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# Effect of short- and long-term physical activities on circulating granin protein levels

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ABSTRACT

*Background:* The classic chromogranin–secretogranin (granin) proteins are produced in the myocardium and throughout the neuroendocrine system, but while chromogranin (Cg) A and B levels are high in the adrenal medulla, secretogranin (Sg) II production is higher in the pituitary gland. Whether these differences may influence the response to physical activity is not known.
*Methods:* We measured circulating granin proteins during (1) a short-term maximal bicycle exercise stress test and (2) a 7 day military ranger course of continuous physical activity and sleep and energy deprivation.
*Results:* In 9 healthy subjects performing the exercise stress test (7 male, age 45 ± 5 y [mean ± SEM], duration 10.13 ± 1.14 min), CgB levels increased from before to immediately after the test: 1.20 ± 0.12 vs. 1.45 ± 0.09 nmol/L, p = 0.013. Metabolic equivalents, representing an index of performed work, were closely associated with the change (Δ) in CgB levels during stress testing and explained 74% of the variability in $_{\Delta}$CgB levels (p = 0.004). CgA and SgII levels were not increased after exercise stress testing. In the second cohort of 8 male subjects (age 25 ± 1 y) participating in the ranger course, CgB levels increased from day 1 and were significantly elevated on days 5 and 7. CgA also increased gradually with levels significantly elevated on day 7, while SgII was markedly increased on day 5 whereas levels on days 3 and 7 were unchanged compared to baseline levels.
*Conclusion:* We demonstrate a heterogeneous response to short- and long-term physical activities among circulating granin proteins with the most potent effect on CgB levels.


## 1. Introduction

The chromogranin–secretogranin (granin) proteins are a group of proteins characterised by a high proportion of acidic amino acids, the ability to form stable aggregates in cytoplasmic vesicles, and pronounced post-translational processing due to several dibasic cleavage sites [1]. Although 8 different granin proteins have been identified, chromogranins (Cg) A and B and secretogranin II (SgII) are considered the classic members of this protein family [1]. The granin proteins were first identified in neuroendocrine tissue [2], but production has later been demonstrated in other tissues, including in the myocardium [3–6]. In the neuroendocrine system, the adrenal medulla is considered a primary source of CgA [7] and CgB productions [8], while SgII production is especially high in the pituitary gland [9–11].

Of the granin proteins, CgA was first identified and has been studied most extensively. CgA is co-released with catecholamines from neuroendocrine tissue [12] and circulating CgA levels have been found to correlate with noradrenaline and adrenaline levels in situations of enhanced adrenergic drive [13–15]. An inverse correlation between CgA levels and heart rate variability has also been reported [16], thus supporting CgA as an index of neuroendocrine activity. The association with neuroendocrine activity may at least partly explain the powerful prognostic information obtained by measuring CgA levels in patients with acute cardiac disease [17–19] or severe sepsis [20]. In contrast, although adrenal medulla CgB levels have been found to parallel CgA levels in humans

*Abbreviations:* CgA, chromogranin A; CgB, chromogranin B; SgII, secretogranin II; SEM, standard error of mean; ECG, electrocardiogram; BMI, body mass index; METs, metabolic equivalents; $VO_2$ max, maximal oxygen uptake; RIA, radioimmunoassay; HPLC, high-performance liquid chromatography; NT-proBNP, N-terminal pro-B-type natriuretic peptide; mRNA, messenger-RNA.

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[11], no information is currently available on the association between adrenergic tone and circulating CgB or SgII levels. Physical activity is associated with enhanced adrenergic tone [21] and alterations in protein levels after exercise could serve as a model to explore the association between adrenergic tone and granin proteins. Physical activity may also induce myocardial release of granin proteins, e.g. due to acute myocardial wall stress, analogous to the postulated main stimulus for pro-B-type natriuretic peptide secretion from the myocardium [22]. Accordingly, as several organs may release granin proteins to the circulation during exercise, in this study we wanted to assess the influence of short- or long-term strenuous physical activities on circulating granin protein levels.

## 2. Methods

### 2.1. Study cohort #1: Short-term strenuous physical activity

Nine adult (>18 y) subjects with no history of cardiovascular disease or other concurrent disease, no current symptoms of cardiovascular disease as evaluated by one investigator (HR), and no use of medication, including proton-pump inhibitors (PPIs), were recruited from outside the hospital to perform a maximal bicycle exercise stress test. All tests were performed in the morning (8–10 a.m.). The self-reported physical capacity of the participants ranged from well trained (daily training) to sedate (only walking during daily activities). We started the exercise stress test at 100 W, increased by 50 W/4 min, and encouraged the participants to continue the test to physical exhaustion as determined by ≥18 on the Borg scale. We recorded symptoms, heart rate, blood pressure, and a 12-lead ECG before, during, and after exercise stress testing. Predetermined criteria to terminate the stress test were physical exhaustion or severe chest pain, >2 mm horizontal or downsloping ST segment depression, >20 mm Hg decrease in systolic blood pressure, or sustained ventricular arrhythmias. We recorded the duration of the stress test, workload (W) achieved, and calculated metabolic equivalents (METs = [12 ∗ workload (W) + 300] / [weight (kg) ∗ 3.5]). The exercise ECGs were reviewed by a cardiologist (JEH) with no knowledge of granin, NT-proBNP, or catecholamine levels, and >0.1 mV horizontal or downsloping ST segment depression 0.08 s after the J-point and/or characteristic symptoms were considered as indicative of reversible myocardial ischaemia. We recorded weight and height of the subjects before the start of the exercise test and body mass index (BMI) was calculated by weight (kg) / [height (m)]$^2$.

