

# Reduced NGF-Serum Concentrations in Coronary Artery Disease Patients Increase after Coronary Artery Bypass Grafting

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## Abstract

**Background:** Coronary Artery Disease (CAD) is the leading cause of death worldwide, but neurological deficits are the most devastating complications of its treatment, Coronary Artery Bypass Surgery (CABG). As Nerve Growth Factor (NGF) has been implicated in the modulation of inflammatory and fibroproliferative mechanisms leading to atherosclerosis as well as in neuroprotection and survival of neurons, the time course of NGF serum concentrations during CABG has been studied.

**Methods:** Twenty-six male patients were scheduled for coronary artery bypass grafting and NGF levels have been measured 1 hour before the operation started and 2, 5 and 120 hours postoperatively. Pre-operation values of NGFs were compared to healthy controls.

**Results:** We found significantly lower NGF serum concentrations in coronary artery disease patients (n=26, age: 68.8 ± 5.5 years, mean NGF: 13.04 ± 32.1 pg/ml) as compared to age-matched healthy controls (n=20, age: 64.35 ± 4.15, mean NGF: 29.54 ± 24.23 pg/ml). A significant increase of NGF was found 120 hours after operation (Z=3.26, p=0.001).

**Conclusion:** NGF seems to be decreased in coronary artery disease and is increased by operative coronary revascularization procedures. Changes occurring during cardiac surgery may indicate beneficial regenerative processes but may also implicate neuronal alterations induced by operative procedures.

**Keywords:** Nerve Growth Factor (NGF); Atherosclerosis; Surgery; Coronary artery syndrome

## Introduction

Coronary Artery Disease (CAD) and Myocardial Infarction (MI) remain major causes of morbidity and mortality worldwide [1]. Reduced blood flow (ischemia) causes progressive cardiomyocyte and endothelial cells (ECs) depletion by apoptotic death, which contributes to cardiac dysfunction. Acute coronary syndrome occurs as a consequence of coronary plaque rupture and superimposed thrombus following a process of atherosclerosis. Basic animal and clinical human studies have implicated inflammatory as well as fibroproliferative mechanisms in atherosclerosis [2]. Primarily initiated by endothelial dysfunction, this disease develops as a result of a complex interplay between various growth factors, cytokines, vascular smooth muscle cells and immune cells [3]. Nerve Growth Factor (NGF) is a powerful endogenous mediator that plays a prominent role in differentiation, survival and regeneration of sympathetic and sensory peripheral nerve cells [4]. It also plays this role in a variety of non-neuronal cells, including lymphocytes, mast cells and vascular smooth muscle cells [3,5], in which it induces cell migration and growth [3]. In addition, NGF is a major regulator of sympathetic innervations in the adult heart [6-8], where it acts on the nerves primarily in the cardiac atrium and ventricle [7] and coronary arteries [9]. NGF is expressed in the heart and other sympathetic targets and its concentration correlates with the density of sympathetic innervation [10]. The quantity of NGF may affect the sympathetic nerve survival and synaptic transmission between neurons and cardiac myocytes [11]. NGF elicits its biological effects mainly by binding the high-affinity TrkA receptor (tropomyosin-related receptor A, which is a tyrosine kinase). It was previously demonstrated that NGF, via TrkA, promotes angiogenesis and endothelial cells (ECs) survival through a mechanism involving the serine/threonine kinase Akt (also known as protein kinase B) [12]. Recently, it was shown

that NGF elicits pleiotropic beneficial actions in the post-myocardial infarction heart [13]. There is increasing evidence that there are two different trends in NGF expression after MI: an increase in the first phase within the first few hours and an opposite profile (decrease) after presentation of heart failure [14]. The failing heart in general shows a differential expression of cardiac neurotrophic factors including NGF and brain-derived neurotrophic factor (BDNF) [15]. For example, an NGF-depletion induced by Norepinephrine causes cardiac sympathetic denervation in severe heart failure and has been reported recently [16]. Additionally, there is evidence that the development and regulation of the cardiac sensory nervous system are dependent on NGF. NGF-depletion during diabetes mellitus is well known [17,18] and causes cardiac sensory neuropathy [19].

