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L-arginine, NO and asymmetrical dimethylarginine in hypertension and type 2 diabetes

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1. ABSTRACT

Both hypertension and type 2 diabetes mellitus are common and there are no reliable markers either to predict their development or complications. High fat diet and carbohydrate-rich diet enhance serum asymmetrical dimethylarginine (ADMA) levels, an endogenous inhibitor of nitric oxide synthesis. ADMA levels are elevated in patients with hypertension, poor control of hyperglycemia, diabetic microangiopathy and macroangiopathy and dyslipidemia. One of the earliest signs of vascular dysfunction and insulin resistance, which are present in hypertension and type 2 diabetes mellitus, is an elevation in serum ADMA levels. Displacing plasma ADMA by oral supplementation of L-arginine restores endothelial dysfunction by augmenting endothelial nitric oxide generation. Strict control of hyperglycemia decreases serum ADMA levels. These and other studies suggest that serum ADMA levels could be used to predict the development of hypertension and type 2 diabetes mellitus in those who are at high-risk to develop these diseases.

2. INTRODUCTION

Nitric oxide (NO) is a free radical that is involved in the regulation of a vast number of biological processes. NO seems to have a role in the nervous system, cardiovascular and immune systems, and is participates in apoptosis, inflammation, and modulates kidney function and has a role in the pathophysiology of diabetes mellitus, oxidative stress and aging.

NO is synthesized from the semi-essential amino acid, L-arginine in an oxidative reaction that consumes molecular oxygen and reducing equivalents in the form of NAD(P)H. The products of the reaction are NO, NADP(+), and citrulline. NO is not stored and is formed and released as needed. The enzyme involved in the synthesis of NO is NO synthase (NOS), which requires FAD, FMN, heme, Ca(2+), calmodulin and tetrahydrobiopterin (BH_4) as co-factors. Three NOS iso-enzymes have been characterized and structurally all NOS iso-enzymes consist of a carboxy-terminal reductase domain, which binds the flavin cofactors. A Ca(2+)/calmodulin binding domain lies in the

center followed by an oxygenase domain, with electronic absorption properties similar to P450 enzymes, where binding of heme, O_2, BH_4, and arginine substrate all take place. The three distinct NOS enzymes: neuronal or type 1, nNOS; inducible NOS or iNOS; and endothelial NOS or eNOS, each the product of a unique gene, have been identified and well characterized. Type 1 or nNOS is a Ca(2+) dependent enzyme found in neuronal tissue and skeletal muscle. Four splice variants of full length nNOS have been identified: nNOS Beta, nNOS Gamma, nNOS Mu, and nNOS-2. Type 2 or iNOS is inducible in a variety of cells and tissues in response to cytokine or endotoxin activation. INOS binds Ca(2+)/calmodulin so tightly that at normal physiologic levels its activity is functionally Ca(2+)-independent. Type 3 or eNOS is also Ca(2+) dependent and is myristoylated and palmitoylated at the N-terminus, modifications, which are needed for localization to the plasmolemmal caveolae of endothelial cells. Though both nNOS and eNOS are constitutive enzymes, it is now believed that all three enzymes can be induced, albeit to different levels and by different stimuli.

3. ASYMMETRICAL DIMETHYLARGININE AND NITRIC OXIDE IN ESSENTIAL HYPERTENSION

Selective defect in NO synthesis may be present in hypertension which could be responsible for the impaired endothelium-dependent vasodilatation seen. Vasodilator response to acetylcholine (which stimulates NO release from endothelial cells) was significantly reduced in hypertensives compared with normotensives (1). However, the vasodilator response to isoproterenol (a Beta receptor agonist that stimulates NO synthesis by increasing intracellular cAMP) and sodium nitroprusside, a NO donor, was similar in normotensives and hypertensives, suggesting that the endothelial dysfunction in essential hypertension is due to selective abnormality of NO synthesis. This coupled with the observation that endothelium-dependent vasodilation declines and oxidative stress increases with aging (2) explains why hypertension is common with advancing age.

