The inflammatory biomarker YKL-40 decreases stepwise after exercise stress test

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Background **Serum YKL-40 is an inflammatory biomarker associated with disease activity and mortality in diseases characterized by inflammation such as coronary artery disease (CAD). Exercise has a positive effect on CAD, possibly mediated by a decreased inflammatory activity. This study aimed to compare serial measurements of serum YKL-40 before and after exercise in patients with stable CAD versus controls.**

Materials and methods **Eleven patients with stable CAD verified by coronary angiography (>70% stenosis) and 11 patients with a computer tomography angiography with no stenosis or calcification (calcium score = 0) (controls) performed a standard clinical maximal exercise test. Serum YKL-40 was measured before exercise, immediately after exercise, and every hour for 6 h.**

Results **Cardiovascular risk factors were more prevalent among the CAD patients compared with the controls. CAD patients had higher serum concentration of YKL-40 at baseline compared with controls, median (interquartile range) 94 (52–151) versus 57 (45–79) μg/l. Serum YKL-40 decreased stepwise after exercise, with a median decrease of 16 (13–39) μg/l for the CAD patients and 13 (10–22) μg/l for the controls from baseline to the lowest value. Thereafter, values increased again toward baseline level. Time after exercise was a significant factor for decrease in serum YKL-40 ($P < 0.0001$), but no difference in YKL-40 decrease over time could be demonstrated between the groups ($P = 0.12$).**

Conclusion **Serum YKL-40 is elevated in patients with documented CAD compared with controls, and it decreases stepwise after exercise in both groups, indicating an anti-inflammatory effect of exercise independent of the presence of coronary atherosclerosis.** ***Cardiovasc Endocrinol*** **5:21–27**

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Keywords: biomarkers, coronary artery disease, exercise test, inflammation, serum YKL-40, stable angina pectoris

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Introduction

Coronary artery disease (CAD) is the most common cause of death in western countries [1]. Many factors, including inflammation, are involved in the development of CAD [2]. Inflammatory processes play a pivotal role in all stages of the development of both acute and chronic atherosclerosis – from the initial induction of endothelial dysfunction and plaque formation to plaque destabilization and disruption with superimposed thrombosis leading to acute myocardial infarction or death [3]. Therefore, biomarkers with the ability to monitor inflammatory processes are of incremental importance in CAD.

YKL-40 (also named chitinase 3-like 1 protein, CHI3L1) is a highly conserved heparin-binding glycoprotein produced by several cell types of the immune system [4] and macrophages in atherosclerotic plaques [5,6]. Circulating YKL-40 is regarded as a non-disease-specific biomarker of inflammation and tissue remodeling [7–9]. YKL-40 increases with age [8,10–12] and is elevated in diseases characterized by inflammation, increased extracellular remodeling, and ongoing fibrosis [4], such as cancer [8], heart failure [13–15], and ischemic cerebrovascular disease [16]. YKL-40 is increased in patients with acute myocardial infarction [17,18] and in patients with stable CAD [12,14,18–21] and furthermore related to prognosis [12].YKL-40 has also been found to be a strong predictor of death independent of diagnosis [22]. A recent study demonstrated that plasma CHI3L1 (YKL-40) and muscle CHI3L1 mRNA increase after 1 h of intensive exercise in healthy participants [23].

Physical inactivity is an important risk factor for CAD, and increasing evidence suggests that the beneficial effects of physical activity are mediated partly by reducing vessel wall inflammation [24,25]. The purpose of

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this study was to compare serial measurements of serum YKL-40 before and after exercise in patients with stable CAD and angiographic documented coronary artery stenosis versus controls referred for suspected CAD, but found to have no stenosis nor calcification of the coronary arteries. We hypothesized that serum YKL-40 would decrease after exercise with a potentially different effect depending on the degree of coronary artery stenosis.

Materials and methods

Patients referred to the Department of Cardiology, Hillerød Hospital, Denmark because of stable angina pectoris were screened for inclusion between March 2010 and March 2012 [26]. Patients with a moderate to high a-priori risk for CAD underwent direct invasive coronary angiography (CAG), and the degree of epicardial stenosis was determined by the invasive cardiologist performing the CAG. Patients with a low a-priori risk for CAD had a computer tomography (CT) angiography. The coronary artery calcium score was obtained from the CT angiography according to the method described by Agatston using a threshold of 130 Hounsfield Units (HU) [27]. In the present study, patients with CAG-documented CAD (>70% stenosis in minimum 1 epicardial coronary artery) comprised the CAD patient group, whereas participants with no stenosis or coronary calcification (calcium score = 0) served as the control group.

