Inflammation is strongly associated with cardiorespiratory fitness, gender, BMI and the metabolic syndrome in a self-reported healthy population: HUNT3 Fitness Study

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Abstract

Objective:

To investigate whether C-reactive protein (CRP, a general marker of inflammation), neopterin (activated macrophages), lactoferrin (activated neutrophils), and endothelial function (flow-mediated vasodilation, FMD) are associated with cardiorespiratory fitness (peak oxygen uptake, VO_{2peak}), gender, BMI and the metabolic syndrome in a healthy adult population.

Patients and Methods:

This was a cross-sectional association study based on the population-based HUNT3 Fitness Study performed from 05/15/2007 through 06/23/2008. Seven hundred and forty self-reported healthy respondents (307 women) identified with the metabolic syndrome were age- and sex-matched with 692 controls (327 women) from the same cohort. Associations between the inflammatory biomarkers and VO_{2peak}, FMD, and the metabolic syndrome, were analyzed by multivariate linear and logistic regression.

Results:

CRP was negatively associated with VO_{2peak} ($P<.001$), positively associated with the metabolic syndrome with a stronger effect in men ($P<.001$), positively associated with BMI with a stronger effect in women ($P<.01$), but not with FMD ($P=.34$). Lactoferrin was positively associated with the metabolic syndrome ($P<.001$), but neither neopterin nor lactoferrin were associated with VO_{2peak} or FMD.

Conclusion:

CRP was strongly associated with VO_{2peak} and the metabolic syndrome, but not with FMD. The associations between inflammation, VO_{2peak} and the metabolic syndrome were strongly

influenced by gender and BMI. Our data support that low cardiorespiratory fitness should be considered an etiological factor contributing to systemic inflammation, and that reducing body weight and/or improving VO_{2peak} are methods that may positively affect CRP levels.

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Alphabetical list of abbreviations

BMI	body mass index
CAD	coronary artery disease
CRP	C-reactive protein
FMD	flow-mediated dilation
MetSyn	metabolic syndrome
VO_{2peak}	peak oxygen uptake

Introduction

Systemic inflammation plays a major role in the pathogenesis of atherosclerotic vascular disease [1, 2]. A substantial amount of data has demonstrated a positive association between the general biomarker of inflammation C-reactive protein (CRP) and risk of coronary artery disease (CAD) and vascular mortality [3]. CRP has therefore emerged as a potential surrogate endpoint for interventional studies aimed at reducing inflammation and decreasing vascular event rates [4, 5]. Several other inflammatory markers, reflecting more specific activation of the immune system in atherogenesis, have also been proposed as predictive biomarkers of atherosclerotic vascular disease. Of these, neopterin, released from activated macrophages [6], and lactoferrin, released from activated neutrophil granulocytes are both elevated in patients with CAD [7, 8] and predict fatal CAD in patients with type 2 diabetes mellitus [9, 10]. However, data on these biomarkers in preclinical atherosclerosis and associations with established risk factors are sparse.

Cardiorespiratory fitness and endothelial function have been widely studied as determinants of cardiovascular health. A large amount of evidence has established that high cardiorespiratory fitness measured as peak oxygen uptake (VO_{2peak}) is associated with a reduced risk for cardiovascular disease and all-cause mortality [11], even in subjects that do not meet recommendations for physical activity [12]. Endothelial dysfunction, a precursor of atherosclerosis [13], is often examined non-invasively using ultrasound assessment of brachial flow-mediated dilation (FMD). FMD is correlated with endothelium-dependent dilatation of coronary arteries [14] and cardiovascular events [15], and has therefore been suggested as a useful method to detect increased cardiovascular risk and preclinical pathology.

CRP, VO_{2peak} and FMD are strongly associated with other major cardiovascular risk factors, such as the metabolic syndrome (MetSyn), but their interdependence has not been

established in a healthy population. The aim of the present study was therefore to investigate the associations of three markers of different aspects of systemic inflammation, i.e. CRP, neopterin, and lactoferrin, with VO_{2peak} and FMD in a cohort of self-reported healthy respondents whose medical screening identified MetSyn. We hypothesized that all three biomarkers would be associated with low VO_{2peak} and poor FMD, both in healthy respondents and in respondents who were identified with MetSyn in the study.

