UNIVERSITY OF TROMSØ UIT

Faculty of Medicine
Department of Clinical Medicine
Department of Neurology and Neurophysiology

Prevalence, risk factors for and clinical impact of persistent hyperCKemia in a general population.

The Tromsø Study

Hallvard Lilleng

A dissertation for the degree of
Philosophiae Doctor
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Hallvard Lilleng

Tromsø, October 2012

ABBREVIATIONS

AUDIT	Alcohol use disorders identification test
ADP	Adenosine di-phosphate
ATP	Adenosine tri-phosphate
BMI	Body Mass Index
CK	Creatine kinase
CK- MM	CK- Muscle Muscle (muscle type)
CK- MB	CK- Muscle Brain (heart type)
CK- BB	CK- Brain Brain (brain type)
DBP	Diastolic blood pressure
EFNS	European Federation of Neurological Society
EMG	Electromyography
ENG	Electroneurography
HyperCKemia	Increased serum creatine kinase level
kDa	kilo Dalton
MRC-sumscore	Medical Research Council sumscore
MRI	Magnetic resonance imaging
NCS	Nerve conduction studies
NORIP	Nordic Reference Interval Project
SBP	Systolic blood pressure
Tromsø 6	the 6th survey of The Tromsø Study
U/L	Units per liter
ULN	Upper limit normal
URL	Upper reference limit
WHR	Waist hip ratio

LIST OF PAPERS

I. Lilleng H, Abeler K, Johnsen SH, Stensland E, Løseth S, Jorde R, Figenschau Y, Lindal S, Wilsgaard T, Bekkelund SI.
Variation of serum creatine kinase (CK) levels and prevalence of persistent hyperCKemia in a Norwegian normal population. The Tromsø Study
Neuromuscul Disord 2011;21:494-500.

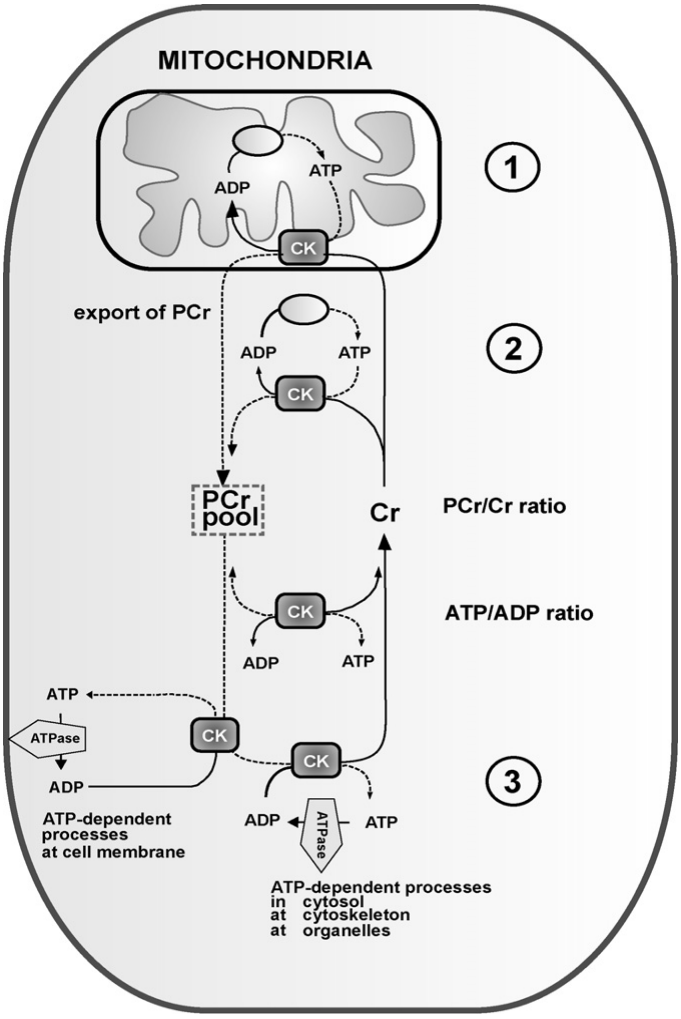
II. Lilleng H, Johnsen SH, Wilsgaard T, Bekkelund SI.
Are the currently used reference intervals for creatine kinase (CK) reflecting the general population? The Tromsø Study
Clin Chem Lab Med 2011 Nov 10;50(5):879-84.