Data were collected via interview with trained health professionals. A transthoracic echocardiography (Vivid 7; General Electric, Horten, Norway) was performed before inclusion, and patients were excluded if the left ventricular ejection fraction was below 50% or in the case of disease of the heart valves. Furthermore, patients were excluded if the CAG revealed three-vessel disease or left main artery stenosis. Further exclusion criteria were as follows: unstable angina pectoris, previous coronary artery bypass graft surgery, history of arrhythmia, renal insufficiency, chronic obstructive pulmonary disease, inability to perform a bicycle exercise test, or inability to provide written informed consent.

Bicycle exercise test

A maximal exercise test was performed in both the CAD patients and the control group. The test was performed on a bicycle ergometer (eBike Basic; General Electric) applying a standard clinical protocol. Work load was gradually increased with 25 W every second minute while patients were monitored with continuous 12-lead ECG and noninvasive blood pressure measurement every minute. The exercise test was terminated when patients reached physical exhaustion or experienced severe chest pain. The following was registered for all participants: symptoms, ST-segment changes, maximal work load, metabolic equivalents, peak blood pressure, and heart rate.

Blood samples

Peripheral blood samples were drawn a total of eight times: at baseline right before the exercise test, immediately after test termination, and once every hour until 6 h after the exercise test had terminated. Serum was extracted and stored at −80°C until analysis was performed. YKL-40 is stable in blood stored at −80°C [10]. Serum concentrations of YKL-40 were measured in November 2013. Samples were determined in duplicates by a commercial enzyme-linked immunosorbent assay (Quidel, Santa Clara, California, USA). The detection limit was 20 µg/l. The intra-assay coefficients of variation were 5% (at 40 µg/l), 4% (at 104 µg/l), and 4% (at 155 µg/l). The interassay variation coefficient of variation was less than 6%.

Ethics

The study was approved by the regional ethics committee with the reference number H-3-2010.013. All included patients gave written consent following oral and written information.

Statistics

Continuous data with a Gaussian distribution are presented as mean ± SD. Continuous data with a non-Gaussian distribution are presented as median (interquartile range). Proportions are presented as number (n) and percentage (%). Serum YKL-40 was logarithmically transformed ($\log_{10}$) because of a non-Gaussian distribution.

An unpaired Student's t-test was performed for within-group comparisons of continuous variables with a Gaussian distribution. A Wilcoxon signed-rank test was performed on the continuous data with a non-Gaussian distribution (YKL-40, pack-years, alcohol, and weekly exercise). Unpaired comparisons of categorical variables were performed with the χ^2-test or Fisher's exact test, if any expected value was below 5.

Differences in YKL-40 between groups were tested using a linear mixed model. Serum YKL-40 was logarithmically transformed ($\log_{10}$) to stabilize the variance.

Statistical analysis was performed using SAS Enterprise Guide 7.1 (SAS institute Inc., Cary, North Carolina, USA). A two-sided P-value below 0.05 was considered statistically significant.

Results

Baseline characteristics

Twelve CAD patients and 12 controls were initially included. One patient with CAD was excluded because of missing serum YKL-40 values. One control was excluded because of very high serum YKL-40 concentrations (i.e. 1644 µg/l and > 20 times above normal levels). Baseline characteristics for the 11 CAD patients and 11 controls are given in Table 1. Patients with CAD

Table 1 Demographic data of the patients and controls

	CAD patients ($N=11$)	Controls ($N=11$)	*P*-value
Demographics			
Age (mean ± SD) (years)	63 (10)	54 (11)	0.052
Males [*n* (%)]	10 (91)	3 (27)	0.008
Diabetes mellitus [*n* (%)]	1 (9)	0	1.000
Hypertension [*n* (%)]	5 (45)	3 (27)	0.66
Dyslipidemia [*n* (%)]	10 (91)	3 (27)	0.008
Current or former smokers	10 (91)	4 (36)	0.024
Pack-years[a] [median (IQR)] [*n* (%)]	30 (25–40)	15 (10–20)	0.004
Alcohol [median (IQR)] (g/week)	10 (5–12)	6 (0–10)	0.26
Weekly exercise [median (IQR)] (h)	3 (1–4)	3 (2–5)	0.56
Systolic blood pressure (mean ± SD) (mmHg)	137 ± 21	128 ± 13	0.25
Diastolic blood pressure (mean ± SD) (mmHg)	89 ± 15	85 ± 18	0.60
Heart rate (mean ± SD) (beats/min)	64 ± 10	79 ± 8	0.001
Previous acute myocardial infarction [*n* (%)]	4 (36)	0 (0)	*
One-vessel disease [*n* (%)]	6 (55)	0 (0)	*
Two-vessel disease [*n* (%)]	5 (45)	0 (0)	*
Cerebrovascular events [*n* (%)]	1 (9)	1 (9)	1.00
β Blockers [*n* (%)]	8 (73)	3 (27)	0.033

CAD, coronary artery disease; eGFR, estimated glomerular filtration rate; IQR, interquartile range.

