical were generated *in vitro*:

- Superoxide anion radical: Xanthine (100 µM) in 5

mM Tris-HCl buffer was incubated with 8 mU/ml of xanthine oxidase to generate superoxide anion.

- Hydroxyl radical: The incubation mixture to generate hydroxyl radical contained, in a total volume of 2 ml, 5 mM Tris-HCl, 100 µM $FeCl_3$, 100 µM EDTA and 100 µM xanthine. Xanthine oxidase (8 mU/ml) was added to initiate the reaction and to produce hydroxyl radicals [11].

Chemiluminescence measurements

Chemiluminescence, as an index of reactive oxygen species production, was measured in a Chronolog Lumivette luminometer (Chronolog Corp., Philadelphia, PA). The assay was conducted in 3 ml glass minivials. The vials were incubated at 37°C prior to measurement and the background chemiluminescence of each vial was checked before use. Samples were preincubated at 37°C for 15 min, and 4µM luminol was added to enhance chemiluminescence. All additions to the vials as well as chemiluminescence counting procedures were performed under dim lighting conditions. Results were examined as counts per unit of time minus background. Chemiluminescence was monitored for 6 min at continuous 30-s intervals [12].

Statistical analyses

Significance between pairs of mean values was determined by Student's *t*-test. $p<0.05$ was considered significant for analysis.

Replicates for the Creighton University free radical inhibition research were conducted four to six times. Replicates for the Brunswick Laboratories analyses were conducted two to three times.

Results

In vitro work done at Creighton University School of Pharmacy and Allied Health Professions (Omaha, NE) matched N-AX (as BioAstin® Hawaiian Astaxanthin from Cyanotech Corporation) against several other well known natural antioxidants such as vitamin C, vitamin E, beta-carotene, Pycnogenol® pine bark extract and S-AX (as Sigma catalogue #9335). N-AX proved to be 14–65 times more potent at eliminating free radicals when compared directly against these other antioxidants, including S-AX. N-AX was approximately 20 times more potent at free radical elimination than S-AX. Results are summarised in Table 1 and Fig. 4.

Antioxidant activity of N-AX (as BioAstin® Hawaiian Astaxanthin by Cyanotech Corporation, Kailua-Kona, HI) and S-AX (as Vivital™ AstaFeed by Divis Laboratories, Morristown, NJ) was measured in a suite of tests by Brunswick Laboratories (Southborough, MA). Results are shown in Table 2. N-AX was

Material	Active material used (mg)	Free radical inhibition in study (%)	Free radical inhibition per mg active material (%)	N-AX relative performance
Vitamin C	100	19	0.19	N-AX 65× stronger
Vitamin E	50	43	0.86	N-AX 14× stronger
Beta-carotene	100	23	0.23	N-AX 53× stronger
Pycnogenol	100	69	0.69	N-AX 18× stronger
S-AX	100	59	0.59	N-AX 20× stronger
N-AX	5	61.7	12.34	N/A

Table 1 Free radical eliminating potency of various antioxidants (N=4–6)

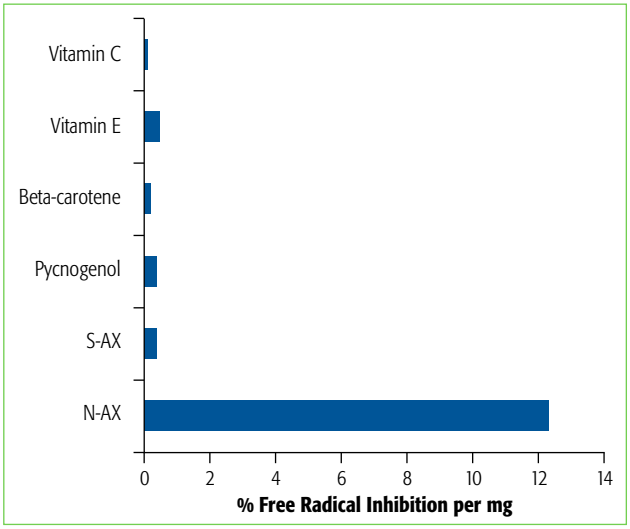

Figure 4 Free radical eliminating potency of various antioxidants

55 times stronger than S-AX in eliminating singlet oxygen *in vitro*. Similar to the results at Creighton University cited above, N-AX was over 20 times stronger than S-AX in eliminating the superoxide ion. N-AX was 3.5 times stronger against peroxyl radicals. N-AX performed significantly worse than S-AX against peroxynitrite, with only 24% of the antioxidant power of S-AX. Peroxynitrite is produced from the diffusion-controlled reaction between nitric oxide and the superoxide anion. Peroxynitrite interacts with lipids, DNA and proteins via direct oxidative reactions or via indirect, radical-mediated mechanisms. However, N-AX decreases nitric oxide production [7] and has very powerful activity against the superoxide anion and hence, would decrease the production of peroxynitrite, rendering this particular result less meaningful. In the final survey by Brunswick Laboratories, antioxidant activity against hydroxyl radicals was measured. Unfortunately, a different procedure from that used at Creighton University was employed, and no result was obtained for N-AX, rendering this test incomparable. Brunswick Laboratories issues a summary score for this suite of antioxidant tests called oxygen radical absorbance capacity (ORAC). Including the hydroxyl test for which the N-AX score was not determined and the peroxynitrite test in which S-AX performed better than N-AX, the summary result found N-AX to be 14 times stronger overall as an antioxidant than S-AX. The results are summarised in Table 2.