NO levels were significantly lower in platelets in hypertensive patients than in normotensives (3). Platelet cytosolic Ca(2+) levels were higher in hypertensive patients compared with normal. An inverse correlation was found between platelet cytosolic Ca(2+) and NO levels indicating that a close link exists between hypertension and altered platelet function (3). Thus, NO abnormality seen in hypertensives is not restricted to the vascular endothelium but may be seen in other tissues as well.

Male Sprague-Dawley rats fed 10 percent d-glucose when were given NO synthase inhibitor for 4 weeks showed not only an increase in systolic blood pressure but also developed hyperinsulinemia and insulin resistance that was more marked in those receiving NO inhibitor compared with glucose alone treated group. Plasma adrenaline concentrations were markedly increased especially in NO synthase fed group, whereas noradrenaline was increased in glucose-treated rats only. Superoxide anion formation was increased in the aorta of both glucose and glucose + NOS inhibitor treated groups without any change in the concentrations of reduced NAD(P)H oxidase activity. These results imply that NO inhibition increases vascular oxidative stress, sympathoadrenal hyperactivity and insulin resistance that contribute to hypertension (4). Endothelial dysfunction was noted in streptozotocin-induced diabetic male Sprague-Dawley rats as evidenced by impaired relaxant response to acetylcholine and reduced vasoconstrictor response to the NO synthase inhibitor L-NAME, whereas the response to nitroprusside and the expression of endothelial NO synthase remained unchanged. An increase in superoxide production and increased expression of the NADPH oxidase was noted in these diabetic rats (5), suggesting that diabetes-induced endothelial dysfunction occurred via enhanced NADPH oxidase-derived superoxide production.

One of the markers that suggest the presence of low-grade systemic inflammation in insulin resistance, atherosclerosis, obesity, type 2 diabetes mellitus, coronary heart disease and hypertension is the presence of elevated C-reactive protein in the plasma (CRP). CRP, produced by hepatocytes, is a good predictor of the development of cardiovascular events in apparently healthy men and women. CRP inhibits endothelial NO generation (6). This can be interpreted to mean that elevated levels of plasma CRP indicates that eNO generation is reduced.

4. SALT, POTASSIUM AND CALCIUM MODULATE ENDOTHELIAL NO GENERATION

NO is synthesized within the kidney and plays a significant role in the regulation of renal hemodynamics and excretory function. Both bradykinin and acetylcholine induce vasodilation by increasing NO synthesis that, in turn, leads to enhancement of diuresis and natriuresis. In contrast, inhibition of basal NO synthesis decreases renal blood flow and sodium excretion (7). NO interacts with the rennin-angiotensin system. Intrarenal inhibition of NO synthesis reduces sodium excretion in response to changes in renal arterial pressure without any effect on renal autoregulation, suggesting that NO has a role in pressure natriuresis. NO released from macula densa affects afferent arteriolar constriction. NO produced in the proximal tubule influences the effects of angiotensin on tubular reabsorption. In the collecting duct, NO alters solute transport. Even in the glomerulus, NO pathway has an active role (29). On the other hand, under pathologic conditions such as glomerulonephritis, enhanced NO generation is from the infiltrating macrophages (and so is an inducible NO) that plays a role in proteinuria, mesangial proliferation and other features seen in this condition.

The involvement of NO in renal function is supported by the observation that hypertensive patients whose average 24-hour blood pressure was increased by more than 5 percent by high salt diet (20 to 23 g per day) had decreased plasma nitrate plus nitrite, and increased asymmetrical dimethylarginine (ADMA) concentrations which reverted to normal following salt restriction. The

change in plasma nitrite and nitrate levels correlated inversely with the blood pressure and plasma ADMA levels after salt loading and salt restriction (8). These results suggest that salt intake decreases NO synthesis (9, 10) that could be responsible for salt-induced hypertension via increase in blood ADMA. In contrast, potassium enhances eNO release that explains its anti-hypertensive action (11).

Dietary calcium reduces blood pressure. When calcium intake is adequate, it could stabilize the arterial membranes, block its own entry into the cell, and render the arterial smooth muscles less likely to contract (12). Dietary calcium enhances NO synthesis and thus, reduces blood pressure (12, 13). Thus, salt-induced hypertension can be related to reduced NO generation that supported by the observation that adequate supplementation of L-arginine reduces high salt intake-induced hypertension (14-16). Vasodilation to acetylcholine increases with potassium chloride supplementation in patients with hypertension (17), indicating that potassium enhances NO synthesis and release (9, 10, 12) and thus, reduces blood pressure. In view of these findings, previously I suggested that at optimum physiologic concentrations of sodium, potassium, calcium, and magnesium the synthesis and release of NO and other vasodilators such as PGE_1 and PGI_2 will remain adequate to maintain normal blood pressure (12, 18-20).