Main treatment options of CAD in addition to medication consist of Percutaneous Transluminal Coronary Angioplasty (PTCA) and Coronary Artery Bypass Grafting (CABG). Despite major improvement in surgical techniques, neurological deficits remain

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**Received** November 03, 2012; **Accepted** December 10, 2012; **Published** December 12, 2012

**Citation:** Köhler S, Tugtekin SM, Kappert U, Willbring M, Eckart S, et al. (2013) Reduced NGF-Serum Concentrations in Coronary Artery Disease Patients Increase after Coronary Artery Bypass Grafting. J Clin Exp Cardiol 4:228. doi:10.4172/2155-9880.1000228

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one of the most devastating complications of CABG, with a reported incidence of perioperative neurological injury ranging up to 6% [for review see [20]]. In recent years, off-pump surgery has been successfully established in CABG [21]. Avoiding Extracorporeal Circulation (ECC) seems to be a suitable alternative strategy since ECC is associated with morbidity, particularly in case of a neurological deficit. As neurological deficits and cognitive dysfunction in CABG patients continue to be major sources of complications leading to strokes, perioperative search for prospective markers or signs for neuroprotection is an essential tool for stroke prevention. NGF partially mediates neuroprotection [22], so it was suggested that strokes may modulate peripheral serum neurotrophin levels [23].

Therefore, we initiated this study to find a possible time course of NGF serum levels during CABG to eventually further detect relevant information on the role of this neurotrophin in the modulation of inflammation, atherosclerosis and neurological deficits with particular interest on applied surgical techniques.

## Materials and Methods

We investigated the perioperative course of NGF serum concentrations in 26 patients ( $68.8 \pm 5.5$  years) with isolated coronary artery disease undergoing CABG (Table 1). Patients were divided in two groups: thirteen patients were operated on-pump with the use of ECC and the other thirteen pts were operated off-pump without the use of ECC. None of these patients suffered from acute coronary syndrome. The study protocol was in agreement with the guidelines of the ethics committee of our institution and a written consent was obtained from all subjects in accordance to the Declaration of Helsinki. Inclusion criteria were isolated three vessel coronary artery disease with an indication for elective operative revascularization. Patients with coexisting metabolic, primary renal or hepatic dysfunction or neurological disorders and major psychiatric disorders were not included in this study. Our age-matched healthy control subjects ( $n=20$ , age:  $66.2 \pm 4.8$  years) were selected from a larger sample ( $n=376$ ) on the basis of age, gender and availability of NGF serum concentrations [24]. CAD was excluded in the normal population group (Table 1). We also excluded patients with other heart diseases (e. g. hypertension, heart failure). Controls received no cardiological or psychopharmacological medication.

## Anesthetic technique

All patients received 1-2 mg lorazepam p. o. depending on body weight, at an exact time on the evening before operation. Anesthesia was induced using thiopental, (3-5 mg/kg) and fentanyl (0.03 mg/ kg body weight i.v.). Rocuronium was used as a muscle relaxant (0.6-0.8 mg/kg). A single lumen endotracheal tube was inserted and sevoflurane was used to maintain anesthesia in clinically relevant concentrations. Additional fentanyl was applied when necessary. After harvesting the internal mammary artery, 500 IU/kg body weight was injected i.v. In procedures using extracorporeal circulation, normothermia was used. At the end of the procedure, heparin was always reversed using 400 int.

	Basic characteristics of the study group		
	control group	on-pump	off-pump
n	26	13	13
age, mean	66.2	67.8	69.8
Sex, female, n	12	7	7
Hypertension, n	26	13	2
Diabetes, n	18	8	2
Dyslipidimia, n	23	11	4

Table 1: Basic characteristics of the study group.

IU/kg protamine. Invasive arterial pressure, central venous pressure and 6-lead ECG monitoring were performed during the procedure. For temperature monitoring, an intravesical catheter was used.

## Surgical procedure

Decision regarding the applied surgical technique was left to the operating surgeon. All surgical procedures were performed through a median sternotomy. All on-pump procedures included the use of normothermic ECC and antegrade myocardial protection with blood cardioplegia. The method of exposure of the target coronary vessel and of stabilization for off-pump surgery has been previously reported [25]. For exposure of the circumflex and inferior coronary area, apical suction was applied and stabilization was achieved with the use of the Guidant® system. An intracoronary shunt was used in all coronary target vessels.