Creighton University tests were carried out under the supervision of Debasis Bagchi, the developer of a method of free radical generation and an expert in antioxidant research. Brunswick Laboratories is regarded as a leading antioxidant research laboratory, and while it is unclear why results for hydroxyl radicals were unavailable for N-AX, it is clear that this lab is a competent source for antioxidant testing. One possible reason why the N-AX score in the hydroxyl radical test was not determined is that N-AX may not be soluble in the solvent used in this ORAC test.

Test	N-AX	S-AX	N-AX vs. S-AX
Antioxidant power against singlet oxygen	12,055	220	55× stronger
Antioxidant power against super oxide ion	5,377	258	21× stronger
Antioxidant power against peroxyl radicals	574	165	3.5× stronger
Antioxidant power against peroxynitrite	28	115	0.24× of S-AX's activity
Antioxidant power against hydroxyl radicals	Not determined	538	Not comparable
Total ORACFN antioxidant power	18,034	1,296	14× stronge

Table 2 Antioxidant power against various oxidants of N-AX vs. S-AX (Brunswick Laboratories antioxidant test results; all numbers in moles TE per gram; N=2–3)

Regardless of this minor issue with the ORAC test, the outcome of this research is clear: N-AX is a superior antioxidant to S-AX by more than an order of magnitude. Results range from approximately 14 times stronger in the overall ORAC summary score to more than 20 times stronger in free radical elimination to as high as 55 times stronger in singlet oxygen quenching.

Discussion

N-AX has proven to be exceptionally more powerful than other common antioxidants as well as S-AX; tested against other commonly used antioxidants, it scored a minimum of 14 to a maximum of 65 times higher in free radical elimination. Two separate antioxidant tests were performed directly comparing N-AX with S-AX, one at a leading university and the other at an independent laboratory specialising in antioxidant testing. The results of this testing showed that:

- N-AX is approximately 55 times stronger than S-AX in singlet oxygen elimination.
- N-AX is approximately 20 times stronger than S-AX in free radical elimination.
- N-AX is approximately 14 times stronger than S-AX in the suite of antioxidant tests known as ORAC.

For these reasons, should it be commercialised for human use, S-AX would have to be used at a rate 14–55 times greater than N-AX to obtain the same antioxidant protection. Current dosage recommen-

dations for humans for N-AX range from 2 to 16 mg/day based on extensive human clinical trials showing a wide range of health benefits. Based on this dosage range for N-AX, should S-AX be allowed for human use, the resulting recommended range would be a minimum of 28 mg/day to a maximum of 880 mg/day when considering the differences in antioxidant activity. With an average difference of antioxidant measurements in the range of 20×–30×, and an average human dosage of 8 mg/day, the average dose for S-AX would be in the proximity of 160–240 mg/day. Before release to human consumers, long-range safety trials should be conducted at this dosage level to ensure that, unlike synthetic beta-carotene and synthetic canthaxanthin, there are no concerns with S-AX in areas such as carcinogenesis or retinal crystal formation (see below).

Other nutraceutical supplements that are available in both synthetic and natural forms show safety concerns with their synthetic form. This includes molecules closely related to astaxanthin such as the carotenoids beta-carotene and canthaxanthin as well as other nutraceuticals such as vitamin E. While the exact cause of the differences between natural and synthesised forms of nutraceuticals is not known, one logical theory is that synthesised compounds may not be the most physiologically valuable part of the natural nutrient complex. For example, synthesised vitamin E is exclusively DL-alpha tocopherol, while natural vitamin E is a complex of several mixed tocopherols and tocotrienols. Nutrients may be synergistic, meaning that they may work best when taken in concert with other compounds in their natural forms.

Research has shown that synthetic vitamin E may be inferior to the natural form in its physiological properties. Synthetic E, which is exclusively DL-alpha tocopherol, has a limited ability to yield health benefits. Members of the natural vitamin E complex have essential independent functions. For example, the alpha-tocotrienol component of the natural E complex prevents neurodegeneration. Tocotrienols are not found in synthetic vitamin E [13]. Also, synergy can play an important role in vitamin E's effects. In a study published in the *Journal of the National Cancer Institute*, it was found that alpha-tocopherol, gamma-tocopherol and selenium work in concert to prevent prostate cancer. In other words, benefits increased with the complete vitamin complex versus single synthesised molecules [14]. Carotenoids in their synthetic forms in particular yield very significant safety concerns. The most researched carotenoid to date is beta-carotene. The literature is full of studies demonstrating a variety of health benefits for beta-carotene in areas such as immunity, prevention of cancer and skin health [15]. However, the differences in absorption between the synthetic and natural varieties of beta-carotene are profound; in one study, natural beta-carotene was absorbed ten times better than the synthetic form by rats and chickens [16]. Not only is absorption a concern, but also efficacy. Similar to our results with S-AX versus N-AX in antioxidant potential, synthetic beta-carotene does not have the same antioxidant abilities as its natural cousin. Synthetic beta-carotene is primarily the *trans* form, while natural beta-carotene contains large amounts of the *cis* form. The 9-*cis* beta-carotene form, which is found in high amounts in natural beta-carotene, is a more efficient lipophilic antioxidant than the synthetic *trans* form. The stereochemistry of this carotenoid (similar to the situation with astaxanthin) is important in antioxidant potential as well as absorption and transport [17].