5. ENDOCANNABINOID SIGNALLING IN NO GENERATION AND TYPE 2 DIABETES

Endocannabinoid signaling system is coupled to nitric oxide synthase (NOS)-derived nitric oxide (NO) released in vertebrates, thereby regulating neural, immune, and vascular-like functions. (21) There have been several studies of the interactions of the endocannabinoids (EC) with NOS which suggest that the actions of the EC on NOS may vary in different cell types.

Data suggest that the neuronal CB_1 receptor plays a role in the regulation of NO synthesis. Cannabinoid agonists stimulated NO production through CB(1) receptors, leading to activation of Gi/o protein and enhanced nNOS activity in N18TG2 neuroblastoma cells (22, 23). On the other hand, in cerebellum granule layer cells activation of the CB_1 receptor results in a decreased influx of calcium in response to membrane depolarization, resulting in a decreased activation of neuronal NOS. CB_1 receptor agonists can further reduce NOS activity while the cell itself, via changes in the synthesis or activity of CB_1 receptors themselves, can increase (i.e. disinhibit) NOS activity (24). The findings reported in these studies provide evidences linking to cannabinoid-mediated NO signal transduction in neuronal cells, which has important implications in the ongoing elucidation of the endocannabinoid system in the nervous system. (23).

Furthermore, Endocannabinoid System (ECS) can also play a role in development of hypertension and type 2 diabetes mellitus. This signalling system could be modulated by exogenous manipulation of polyunsaturated fatty acids (PUFAs), precursors of the endocannabinoids. Indeed, exist evidence for the overactivity of the ECS, in terms of up-regulation of either CB1 receptor or EC levels (25), or both during conditions of unbalanced energy homeostasis (e.g. obesity and hyperglycemia). This overactivity occurs at the level of both the hypothalamus and peripheral tissues, including the liver, pancreas, and epididymal adipose tissue in animals fed with a high fat diet (HFD), and in the visceral fat and blood of obese patients.(26, 27). Data from experimental studies indicated that the level of tissue EC can be significantly affected by modulation of dietary FA intake. In these experiments the early elevation of the levels of 2-AG, either as effect of diet or obesity induce over activity of the system in relation with the insulin resistance. Thisin turns might cause a stronger inhibition of AMPK and, subsequently, of glucose uptake and oxidation from the skeletal muscle, where CB1 receptors are present and functionally active with subsequent overall reduced energy expenditure. These effects could bring an increase of glucose levels, with a alteration of the action and response of a second peak of insulin liberation (25, 28). In human beta cells, despite the relatively low abundance of CB1 receptors, their stimulation also enhances insulin release. (26). However the relationship of ECS with obesity, hypertension and type 2 diabetes are very complex and further research is needed in order to integrate all the biological process involucred in these complex interlinked diseases.

6. ANTI-HYPERTENSIVE DRUGS SUPPRESS SUPEROXIDE ANION AND ENHANCE NO GENERATION

If it is indeed true that $O_2^{-\cdot}$ and NO have a role in the pathogenesis of hypertension, then it should be shown that currently available anti-hypertensive drugs have an action on NO system. Following 4-week treatment with lisinopril, an ACE inhibitor, a reduction in blood pressure and increased plasma NO and 6-keto-$PGF_{1\alpha}$, and a tendency for an increase in plasma bradykinin levels was noted (8). Hypertensive patients treated with benidipine, a calcium antagonist, or trandolapril, an ACE inhibitor, showed decreased blood pressure, and normalized NO and cGMP levels following 12 weeks after the treatment (9). Others reported similar results as well (10-13). These results indicate that the beneficial action of various anti-hypertensive drugs currently in use bring about their actions by increasing NO, PGI_2, and bradykinin formation. Our own study showed that NO is a potent inhibitor of ACE activity *in vitro* (12). We also observed that even calcium antagonists and Beta-blockers are potent inhibitors of free radical generation and lipid peroxidation process (1). It is likely that anti-hypertensive drugs inhibit $O_2^{-\cdot}$ generation that, in turn, increases the half-life of NO and at the same time they also enhance NO production. On the other hand, NO is a potent inhibitor of ACE activity whereas angiotensin II induces $O_2(-)$ generation by stimulating NAD(P)H oxidase. Thus there seem to exist a close interaction among NO, $O_2(-)$, ACE activity, angiotensin II, and the regulation of blood pressure (see Figure 1).