Methods

This was a population-based cross-sectional study based on data from the HUNT3 Fitness study [16-18] performed from 05/15/2007 through 06/23/2008. A total of 4,631 self-reported healthy men and women above 20 years of age completed treadmill testing of VO_{2peak} and endothelial function (estimated as flow-mediated vasodilation, FMD). Participants also completed a health-related questionnaire that provided information on medical history and lifestyle factors [18], and underwent a brief clinical examination. Self-reported physical activity was categorized into four groups as previously described [18].

Inclusion criteria were participation in the HUNT3 main study (ntnu.edu/hunt) and written informed consent for the fitness substudy. Exclusion criteria were known cardiovascular disease (including angina pectoris, former myocardial infarction, and cerebral or peripheral artery disease), cancer, asthma, chronic pulmonary obstructive disease, as well as use of vasoactive or antihypertensive medication, or inability to perform an exercise test due to physical impairment. Subjects reporting episodes of obstructive breathing or dyspnea for the past year and subjects with arrhythmias during treadmill testing were also excluded. The study was approved by the Regional Committee for Medical and Health Ethics of Central Norway and conducted according to the Helsinki Declaration.

All participants were asked to fast for four hours before a venous blood sample was drawn, but for logistic reasons strict fasting conditions were only achieved in one fourth of study participants [16]. Blood was analyzed for total cholesterol, high-density lipoprotein cholesterol, triglycerides, glucose, creatinine, and CRP using in-hospital procedures at Levanger and Namsos Hospital, Nord-Trøndelag, Norway. In addition, serum was stored at -80˚C for later analysis of neopterin and lactoferrin. Neopterin was analyzed using ELISA (enzyme linked immunosorbent assay) according to the manufacturer's instructions (Brahms, Henningsdorf, Germany), and lactoferrin was analyzed using ELISA as previously reported [19].

Resting heart rate, systolic and diastolic blood pressure, height, weight, and waist circumference were measured as previously reported [17]. VO_{2peak} was measured using mixing chamber gas analyzer ergospirometry (Cortex MetaMax II, Cortex, Leipzig, Germany) during an individualized treadmill test. Criteria for a maximal test were a respiratory exchange ratio of 1.05 or higher, or when the oxygen uptake did not increase more than 2 $ml \cdot kg^{-1} \cdot min^{-1}$ despite increased workload. However, as 12.6 % of the study participants did not achieve VO_{2peak} based on the abovementioned criteria, the term VO_{2peak} was used [18]. FMD was assessed in the left brachial artery using a 3-point echocardiography system with a 12 MHz transducer (Vivid i, GE Healthcare, Chicago, Il, USA) in the forearm cuff position. Measurements were performed at baseline after 10 minutes of rest, and 1 minute after deflation of the cuff following 5 minutes of arterial occlusion at 250 mm Hg. Brachial artery diameter was measured as the intima-to-intima distance at the R-wave of the electrocardiogram. FMD was defined as the mean of three measurements of percent change in vessel diameter calculated as [post-occlusion diameter minus baseline diameter] divided by baseline diameter. The acquisition and variability of cardiorespiratory variables and FMD data have been described in more detail in previous publications [17, 18].

The MetSyn was defined according to a joint interim statement [20] as the presence of at least three of the following cardiovascular risk factors; increased waist circumference (≥ 94 cm in men, ≥ 80 cm in women), hypertriglyceridemia (≥ 1.7 mmol/L), low high-density lipoprotein cholesterol (< 1.0 mmol/L in men, < 1.3 mmol/L in women), elevated blood pressure (systolic ≥ 130 and/or diastolic ≥ 85 mm Hg), and elevated fasting glucose. To account for suboptimal fasting conditions prior to blood sampling, the glucose criterion was changed from ≥5.6 mmol/L, to ≥7.8 mmol/L as previously discussed [17].

Data are presented as mean with 95 percent confidence intervals in parentheses and compared between participants with and without the MetSyn using Student's t-test, the Mann-Whitney U-test or Pearson's Chi-Square test. To investigate whether the biomarkers were associated with the MetSyn, VO_{2peak}, or FMD, linear regression with robust standard errors was performed including gender, age, smoking (present vs. former or never smoker), and physical activity (none or little vs. moderate or high) as other explanatory variables. If gender and the MetSyn were significant, the interaction term between these two variables was also tested to identify any gender-specific effects. When this interaction term was found significant, a stratified analysis was performed in each gender as a sensitivity analysis. As a second step in the linear regression analysis, body mass index (BMI, calculated as weight (kg) divided by the square of height (m)) was added as an additional explanatory variable. In alternative gender-specific models, the separate categorical variables used to define the MetSyn were included instead of the joint MetSyn variable. Model fit was checked by plotting residuals, and transformation of continuous variables to normality was performed when necessary.