[a]Smokers only, 20 cigarettes/day per year.

*Groups differ by design. *P*-value for differences between groups was obtained by the χ^2-test (or Fisher's exact test if any expected value was below 5) for the categorical variables and by an unpaired Student's *t*-test for the continuous variables. A Wilcoxon signed-rank test was performed on the continuous data with a non-Gaussian distribution (pack-years, alcohol, weekly exercise).

were slightly older than the controls (62.5 vs. 53.5 years), and a higher proportion of CAD patients were male (91 vs. 27% in the control group). The control group fulfilled the inclusion and exclusion criteria, but had a normal CT angiogram. CAD patients were more likely to have dyslipidemia and a history of smoking. Among the smokers in both groups, those with documented CAD had smoked more pack-years (i.e. 20 cigarettes/day per year). Furthermore, CAD patients were more often treated with β blockers and had a lower heart rate at baseline compared with controls. By design, the distribution of cardiovascular disease differed between the two groups. Four (36%) CAD patients had a previous myocardial infarction, six (55%) had one-vessel disease, and five (45%) had two-vessel disease. Angiographic stenosis of the right coronary artery was observed in eight patients (72%), left anterior descending artery in four patients (36%), and in the left circumflex artery in four (36%) patients.

Bicycle exercise test

The exercise test variables were comparable in the two groups, with no difference in duration or work load. Peak heart rate was significantly lower for the CAD patients compared with the controls. The CAD patients reached a significantly lower percentage of predicted heart rate compared with the controls. ECG changes indicating ischemia were not significantly more frequently seen in the CAD patients, but angina pectoris was more frequently experienced by the CAD patients compared with the controls (Table 2). Angina pectoris was the limiting factor leading to termination of the exercise test in five CAD-positive patients and none of the controls ($P=0.04$).

Serum YKL-40

Table 3 and Fig. 1 illustrate serum YKL-40 at baseline right before exercise, immediately after exercise, and during the first 6 h after exercise, as well as the median change between each measurement. CAD patients had a higher level of serum YKL-40 at baseline compared with controls, with a median (interquartile range) of 94 (52–151) versus 57 (45–79) μg/l. Serum YKL-40 at baseline was above the 95th percentile for healthy individuals according to the mean age of the group in two (18%) of the CAD patients and in one (9%) of the controls. Overall serum YKL-40 was higher in CAD patients compared with the controls, but the difference was only borderline significant 6 h after exercise ($P=0.063$). Men had a higher, although not significant, serum YKL-40 at baseline compared with women [93 (52–97) vs. 76 (45–79) μg/l, $P=0.48$]. Furthermore, the proportion of patients who had CAD was also higher among the men compared with the women (77 vs. 11%).

As illustrated in Figs 1 and 2, serum YKL-40 decreased stepwise after exercise, reaching the lowest values 2 h after exercise for the controls [48 (35–63) μg/l] and 4 h after exercise for the CAD patients [72 (45–119) μg/l]. There was no significant difference between the two groups with regard to change in serum YKL-40 at any of the different time points (Table 3). CAD-positive patients had a median decrease in YKL-40 of 16 (13–39) μg/l, whereas the controls had a median decrease of 13 μg/l [8,10–21] from baseline to the lowest observed serum YKL-40 value ($P=0.212$). Thereafter, values increased again toward baseline serum YKL-40 level (Table 4).

In the mixed model, time after exercise was a significant factor for the decrease in serum YKL-40 overall ($P<0.0001$), but no difference in YKL-40 decrease over time could be demonstrated between the two groups ($P=0.12$). There was no difference of the effect of time on YKL-40 between the groups ($P=0.76$), as illustrated in Fig. 3.