Perhaps the most significant difference found in the literature between natural and synthetic forms of beta-carotene was demonstrated in the famous "Finnish Smokers Study" in the 1990s. After scores of epidemiological studies, *in vitro* and preclinical animal trials demonstrated that natural beta-carotene has cancer-preventative properties [15]. A study of men from Finland who smoked on average three packs of cigarettes per day found an unexpected outcome: when supplemented with synthetic beta-carotene, there was a slight increase in cancer among the treatment group versus the

placebo group [18]. This study was very troubling to many consumers who were taking beta-carotene as a cancer preventative supplement. Further research comparing synthetic beta-carotene with natural beta-carotene extracted from *Dunaliella salina* microalgae found that synthetic beta-carotene may be involved in the formation of cancer. This same study concluded that natural beta-carotene could be valuable in tumour prevention and supplementary treatment [19]. The possibility that synthetic beta-carotene may cause cancer while natural beta-carotene may prevent cancer is the most grave concern of all when considering the introduction of S-AX, a related carotenoid, as a supplement for human use.

Synthetic canthaxanthin taken as a supplement has also yielded grave concerns. This is particularly relevant to our discussion in this paper since canthaxanthin is in the same carotenoid family as beta-carotene and astaxanthin, but is even more closely related to astaxanthin than beta-carotene is. Canthaxanthin, like astaxanthin, falls into the xanthophyll subgroup since it has hydroxyl groups attached to its molecules. Natural canthaxanthin is not currently available commercially since sources for the natural form are limited. Canthaxanthin is, however, available in its synthetic form, and is used as an addition to animal feeds similar to S-AX. It is important to note that governments around the world consider synthetic canthaxanthin a safety concern, and limit or prohibit its use in animal feeds [20,21]. The safety concern centres on crystallisation in the retina due to supplementation with synthetic canthaxanthin. In the late 1980s, synthetic canthaxanthin was marketed as an internal tanning pill for people who wished to appear sun-tanned without going out in the sun. The product was abruptly taken off the market when golden crystals were found in consumers' retinas. The crystallisation disappeared over time after discontinuing consumption of synthetic canthaxanthin. But it is disconcerting to note how long reversal took: follow-up research published in 2011 found that complete disappearance of the golden crystals took approximately 20 years [22]. The differences in regards to safety between natural and synthetic forms of nutraceutical supplements raise concern for the introduction of new synthetic versions of supplements. Particularly worrisome are the safety concerns with synthetic carotenoids. Synthetic beta-carotene's increase of cancer rates in smokers and synthetic canthaxanthin causing unnatural retinal crystallisation are clear evidence that extensive, long-range safety testing of S-AX and other synthetic carotenoids are necessary before release to human consumers. Additionally, serious questions of efficacy exist with synthetic compounds such as synthetic vitamin E, synthetic beta-carotene and synthetic canthaxanthin when compared to their natural forms. The lack of efficacy and safety in synthetic supplements are most likely due to the profound differences between synthetically produced nutraceutical compounds and their naturally occurring counterparts. For example, in the case of astaxanthin, far-ranging, extensive differences in the shape of the molecule; the esterification of the molecule; and the presence of other naturally occurring carotenoids in their natural form in N-AX lead us to the conclusion that S-AX and N-AX, although both called "astaxanthin", must be considered completely different substances. For these reasons, the authors recommend against the use of S-AX in human nutraceutical supplements until extensive, long-range safety parameters are established and human clinical trials showing health benefits are conducted. In the event that S-AX attains these two milestones, due to the extensive differences between the two molecules, it should be distinctly labelled as "synthetic astaxanthin" on consumer product labels, and dosage levels should be approximately 20–30 times those of N-AX in order to obtain similar antioxidant activity.

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Conflict of interest

Bob Capelli and Gerald R. Cysewski are employees and stockholders of Cyanotech Corporation, the company that sponsored this research. Debasis Bagchi is a professor at the University of Houston College of Pharmacy and was formerly a professor at Creighton University where he oversaw research described herein. He is independent and has no conflict of interest.

Human and Animal Rights

This article does not contain any studies with human or animal subjects performed by any of the authors.

An extensive compilation of published research on astaxanthin is available from the authors. Please contact us at info@cyanotech.com.

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