The left internal thoracic artery was used for revascularization of the anterior wall; further revascularization was performed with saphenous vein grafts. In the off-pump group, a no-touch technique to the aorta was applied in all pts using T-grafts to avoid partial clamping of the aorta.

## Measurements of NGF concentrations

Vein blood samples of 5 ml were taken 1h preoperatively, and 2 h, 5 h, and 120 h postoperatively.

Blood samples were collected in chilled plastic tubes, immediately placed on ice and centrifuged within 20 min. for 15 min. at 3500 rev/min at room temperature. Serum was then stored at  $-80^{\circ}\text{C}$  until assayed. NGF concentrations in the re-thawed serum were determined by a highly sensitive and specific fluorometric two-site ELISA with a detection limit of 12.5 fg NGF/assay as described in detail elsewhere [24,26]. Determinations of recovery, specific and unspecific NGF binding (the latter against mouse IgG<sub>1</sub> obtained from MOPC 21, Sigma Chemicals, Deisenhofen, Germany) involved quadruplicate fluorescence determinations for each serum sample. NGF serum values are shown in pg/ml (mean values  $\pm$  standard deviation) [24].

## Data analysis

Kolmogorov-Smirnov test was employed to evaluate whether NGF level is a normally distributed trait. The differences between the four individual NGF levels were tested by a nonparametric one way analysis of variance for dependent measures (Friedman test). Correlations with clinical parameters were determined with Spearman's rank correlation. Pair differences were tested by Wilcoxon's matched pair signed rank test. NGF changes were measured using Wilcoxon's test for paired matches. Results are presented as means  $\pm$  one standard deviation. Analyses were computed using statistical software (SPSS 16.0®). A p value of  $p<0.05$  was considered significant while  $p<0.10$  was accepted in order to detect trends. Differences in gender between both groups were calculated using Chi-square tests.

## Results

All patients survived the procedure. In both groups, no perioperative myocardial infarction and no neurologic deficits were documented. No patient received inotropic support during the operation. No secondary complication of bleeding or wound infection occurred.

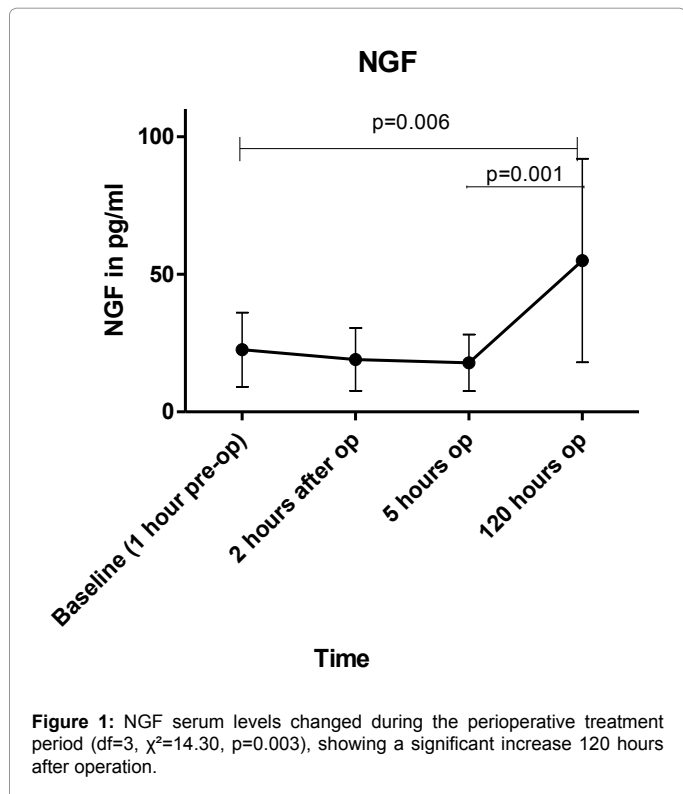
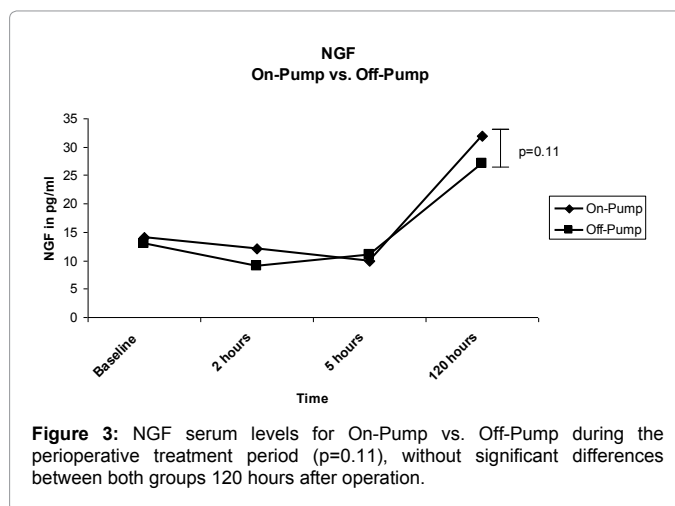
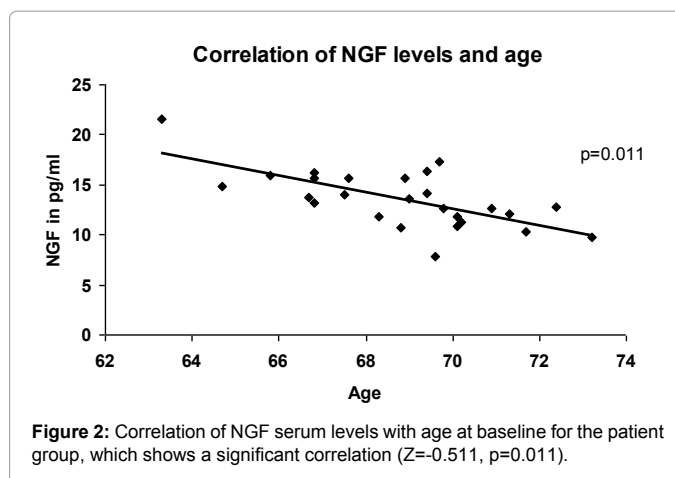
Kolmogorov-Smirnov test ( $Z=2.232$ ,  $p=0.0001$ ) showed that NGF serum concentrations in our sample were not normally distributed, which is in line with our previous publications [24,27]. NGF serum