Discussion

In this study, we investigated the effect of exercise on the inflammatory biomarker serum YKL-40 in patients with CAD versus controls. We found that serum YKL-40 was higher at baseline for the CAD patients compared with the controls, and in both groups serum YKL-40 decreased after exercise with a slightly steeper decrease for the CAD patients than the controls. Time after exercise was a significant factor for decrease in serum

Table 2 Exercise test variables

	CAD ($N=11$)	Controls ($N=11$)	*P*-value
Exercise test performance			
Duration (mean ± SD) (min)	12 ± 4	13 ± 5	0.39
Maximum strain (mean ± SD) (W)	155 ± 47	177 ± 67	0.37
Maximum work load (METS) (mean ± SD)	7 ± 2	9 ± 3	0.15
Peak systolic blood pressure (mean ± SD) (mmHg)	203 ± 33	203 ± 23	0.99
Peak diastolic blood pressure (mean ± SD) (mmHg)	105 ± 28	94 ± 14	0.31
Peak heart rate (mean ± SD) (beats/min)	136 ± 13	169 ± 15	**< 0.001**
Predicted heart rate (mean ± SD) (%)	87 ± 12	101 ± 8	**0.006**
Evidence of ischemia [*n* (%)]			
ST-elevation	1 (9)	0 (0)	1.000
ST-depression	5 (45)	1 (9)	1.000
Angina pectoris	6 (55)	0 (0)	**0.012**

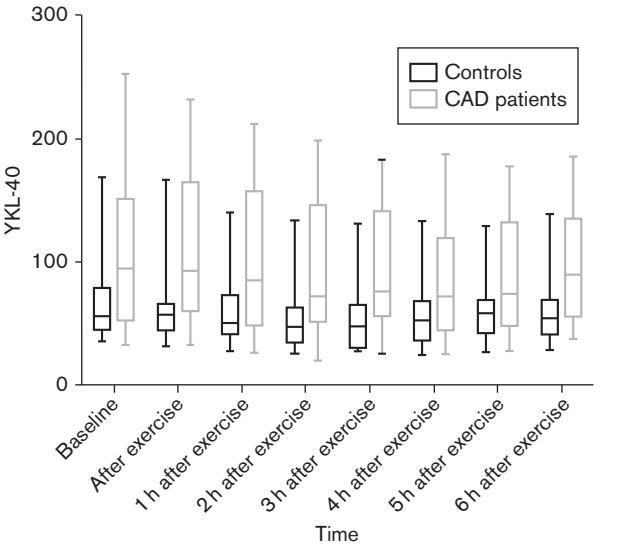
CAD, coronary artery disease; METS, metabolic equivalents of task.
ST-elevation indicates elevation ≥ 0.1 mV; ST-depression indicates horizontal or down-sloping ST-depressions ≥ 0.1 mV.
Bold values are represented as $P < 0.05$.
A $P < 0.05$ indicates significant differences between the CAD patients and controls.
P-value for differences between groups was obtained by the χ^2-test (or Fisher's exact test if any expected value was below 5) for the categorical variables and by an unpaired Student's *t*-test for the continuous variables.

Table 3 Serum YKL-40 concentrations and changes before and after exercise test

	CAD ($n=11$)	Controls ($n=11$)	*P*-value
Before exercise test [median (IQR)] (µg/l)	94 (52–151)	57 (45–79)	0.14
Change[a]	÷2 (÷14–1)	1 (÷9–5)	0.58
After 0 min [median (IQR)] (µg/l)	93 (60–165)	57 (44–66)	0.09
Change[a]	12 (7–20)	10 (4–13)	0.41
After 1 h [median (IQR)] (µg/l)	85 (48–158)	51 (41–73)	0.11
Change[a]	8 (÷3–16)	3 (0–6)	0.41
After 2 h [median (IQR)] (µg/l)	72 (51–146)	48 (35–63)	0.11
Change[a]	÷2 (÷8–6)	1 (÷1–6)	0.45
After 3 h [median (IQR)] (µg/l)	76 (56–141)	48 (30–30)	0.16
Change[a]	3 (÷3–9)	÷2 (÷9–5)	0.21
After 4 h [median (IQR)] (µg/l)	72 (45–119)	52 (38–66)	0.16
Change[a]	÷4 (÷10–7)	÷2 (÷5–3)	0.78
After 5 h [median (IQR)] (µg/l)	74 (49–132)	58 (42–69)	0.12
Change[a]	÷5 (÷8–0)	÷2 (÷3–1)	0.62
After 6 h [median (IQR)] (µg/l)	89 (61–128)	55 (41–69)	0.06
Maximum decrease [median (IQR)] (µg/l)	÷16 (13–39)	÷13 (10–22)	0.21

Serum YKL-40 is given in µg/l.
CAD, coronary artery disease; IQR, interquartile range.
[a]Change between samples above and below.
÷ indicates decrease in values.
P-value for differences between groups was obtained by a Wilcoxon signed-rank test because of non-Gaussian distribution.