III. Lilleng H, Abeler K, Johnsen SH, Stensland E, Løseth S, Lindal S, Wilsgaard T, Bekkelund SI.
Clinical impact of persistent hyperCKemia in a Norwegian general population.
A case control study
Neuromuscul Disord 2012, DOI: 10.1016/j.nmd.2012.07.008

1. INTRODUCTION

1.1. Mechanism of action and subtypes of CK

CK is a dimeric globular protein consisting of two subunits with a molecular mass of 43 kDa. It buffers cellular ATP and ADP concentrations by catalysing the reversible exchange of high-energy phosphate bonds between phosphocreatine and ADP produced during contraction [1,2] (Figure 1).



1. **Connected to oxydative phosphorylation in mitochondria**
2. **Connected to glycolysis**
3. **Connected to cellular uptake of creatine**

Figure 1.
Schematic drawing of the creatine kinase/phosphocreatine (CK/PCr) shuttle in cells
Adopted from Andres R.H. et al. [3] *with permission from Elsevier Limited, May 14, 2012*

At least five isoforms of CK exist: three in cytoplasm, CK-MM (muscle type), CK-MB (heart type), and CK-BB (brain type) and two in mitochondria [4,5].

CK isoenzymes can give specific information on injured tissue because of their tissue distribution. CK-MM is found in several domains of the myofiber where ATP consumption is high, and elevated concentrations in serum due to leakage is considered a marker of muscle disease [6]. CK-MB increases in acute myocardial infarction [7], and CK-BB increases in brain damage [8].

CK-MM is specially bound to the myofibrillar M-line structure located in the sarcomere (Figure 2).

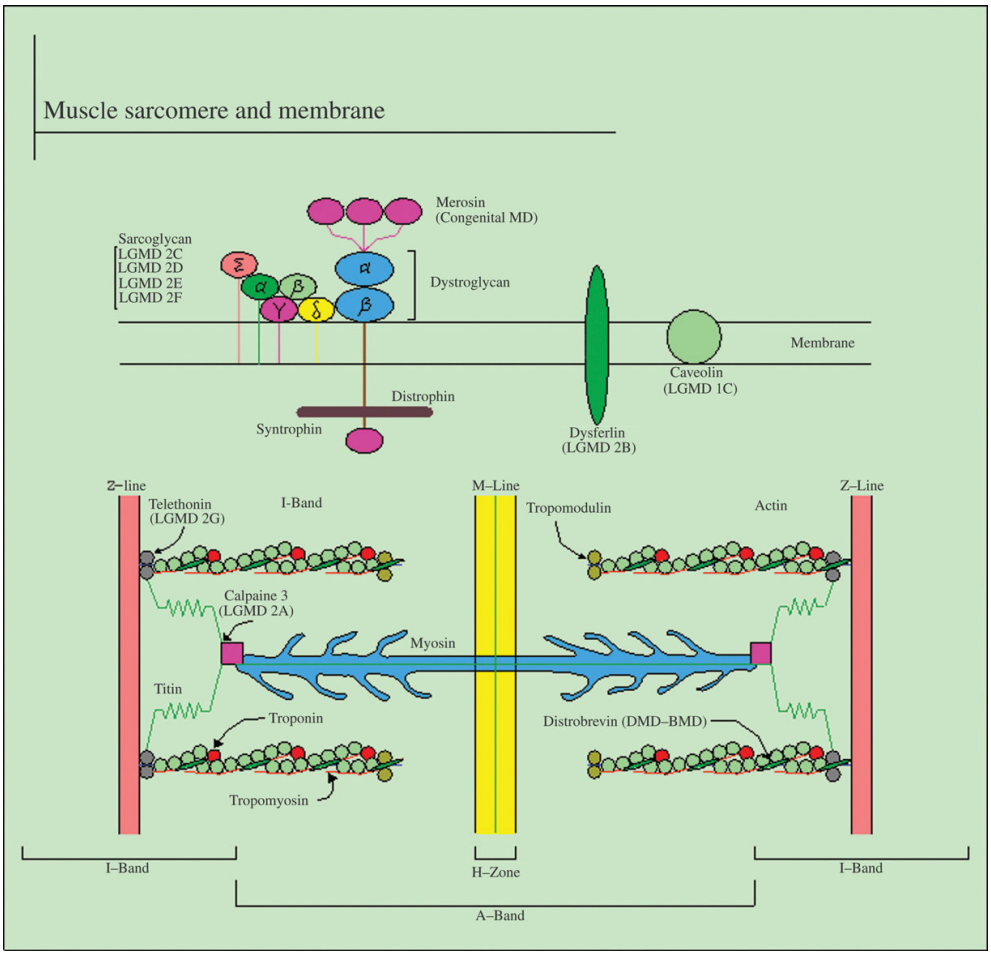


Figure 2.