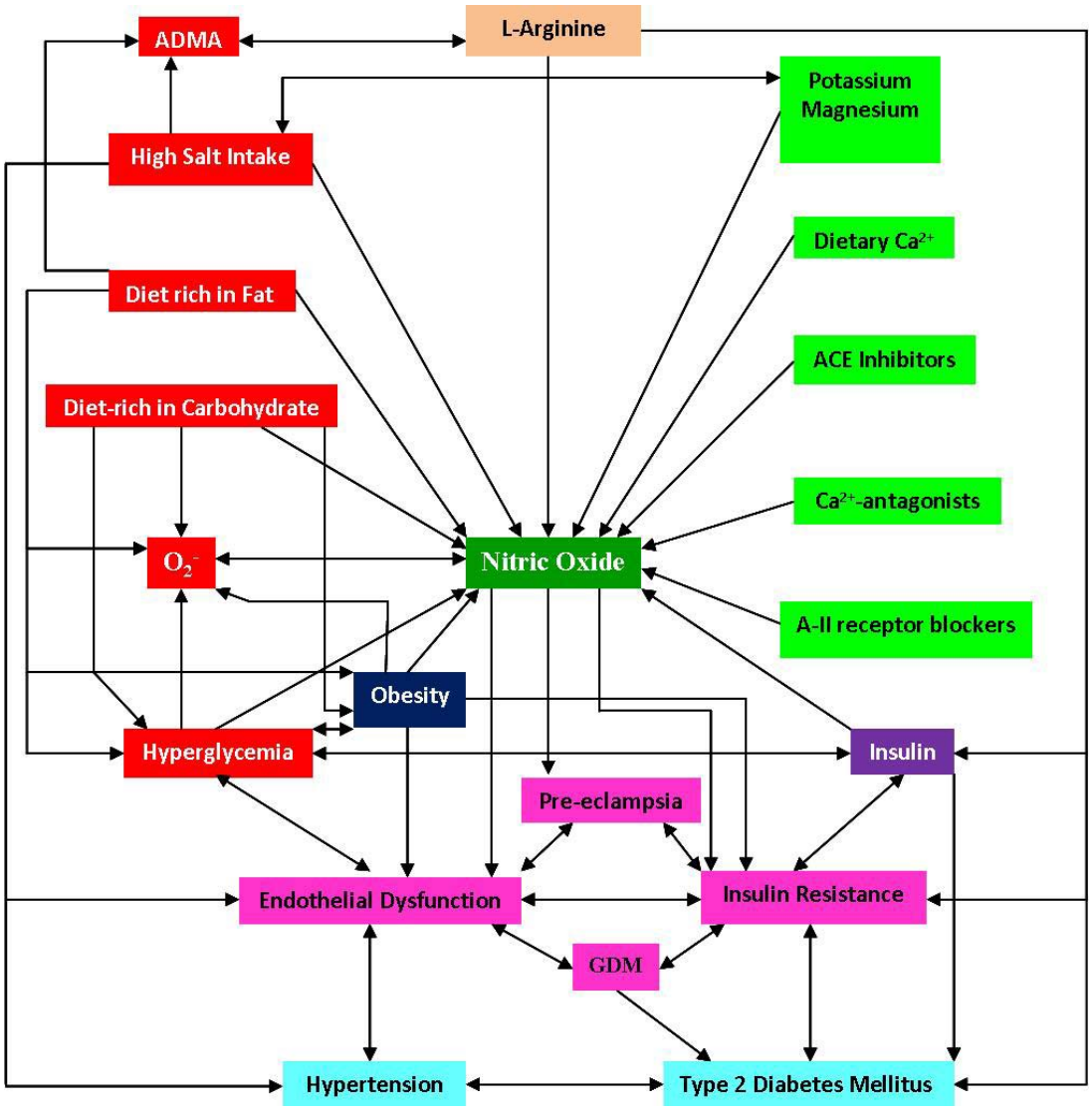

Figure 1. Scheme showing relationship between dietary factors, ADMA, NO, endothelial dysfunction, insulin resistance and blood pressure and type 2 diabetes mellitus.