Fig. 1



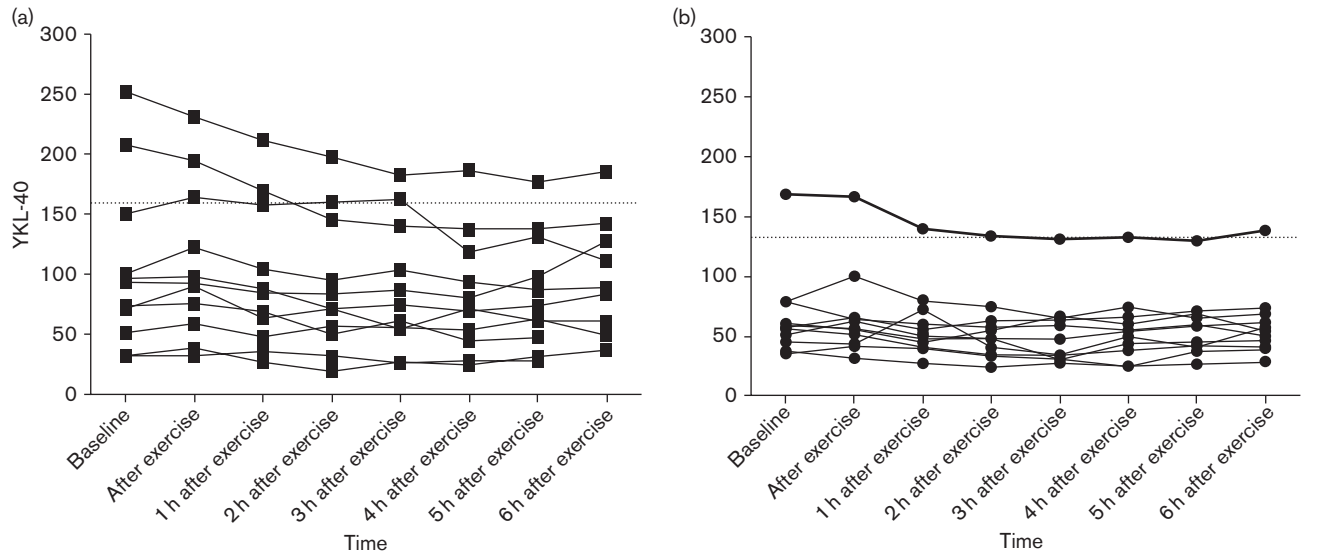
Concentrations of serum YKL-40 (µg/l). Serum YKL-40 was measured at baseline, immediately after exercise, and once every hour for 6 h in patients with documented CAD (gray) and controls (black). Data are presented as median (line), interquartile range (box), and minimum and maximum observations (whiskers).

YKL-40 overall, but we did not observe a difference in serum YKL-40 decrease over time between the two groups, indicating that exercise has a beneficial effect on the inflammatory level independent of atherosclerotic degree.

Serum YKL-40 is a new inflammatory biomarker, which potentially could be of interest in monitoring treatment efficacy, and as a prognostic factor in patients with CAD. Earlier studies suggest that serum YKL-40 could be a new biomarker of acute and chronic inflammation in patients with stable CAD. Circulating serum YKL-40 may reflect the total burden of coronary atherosclerosis or identify high-risk atherosclerosis with ongoing inflammation and atherosclerotic plaque formation.

In this study, it was interesting that we did not see a difference between CAD patients and controls with respect to YKL-40 decrease after exercise. It has already been established that the formation of fatty streaks and atherosclerosis begins early in life [28,29] and advanced atherosclerotic lesions may already appear in young adulthood [29,30]. Our control group had a mean age of 53.5 years and were therefore likely to have developed atherosclerosis despite the fact that they did not have stenosis nor coronary artery calcification on CT angiography. Inflammation plays a central role in atherosclerosis [2], and this could explain why we observed a

Fig. 2



Individual changes in serum YKL-40 (μg/l) after exercise test. Serum YKL-40 was measured at baseline, immediately after exercise, and once every hour for 6 h after exercise. The lines represent each individual. (a) CAD patients; (b) controls. The horizontal dotted line represent 95th percentile (YKL-40 concentration) according to the mean age for each group [10]. CAD, coronary artery disease.