Because some variables had missing data, another sensitivity analysis was performed using multiple imputations (m=50) by chained equations under the assumption that the data were missing at random, given the observed data [21].

Statistical analyses were performed using STATA (version 13.1, College Station, Texas, USA). P-values below .05 were considered significant.

Results

Seven hundred and forty self-reported healthy participants were identified as having the MetSyn, corresponding to 16 % of the total HUNT3 Fitness cohort. These respondents were matched with healthy controls from the same cohort by the HUNT Research Center. A gender-based 1:1 matching to the nearest year of age was used. Due to missing serum in some of the identified healthy controls, 692 age- and sex-matched healthy individuals were included. Thus, 1,432 subjects were analyzed in the study. Baseline characteristics of the study population stratified by the presence of the MetSyn (yes/no) are shown in Table 1. All parameters but age, gender, FMD, smoking habits, and a family history of cardiovascular disease were significantly different between the two groups (P<.05).

CRP was strongly negatively associated with VO_{2peak} (Figure 1, P<.001), with an interaction between the MetSyn and gender (P<.001), and between BMI and gender (P<.01) (Figure 2). Due to these interactions, the influence of low fitness on CRP concentrations when not considering BMI was stronger in men, whereas the influence of BMI was stronger in women, especially at low fitness. The association between VO_{2peak} and BMI was similar when VO_{2peak} was not normalized to body weight. Present smoking (P=.02) and low self-reported physical activity (P=.01) were also significant, but not FMD (P=.34). The results were essentially unchanged following multiple imputation of missing values (data not shown). In the gender-stratified sensitivity analyses, smoking was only associated with CRP in men (P<.001).

Low HDL-cholesterol was the only single component in the MetSyn that was associated with CRP both in men (P=.001) and women (P=.04). High blood pressure was

significantly associated with CRP in men (P=.002), but not in women (P=.71), whilst high triglycerides (P<.001) and a large waist circumference (P=.02) were significantly associated with CRP in women, but not in men (P=0.99 and P=.14, respectively). All the significant associations were positive.

Neopterin was significantly positively associated with age (P=.001) and creatinine (P<.001), and there was a trend towards a significant association with VO_{2peak} (P=.05). Neither FMD (P=.64) nor the MetSyn (P=.46, single components of the MetSyn not analyzed) were associated with neopterin. The association with creatinine was expected due to renal elimination of neopterin. Creatinine was missing in 230 subjects. Following imputation of missing values, both creatinine and age remained significantly associated with neopterin (P<.001), and gender also became significant with higher concentrations in men (P=.001).

Lactoferrin was positively associated with the MetSyn (P<.001), but not with other variables. The results were confirmed following multiple imputation of missing values. Of the single components in the MetSyn, lactoferrin was significantly positively associated with high blood pressure (P=.03) and high triglycerides (P=.002), but neither with waist circumference (P=.11), glucose (P=.48) nor HDL-cholesterol (P=.07).

Discussion

The main findings in this case-control study within the HUNT population-based study were that low-grade systemic inflammation, measured as CRP, was strongly negatively associated with VO_{2peak} and positively associated with BMI, but not with FMD. The negative association between CRP and VO_{2peak} was present both in subjects with and without the MetSyn, and the influence of high BMI was stronger in women than in men. The neutrophil marker lactoferrin

was associated with the MetSyn, whereas the macrophage marker neopterin gave no additional information.

Coinciding with our hypothesis, we found that the negative association between CRP and VO_{2peak} was present both in subjects with and without the MetSyn. The demonstration of higher CRP concentrations in individuals with the MetSyn at a low VO_{2peak} level compared to more fit counterparts are in agreement with earlier findings [22, 23]. Compared to the present study, there were some methodological differences in the previous studies, such as approximated estimation of fitness and division of CRP concentrations into tertiles. However, the similarity in results supports the robustness of these findings. Our results also extend the validity of these findings to the general population. Furthermore, the demonstration of a strong negative association between CRP and VO_{2peak} supports that low cardiorespiratory fitness should be considered an important factor contributing to systemic inflammation. This is essential as growing evidence supports that the addition of cardiorespiratory fitness to traditional cardiovascular risk factors improves the reclassification of patients with respect to the risk of cardiovascular events [11].