7. L-ARGININE, NO AND ASYMMETRICAL DIMETHYLARGININE IN HYPERTENSION AND PRE-ECLAMPSIA

There is reasonable evidence to suggest that reduced synthesis and release of NO is one of the main, if not the sole, mechanisms of development of hypertension. One endogenous factor that could interfere with NO synthesis is asymmetrical dimethylarginine (ADMA). Higher concentrations of ADMA are associated with endothelial dysfunction in hypercholesterolemic individuals (29-31). In hemodialysis patients, plasma ADMA is a strong and independent predictor of overall mortality and cardiovascular outcome (32). Increased concentrations of ADMA have also been reported in hypertension and pre-eclampsia (30, 33-38). High serum concentrations of ADMA have been associated with an increased risk of acute coronary events suggesting that endothelial dysfunction promotes coronary heart disease (CHD) (39). Hence, displacing ADMA with excess L-arginine may be useful in hypertension, pre-eclampsia, and CHD. This is supported by the observation that in offspring of patients with essential hypertension, endothelial dysfunction due to impaired basal production of NO is present and vasodilation to acetylcholine could be enhanced in them by intra-brachial L-arginine infusion (40, 41). These evidences suggest that estimating the plasma levels of L-arginine and ADMA could be used to predict the development of hypertension and pre-eclampsia and oral supplementation of adequate amounts of L-arginine may prevent the development of hypertension in high risk subjects and pre-eclampsia. The adequacy of the L-arginine supplemented can be determined by estimating plasma L-arginine, ADMA and NO levels. If the supplementation of oral L-arginine is adequate, plasma levels of ADMA will decrease, L-arginine content and NO levels would increase

accompanied by decrease in blood pressure. Since L-arginine is a semi-essential amino acid and is relatively safe, it can be supplemented for long periods of time both to patients and those who are high risk of developing hypertension and pre-eclampsia.

8. L-ARGININE, ASYMMETRICAL DIMETHYLARGININE AND NO IN TYPE 2 DIABETES MELLITUS

In this context, it is noteworthy that even in type 2 diabetes mellitus an increase in plasma ADMA was reported (42, 43). In fact, it was found that in patients with type 2 diabetes mellitus plasma ADMA increased from 1.04 +/- 0.99 to 2.51 +/- 2.27 micromol/L (P:less than 0.0005) 5 hours after ingestion of a high-fat meal (44) and this was associated with a decrease in brachial arterial vasodilation after reactive hyperemia, a NO-dependent function, from 6.9+/-3.9 percent at baseline to 1.3+/-4.5 percent (P:less than 0.0001). The increase in plasma ADMA in response to a high-fat meal was significantly and inversely related to the decrease in percent vasodilation. These changes occurred in association with increased plasma levels of triglycerides and very low density lipoprotein triglycerides, with reduced low density lipoprotein cholesterol and high density lipoprotein cholesterol, and with no changes in total cholesterol. On the other hand, no significant changes in the brachial artery flow responses or in plasma ADMA were observed 5 hours after ingestion of a nonfat isocaloric meal, suggesting that ADMA contributed to abnormal blood flow responses.