levels in the patients amounted to  $13.0 \pm 32.1$  pg/ml (n=26) at baseline (1 hour preoperatively), 2 hours after operation it was  $11.0 \pm 27.1$  pg/ml and 5 hours after operation it was  $10.5 \pm 25.1$  pg/ml and after 120 hours it was  $28.8 \pm 81.2$  pg/ml. NGF serum levels changed during the perioperative treatment period (df=3,  $\chi^2=14.30$ ,  $p=0.003$ ), showing a significant increase at day 5 when compared to 5 hours after operation ( $Z=3.26$ ,  $p=0.001$ ) or when compared to preoperative values ( $Z=3.26$ ,  $p=0.006$ ) (Figure 1). A negative correlation between NGF concentrations and age ( $Z= -0.511$ ,  $p=0.011$ , Figure 2) has been detected in the same sample at baseline. Mean preoperative NGF serum levels were significantly reduced as compared to age-matched normal healthy controls (n=20, age:  $64.4 \pm 4.2$ ,  $29.5 \pm 24.2$  pg/ml) [17] ( $Z= -4.64$ ,  $p=0.0001$ ).

Separation between on-pump and off-pump patients showed significantly increased NGF values of on-pump patients at day 5 (n=13;  $\chi^2=8.82$ ;  $p=0.032$ ), which remained a trend also for off-pump patients (n=13,  $\chi^2=7.163$ ,  $p=0.067$ ). A significant difference of NGF values after 120 hours could not be detected between on-pump and off-pump patients (Figure 3).

### Discussion

We found significantly lower NGF serum levels in patients with coronary artery disease when compared to age matched healthy controls, which significantly increased after cardiac surgery. This increase was even more pronounced in patients receiving on-pump operation compared to the off pump patients group, but this difference was not significant. Our observation of decreased NGF serum concentrations in patients with coronary artery syndrome is in line with findings of decreased plasma concentrations of NGF in patients with acute coronary syndromes [28] and post mortem probes that showed decreased NGF levels in atherosclerosis-lesioned arteries [3].