### 2.2. Study cohort #2: Long-term strenuous physical activity

Eight adult (>18 y) male subjects with no history of cardiovascular disease or other concurrent disease, no current symptoms of cardiovascular disease or intercurrent illness as evaluated by one investigator (PKO), and no use of medication, including PPIs, were recruited from a military ranger course. The subjects were all students at the Norwegian Military Academy participating in the compulsory, one week ranger course in June during their second year of training. Physical exercise is an integrated part of the student curriculum and all participants were well trained. The experimental protocol of the ranger course has been reported previously, but in short the participants were subjected to 24 h continuous physical activities with total energy expenditure of >25 MJ/day, which accounts to 4–5-fold higher expenditure than at the resting metabolic rate [23]. The participants were also subjected to severe sleep and energy deficiency with no time for prolonged sleep allowed during the course and only minimal food provided (~2500–3400 kJ per subject in total). The subjects were allowed free access to water. Weight and height of the subjects were measured before the start of the study and BMI calculated as previously reported. Weight was also recorded on days 3, 5, and 7.

### 2.3. Blood sampling and laboratory analyses

We inserted an intravenous line in an antecubital vein before the start of the exercise stress test and collected blood in EGTA glutathione, EDTA, and Li-heparin plasma tubes before and immediately after the exercise stress test. Processing of the EGTA glutathione plasma was completed within 15 min of collection and the tubes were not exposed to sunlight. The Li-heparin and EDTA samples were processed within 30 min of collection.

Blood samples from the ranger course were obtained in Li-heparin tubes before the start of the ranger course, and then later at day 3, day 5, and day 7. Sampling was performed at 7 a.m. in the field after the participants had rested for 5 min. The tubes were immediately frozen on dry ice and on the same day they were transported to Akershus University Hospital for processing. All samples were stored at −80 °C before analyses. CgA was measured by a commercial available radioimmunoassay (RIA) [EuroDiagnostica AB, Malmö, Sweden] [24], CgB [25] and SgII [26] with in-house RIAs, adrenaline and noradrenaline by high-performance liquid chromatography (HPLC) as previously reported [27], and N-terminal pro-B-type natriuretic peptide (NT-proBNP) levels were measured by the proBNP II assay (Roche Diagnostics, Penzberg, Germany).

### 2.4. Statistics

Distribution of the granin proteins, catecholamines, and NT-proBNP levels were assessed by the Kolmogorov–Smirnov one sample test and found to be normally distributed. Data are presented as mean ± SEM and differences between baseline levels and later time points were assessed by the Student's *t* test for paired samples. Correlation coefficients were examined by Pearson correlation. Variables influencing alterations in granin protein, catecholamine, and NT-proBNP levels during the bicycle exercise stress test were examined by multivariate linear regression analysis with age; gender; BMI; baseline heart rate; baseline diastolic and systolic blood pressure; baseline levels of the granin proteins, catecholamines and NT-proBNP levels; and METs included in the model. Corresponding linear regression models examining factors associated with the largest increment in granin protein and NT-proBNP levels during the ranger course included age; BMI before the start of the course; baseline levels of granin proteins and NT-proBNP; and weight loss during the course. We did not adjust for multiple comparisons and a p value < 0.05 was considered significant for all analysis. Statistical analyses were performed by PASW for Windows version 19.0 (PASW, IBM, Chicago, IL).

The study was conducted according to the Declaration of Helsinki, approved by the local Ethics Committee, and all participants provided informed consent before study commencement.

## 3. Results

### 3.1. Patient characteristics

We recruited 7 male and 2 female subjects for the exercise bicycle test and 8 male subjects for the ranger course. All participants were considered healthy based on medical history and clinical examination. Subjects performing the exercise bicycle test were 45 ± 5 y and participants of the ranger course 25 ± 1 y. BMI was 26 ± 1 in both cohorts.

### 3.2. Granin proteins during short-term strenuous physical activity

Heart rate and systolic blood pressure increased during the exercise stress test (Table 1). All participants continued the exercise stress test to exhaustion (≥18 on the Borg scale), no participant experienced any chest pain or symptom of cardiovascular disease, and all ECGs were considered normal. The granin proteins were not significantly correlated before the start of the exercise stress test, while there was a close