Importantly, the demonstration of a strong positive association between BMI and CRP, show that the negative relationship between CRP and VO2peak may be strongly confounded by BMI if not included in the analysis. It is well-known that adipose tissue releases interleukin-6 that stimulates hepatic cells to produce CRP, and that visceral fat produces more IL-6 than does subcutaneous fat [24]. Another study showed that visceral fat area was positively associated with CRP levels [25].

Previous studies seem to indicate that exercise may provide a larger lowering of CRP in those with higher inflammation at baseline and that the changes are only partly related to alterations in body weight and/or fat [26]. One study has even demonstrated that the lowering of CRP by exercise intervention was similar whether or not it was accompanied by weight

reduction [27]. One explanation could be that VO_{2peak} is often not included when analyzing studies regarding CRP and BMI, which is important as demonstrated by the findings of the present study. Because the association between low VO_{2peak} and high BMI was stronger in women than in men, our data suggest that intervention against these modifiable risk factors may be even more important in women.

Our study demonstrated that CRP was a useful marker related to VO_{2peak} in the MetSyn. Of the other tested plasma biomarkers, only lactoferrin was associated with the MetSyn and specifically with the components blood pressure and triglycerides, but not with VO_{2peak}. We are not aware of previous studies regarding associations of lactoferrin with the MetSyn or VO_{2peak}. However, there seems to be a convoluted relationship among circulating lactoferrin, BMI, and insulin resistance, where adiposity is associated with lower lactoferrin concentrations and insulin resistance with higher concentrations [28]. These factors may contribute to explaining the association of lactoferrin to the MetSyn. The lack of an association between neopterin and the MetSyn is supported by recently published data from the European Prospective Investigation into Cancer and Nutrition study [29].

Inflammation is a complex and multifaceted process, involving a large number of mediators. It is therefore not surprising that some, but not all circulating biomarkers of inflammation are associated with VO_{2peak} and influenced by the MetSyn. Importantly, from our population-based perspective, CRP seems like a more useful marker than lactoferrin or neopterin in healthy populations, even if these two markers have been stronger than CRP as predictors of atherosclerosis in general or fatal coronary artery disease in diabetes patients when directly compared [8-10]. However, due to the complex relationship between inflammation and VO_{2peak}, CRP measurement cannot be used as a surrogate to predict VO_{2peak} instead of actually measuring it. Our study demonstrated an association, and was not designed to clarify the potential mechanisms linking CRP and VO_{2peak}.

Previous studies have also shown that CRP is not correlated to FMD [30]. One study reported an inverse correlation between neopterin and FMD in hypertensives and a control group [31], whereas another study found no correlation of FMD with neopterin in patients with coronary artery disease [32]. However, both these studies were small, including 54 and 30 participants, respectively. We have not found relevant previous studies regarding associations between lactoferrin and FMD. A systematic review involving 4,159 subjects from 56 publications showed that endothelial function was significantly associated with fitness in 59% of the performed tests, independent of the health status of the participants. Taken together, our observations in healthy respondents indicate that the strong association between inflammation and aerobic fitness is independent of endothelial function measured as FMD.

Strengths and limitations

A significant strength is the large number of participants undergoing direct measurement of cardiorespiratory fitness as VO_{2peak}.The most important limitation is the lack of strict fasting conditions in the majority of patients. To account for this, we used a conservative criterion for dysglycemia in the definition of the MetSyn, possibly leading to an underestimation of the number of subjects fulfilling the MetSyn criteria.

Conclusions

Systemic inflammation, estimated as CRP, was associated with VO_{2peak} and the metabolic syndrome, but not with FMD, in healthy individuals. The associations between inflammation, VO_{2peak} and the metabolic syndrome were also strongly influenced by gender and BMI. Specific biomarkers for macrophages (lactoferrin) and neutrophils (neopterin) gave no additional information. These results are of importance to understand the associations among central lifestyle-related risk factors and how to evaluate measurements of inflammatory

markers in the context of cardiovascular health and disease. Our data support that low cardiorespiratory fitness should be considered an etiological factor contributing to elevated CRP. Reducing body weight and/or improving cardiorespiratory fitness are methods that may positively affect CRP levels both in healthy individuals and in individuals with the metabolic syndrome.