It was reported (45) that serum ADMA concentrations were significantly elevated in type 2 diabetic patients compared with healthy subjects (3.44 +/- 0.40 vs 1.08 +/- 0.14 micromol/L, P less than 0.01) and were much higher in those macroangiopathy than the patients without macroangiopathy. But no difference was observed in serum ADMA concentrations between groups of patients with different diabetic duration. The elevation of ADMA was accompanied by impairment of endothelium-dependent relaxation and poor metabolic control in diabetes, suggesting that the extent of elevation in serum ADMA in patients with diabetes is not proportion with the length of their diabetic duration but rather with the metabolic control. Hence, it can be argued that elevated endogenous ADMA may be implicated in diabetes-induced endothelial dysfunction and macroangiopathy. But, a word of caution is in place at this juncture since, diabetic patients with a normal or slightly increased GFR have lower circulating ADMA concentrations than nondiabetic control subjects (46). It was also reported that plasma ADMA levels are associated with macrovascular disease independent of total homocysteine and traditional cardiovascular risk factors in patients with type 2 diabetes mellitus (47), though some studies are not in supportive of such data (46). Since, a close relationship between the inflammatory markers and plasma ADMA levels (48) and intensive treatment of risk factors in patients with type-2 diabetes mellitus was found to be associated with improvement of endothelial function coupled with a reduction in the levels of plasma ADMA (49), it is probably worthwhile to use ADMA a marker of the effectiveness of the treatment given to the patients.

When serum ADMA level and forearm blood flow responses to acetylcholine were measured in overweight and non-overweight women with previous gestational diabetes mellitus, it was noted that ADMA levels were higher and the vasodilation of forearm vessels to ACh was impaired in overweight women when compared with non-overweight women (P less than 0.05). In addition, a significant relationship between vascular responsiveness to Ach, body-mass index, serum ADMA concentrations and stimulated glucose levels (all P less than 0.05) was noted. ACh responses and ADMA levels in non-overweight women were similar to those of healthy controls (50). These results indicate that obesity, increased ADMA levels and insulin resistance appear are strong contributors to endothelial dysfunction observed in women with gestational diabetes mellitus.

It is rather interesting to note that angiotensin-converting enzyme inhibitors (51) but not glitazones (52) reduced plasma ADMA levels in type 2 diabetes mellitus, despite the fact that glitazones could improve endothelial function and reduce inflammatory markers. These results emphasize the fact that ADMA alone is not the marker of endothelial dysfunction. In contrast, Beta-blocker metoprolol produced a significant increase in serum ADMA levels (53).

NO metabolites were higher in subjects with glucose intolerance and type 2 diabetes mellitus; while nitrotyrosine was higher only in the type 2 diabetics; whereas N-acetyl-beta-glucosaminidase (NAGase) , thiols, ADMA and GSSG were elevated both in glucose intolerance and type 2 diabetes mellitus (54). These results indicate that NO metabolites, ADMA, thiols and NAGase GSSG could be used as early markers of endothelial dysfunction and oxidative stress in the early stages of impaired response to insulin.

9. CONCLUSIONS

Endothelial cells produce the potent vasodilator and platelet anti-aggregator NO that is reduced in patients with hypertension and type 2 diabetes mellitus. Diets rich in carbohydrate and saturated fat reduce eNO generation and thus, cause endothelial dysfunction and increase peripheral vascular resistance. Salt decreases whereas potassium and calcium enhance eNO synthesis. Currently available anti-hypertensive drugs suppress superoxide anion and enhance eNO generation and thus, seem to bring about some, if not all, of their beneficial actions. Blood ADMA levels are increased in subjects with obesity, dyslipidemia, hypertension, type 2 diabetes mellitus, gestational diabetes mellitus and pre-eclampsia. Increase in blood ADMA levels seem to precede the development of hypertension and type 2 diabetes mellitus. Elevated blood ADMA levels correlated with uncontrolled hyperglycemia, diabetic micro and macroangiopathy. Supplementation of L-arginine decreased endothelial dysfunction and restored peripheral vascular resistance to normal by lowering blood

ADMA and enhancing eNO generation. These results suggest that ADMA could serve as a useful marker to predict endothelial dysfunction, insulin resistance, glucose intolerance, poor metabolic control in type 2 diabetes mellitus and coronary heart disease (see Figure 1). It appears that serum ADMA levels may also indicate patient's response to the treatment given and thus, may serve as a prognostic marker.

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Abbreviations: NO: nitric oxide, ADMA: asymmetrical dimethylarginine, NOS: nitric oxide synthase, BH_4: tetrahydrobiopterin, PUFA: Polyunsaturated fatty acid, EC: endocannabinoid, ECS: endocannabinoid system, 2-AG: 2-arachidonoylglycerol, CHD: coronary heart disease, NAGase: N-acetyl-beta-glucosaminidase

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