Table 4 Research on YKL-40 in cardiovascular disease

Known facts about YKL-40 and CAD	Findings from this research
YKL-40 is an inflammatory biomarker	YKL-40 decreases after exercise, indicating a beneficial effect of exercise on the inflammatory level
YKL-40 increases with age [8,10–12]	Time after exercise is significant for the decrease in YKL-40
YKL-40 is increased in patients with CAD [12,14,18–21]	
In patients with CAD, YKL-40 is related to prognosis [12] and a predictor of death [22]	

CAD, coronary artery disease.

decrease in both groups and why exercise had a positive effect on inflammation in both CAD patients and controls.

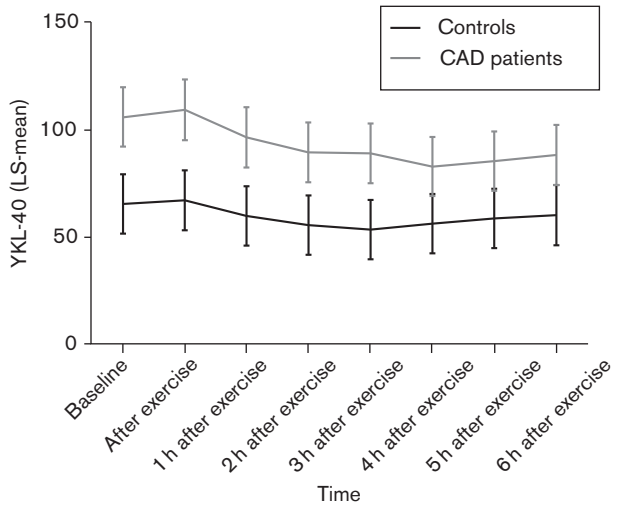
A recent study demonstrated increasing plasma YKL-40 and muscle tissue YKL-40 mRNA values after 1 h of intensive exercise [23]. Another recent study showed that marathon running increased the levels of circulating YKL-40 by 56% [31]. However, yet another study observed no effect on serum YKL-40 after physical exercise [32]. It is possible that a higher intensity and/or a longer duration are necessary for induction of increases in circulating YKL-40 levels in response to exercise. However, none of the mentioned studies considered the changes in YKL-40 after exercise as did the present study.

Inflammation is an important factor in the pathogenesis of atherosclerosis, and several markers of inflammation have been associated with an increased risk of cardiovascular events. Physical activity may lower the risk for CAD by decreasing inflammation. Other studies have shown that exercise decreases inflammation by decreasing inflammatory markers such as C-reactive protein and VCAM-1 [33]. In a study of 177 inactive patients and overweight patients, a single measurement of the inflammatory marker high-sensitivity C-reactive protein was inversely correlated to physical fitness independent of other cardiovascular risk factors. The authors conclude that this indicates the beneficial effects of physical activity on inflammation [34].

Strengths and limitations

To our knowledge, this is the first study to compare serial measurements of serum YKL-40 after exercise in a population of patients with documented CAD and a control group with suspected stable angina but with normal coronary arteries. The cohort was consecutively collected and was representative of CAD patients. Some important limitations in this study need to be addressed. First of all, there were a limited number of participants, which has an impact on the possible generalizability of the results. The borderline significant difference in age

Fig. 3



Mixed model for the interaction of time after exercise test and group (CAD patients vs. controls). *y*-axis: YKL-40 (LS-mean). *x*-axis: time after exercise test. CAD, coronary artery disease; LS, least square.

between the two groups represents a possible confounder, as age is known to be associated with serum concentrations of YKL-40. The choice of the control group can also be discussed, as these were referred because of stable angina pectoris and possible CAD, but were found to have no stenosis nor calcification on CT angiography. Ideally, the control group should be free from symptoms, and it is possible that the control group could represent a population with stable microvascular disease causing them to have angina pectoris. Furthermore, coronary artery pathology was assessed with different methods in the CAD patients and the controls, because of different a-priori risk for CAD. This, however, represents a daily clinical practice scenario, and a CT angiography has a high negative predictive value and therefore it is unlikely that the difference method has affected the results.

Conclusion

Serum YKL-40 was higher in CAD patients compared with controls, but both groups experienced declining values after exercise, indicating that exercise has an anti-inflammatory effect independent of the degree of the atherosclerotic burden.

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Conflicts of interest

There are no conflicts of interest.

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