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Potential competing interests:

The authors report no competing interests.

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Figure legends

Figure 1: Predicted mean CRP concentrations by peak oxygen uptake and gender

Dark line: subjects with metabolic syndrome, dark dotted line: subjects without metabolic syndrome. Light dotted lines represent 95% confidence intervals. Due to a significant interaction with gender, the influence of low fitness on CRP concentrations when not considering BMI was stronger in men. For example, the predicted CRP concentration difference between men with the metabolic syndrome with a VO_{2peak} of 45 $ml \cdot kg^{-1} \cdot min^{-1}$ and 25 $ml \cdot kg^{-1} \cdot min^{-1}$ was 1.4 mg/L, whereas the corresponding difference in women was 0.9 mg/L. The model was adjusted for smoking (yes/no) and physical activity (high/low). Data were back-transformed to the original scale and overlap of confidence intervals cannot be used to assess significance in the multivariate model.

Figure 2: Predicted mean CRP concentrations by body mass index and peak oxygen uptake

Dark line: subjects with metabolic syndrome, dark dotted line: subjects without metabolic syndrome. Light dotted lines represent 95% confidence intervals. Due to a significant interaction with gender, the curves increase more steeply with higher BMI in women than in men. The model was adjusted for smoking (yes/no), physical activity (high/low) and VO_{2peak}. Data were back-transformed to the original scale and overlap of confidence intervals cannot be used to assess significance in the multivariate model.

Panel A: Low fitness, i.e. VO_{2peak} at mean of lowest tertile (28.8 mL/(kg*min).

Panel B: High fitness, i.e. VO_{2peak} at mean of highest tertile (46.5 mL/(kg*min).