We also demonstrated a significant increase of NGF at day 5 compared to 5 hours after operation or preoperative values. This is in line with recent findings of NGF-expression following myocardial infarction in a mouse model. In the same study, Meloni and colleagues reported that NGF overexpression sustained its capacity to improve cardiac function and to promote angiogenesis 14 days after MI, only under experimental conditions however [13]. Another clinical study demonstrated that NGF level is significantly low in human coronary arteries with advanced atherosclerotic lesions [3] and reduced NGF levels were postulated to contribute to the pathogenesis of heart failure [29,30]. Therefore, an improvement of cardiovascular functioning could be indicated by increasing NGF values.

It is known that heart cells secrete NGF and express TrkA in normal conditions. The interaction between NGF and its receptor in cardiomyocytes causes a prosurvival signal of transcriptional factors [31]. For several cardiac diseases (MI, CAD) Rana and colleagues reported that cellular stretch leads to a decrease of NGF mRNA and protein expression in cardiomyocytes [32]. Therefore CABG, which aims to create a new pathway for blood flow that ensures the delivery of oxygen and nutrients to the heart muscle, could improve these conditions in patients with CAD.

However, there could be different explanations for the increase in postoperation NGF levels. Neurological deficits and cognitive dysfunction in patients undergoing coronary artery bypass grafting

with cardiopulmonary bypass continue to be a major source of complications, with a reported incidence of perioperative neurological injury ranging up to 6% [20]. In this context, the large NGF-increase after operative procedures could signal neurologic impairment or the beginning of cerebral deterioration. This explanation is supported by studies that have postulated increased levels of NGF to be associated with central nervous injury as stroke [23], multiple sclerosis [33] traumatic head injury [34], Alzheimer's disease [24] and epileptic seizures [35]. Increased serum NGF could hereby serve as a marker for arising neurologic complications, playing a role in an intrinsic attempt to compensate nervous injury. In light of this, it is important to consider that NGF was effective in prevention of neuronal death in focal cerebral ischemia in a rat model [36] and infarct volume after intranasal NGF was markedly reduced by 38.8% in rats [28]. NGF has also been implicated in hypoxic ischemic brain injury as a possible intraventricular treatment option [37]. Recent trials demonstrated that intraventricular NGF administration improves the cerebral perfusion and stimulates the pathway of neurogenesis differentiation [38].

However, no clinically obvious neurological complications appeared in our sample during the study period and we did not detect emboli or perform neurocognitive testing, which we will follow up with in further observations.

Another explanation for increasing NGF concentrations could be the proposed role of NGF as an important component of healing wounds and tissue repair processes in vivo and in vitro [39,40]; therefore, postoperative increase of NGF could indicate augmented wound closure in several patients.

Also, direct detrimental effects of surgery could change NGF levels, as in the rat aortic balloon de-endothelialization model of vascular injury in which the expression of NGF increased dramatically in the area of injury within 3 days and persisted during the formation of the neointima [5].

## Limitations

In this naturalistic study we did not control for the complex medication in the treatment group. This might have had influence on our results as we cannot exclude that changes of NGF-levels are caused to pharmacological reasons in the severe and complex treated patient group, especially compared to the control group. In general, we did not analyse the cardiovascular profile of the treatment group in detail (for example ejection fraction of the heart, blood cholesterol, inflammation). So there is the possibility that different factors next to CABG had influence on our results. We also did not control for smoking status in the treatment and control group, which may influenced NGF-levels. We also investigated NGF levels only for a short time after operation. To see how NGF-levels develop in the long-run, in future research measurement of NGF should be continued for a longer time period. Therefore, the association of increasing NGF-levels and clinical improvement is highly relevant and should be investigated. It should also be mentioned that reports suggested a gender dependent difference in the NGF and BDNF serum levels [41]. Additionally, due to the small study size, this study may have been underpowered for some comparisons. In different studies, it could be demonstrated that plasma BDNF significantly correlates with multiple risk factors for metabolic syndrome and cardiovascular dysfunction. Because NGF and BDNF belong to the same protein family, it should be taken into consideration that our findings of decreased NGF in CAD are possibly not specific [42].

## Conclusion

NGF serum levels are altered by coronary artery disease and are affected by operative coronary revascularization procedures. Changes occurring during cardiac surgery may indicate beneficial regenerative processes as NGF is critical for cardiac sensory and sympathetic innervation [19]. However, there are several factors which may influence NGF-level. Further research is necessary to clarify the impact of NGF in CAD and MI. Thus, NGF should be considered as a candidate for therapeutic cardiac regeneration.

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