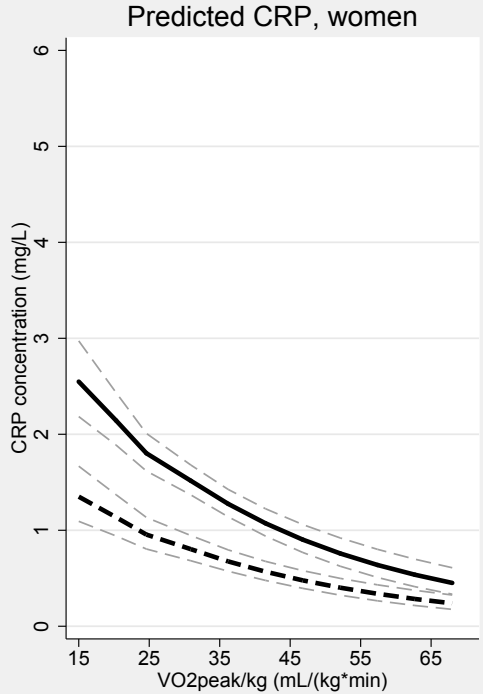
Table 1 Baseline characteristics

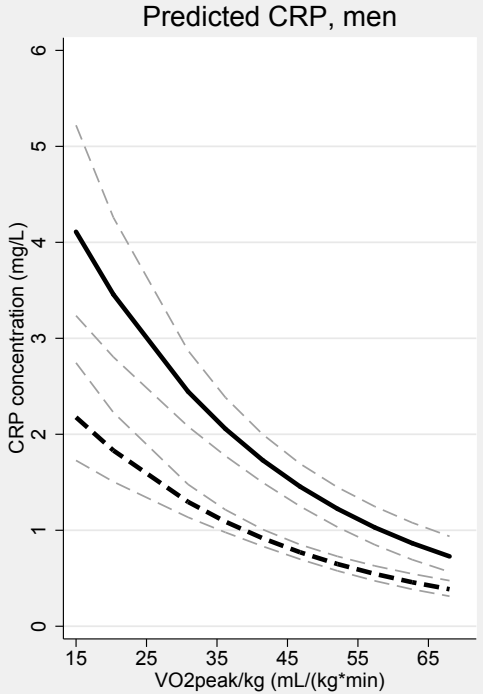
	Metabolic syndrome (n=740)	Controls (n=692)	p-value
Age (years)	54.0 (53.1-54.9)	53.8 (52.9-54.7)	P=.72
Gender (women/men)	307/385 (44/56 %)	327/413 (47/53 %)	P=.95
Waist cirumference (cm)	99.2 (98.5-99.8)	90.1 (89.3-90.9)	P<.001
Weight (kg)	86.0 (85.1-87.0)	77.0 (76.1-78.0)	P<.001
BMI (kg/m^2)	28.9 (28.6-29.1)	25.7 (25.4-25.9)	P<.001
SBP (mmHg)	139.4 (138.3-140.4)	128.8 (127.5-130.1)	P<.001
DBP (mmHg)	79.6 (78.9-80.3)	73.4 (72.7-74.2)	P<.001
Glucose (mmol/L)	6.1 (6.0-6.3)	5.3 (5.3-5.4)	P<.001
Creatinine (µmol/L)	83.3 (82.3-84.3)	81.7 (80.7-82.8)	P=.03
Cholesterol (mmol/L)	5.9 (5.8-5.9)	5.6 (5.5-5.7)	P<.001
HDL-cholesterol (mmol/L)	1.1 (1.1-1.1)	1.5 (1.4-1.5)	P<.001
Triglycerides (mmol/L)	2.6 (2.5-2.7)	1.3 (1.3-1.4)	P<.001
CRP (mg/L)	2.56 (2.27-2.85)	1.82 (1.49-2.15)	P<.001
Neopterin (nmol/L)	5.66 (5.49-5.84)	5.37 (5.20-5.55)	P<.01
Laktoferin (µg/L)	134.84 (127.94-141.74)	110.69 (103.97-117.42)	P<.001
FMD (% dilation from baseline)	4.4 (4.1-4.7)	4.7 (4.3 -5.0)	P=.60
VO_{2peak} ($ml \cdot kg^{-1} \cdot min^{-1}$)	35.7 (35.1-36.3)	39.2 (38.5-40.0)	P<.001
Resting HR (beats per minute)	71.5 (70.6-72.3)	65.7 (65.0-66.5)	P<.001
Smoke (never, former, current)	342/200/179	332/188/161	P=.85
Self-reported PA (1/2/3/4*)	91/146/233/211	150/186/216/176	P<.001
Family history of CVD (yes/no)	126/528 (17/83 %)	141/557 (20/80 %)	P=.67

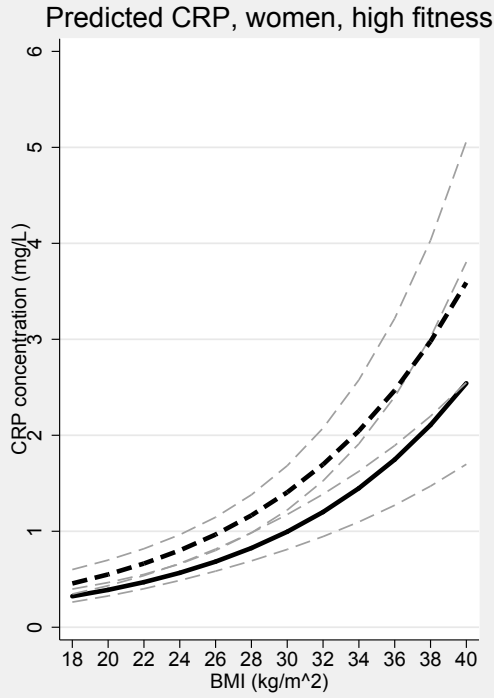
Numbers are gives as mean with 95 percent confidence interval in parenthesis.

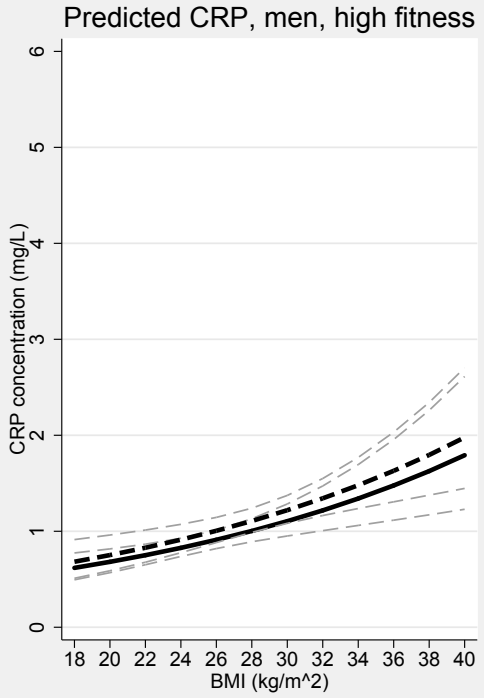
BMI; body mass index, SBP; systolic blood pressure, DBP; diastolic blood pressure, HDL; high-density lipoprotein, CRP; C-reactive protein, FMD; flow-mediated dilation, VO_{2peak}; peak oxygen uptake, HR; heart rate, PA; physical activity, CVD; cardiovascular disease.

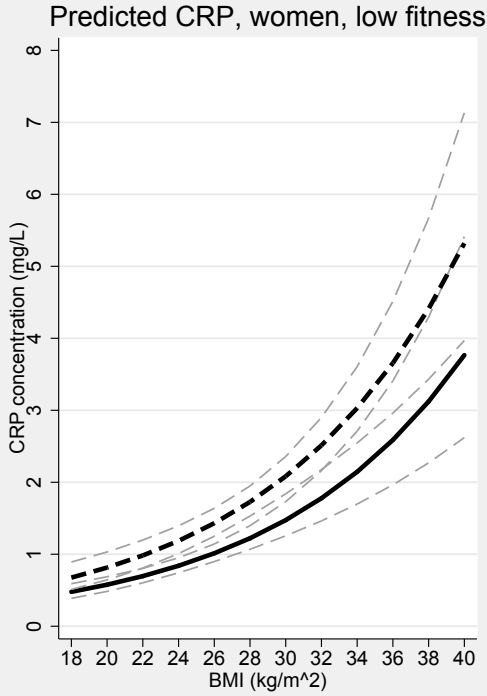
*1: Inactive, zero points on physical activity score, 2: Low physical activity score, 3: Medium physical activity score, 4: High physical activity score. Based on *Aspenes et al. Peak Oxygen Uptake and Cardiovascular Risk Factors in 4631 Healthy Women and Men. Medicine & Science in Sports & Exercise 2011.*

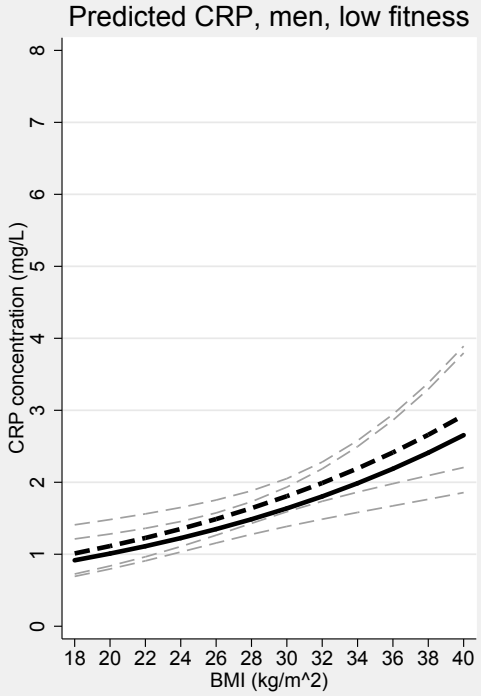

Predicted CRP, women
CRP concentration (mg/L)
0
1
2
3
4
5
6
15
25
35
45
55
65
VO2peak/kg (mL/(kg*min)


Predicted CRP, men
CRP concentration (mg/L)
0
1
2
3
4
5
6
15
25
35
45
55
65
VO2peak/kg (mL/(kg*min)


Predicted CRP, women, high fitness
CRP concentration (mg/L)
0
1
2
3
4
5
6
18 20 22 24 26 28 30 32 34 36 38 40
BMI (kg/m^2)


Predicted CRP, men, high fitness
CRP concentration (mg/L)
0
1
2
3
4
5
6
18 20 22 24 26 28 30 32 34 36 38 40
BMI (kg/m^2)


Predicted CRP, women, low fitness
CRP concentration (mg/L)
0
1
2
3
4
5
6
7
8
18 20 22 24 26 28 30 32 34 36 38 40
BMI (kg/m^2)


Predicted CRP, men, low fitness
CRP concentration (mg/L)
0
1
2
3
4
5
6
7
8
18 20 22 24 26 28 30 32 34 36 38 40
BMI (kg/m^2)