Proteins related to muscular dystrophies and localization in the sarcomere.

Adopted from Brancaccio P. et al. [1] *with permission from Oxford University Press, March 22, 2012*

The M-line region appears to be the only myofibrillar structure which connects thick filaments (myosin) directly to each other, providing physical stability between thick filaments during contraction. The presence of CK-MM suggest that the M-line has a structural and enzymatic role to regenerate ATP at sites of high-energy consumption, thus providing myosine with

sufficient ATP to work even under strenuous condition [9]. High serum levels of CK depend on sarcomeric damage arising either from strenuous exercise or from muscular pathology of various mechanisms. About 90% of total CK in normal serum is CK-MM, mainly provided by the skeletal muscles.

1.2. Causes of increased CK

Serum CK activity varies with physiological variables such as age, sex, race, muscle mass and physical activity [10-14]. Women have lower CK activity at rest and a slightly increase with age, in contrast to men which have a higher CK activity at rest and a slightly decline during life [12]. Black men and South Asians usually have higher CK values at rest than Caucasians [10,12,15].

Additionally, increased serum CK levels (hyperCKemia) is considered an important marker of neuromuscular diseases, but CK may also increase in a number of other diseases such as cardiac diseases, malignancies, systemic metabolic disorders, thyroid, parathyroid and haematological diseases [16-18]. Alcohol, drug abuse and medications (especially statins) are also related to hyperCKemia. These make the test less specific, and the investigations of test reliability important [11,12,19].

Symptoms from the musculoskeletal system are a major health problem in many industrialized countries [20]. Prevalence of symptoms from skeletal muscle varies from 11% to 50% in different population [21-23], but only a minority of patients will expose an underlying muscle disease after diagnostic work- up.

Previous studies focusing on causes of hyperCKemia are mainly based on selected patients referred to specialized centres, or retrospective groups of patients. Small samples and lack of standardized criteria for identification of groups at risk of underlying neuromuscular disorders makes it difficult to extrapolate these results to the general population [24-26].

Information about the prevalence of marked elevated CK (> 5000 U/L) in unselected, normal populations is limited. Knowledge about CK-distribution and prevalence of persistent hyperCKemia in the general population may therefore be informative for both clinical and scientific purposes. In patients with hyperCKemia, it is also important for the clinician to know how CK varies with different physiological conditions.

Prevalence studies of persistent hyperCKemia in the general population has previously not been done.

1.3. Variation of CK and reference intervals

Recent data suggest that the variation in CK values in normal populations is wider than reflected in hospital reference intervals, which may have clinical implications [27,28].
High CK is common in the general population, supporting the view that defining a cut-off for normality should be performed in large general populations, i.e. by using epidemiological samples [10,27]. Selecting representative reference individuals is an important and crucial step in defining reference values [29,30]. The Nordic Reference Interval Project (NORIP), a collaboration of 102 Nordic laboratories, has established biological reference intervals for 25 frequently requested biochemical quantities [31]. Even though the authors acknowledged that reference individuals should be randomly selected from the normal background population, they recruited them from the laboratory staff, their relatives or acquaintances, and among blood donors, using a set of inclusion criteria [31,32].
A fully randomized selection of reference individuals from the entire population is however a huge task and not easily carried out for each clinical laboratory due to costs and resources, and in practice seldom applied [31,32].
Properly established ULN CK values reflecting the reference population could reduce the burden of false positively CK values.

1.4. Guidelines and clinical impact

In routine clinical practice, incidental detection of asymptomatic CK values above the upper reference limit (URL, 97.5th percentile) occurs frequently. Values slightly above URL are often ignored; presumably reflecting that by definition 2.5% of normal individuals will fall into this category, especially in asymptomatic or oligosymptomatic individuals [33]. However, the safety of this approach is not assessed in controlled studies.
Guidelines on the diagnostic approach to pauci- or asymptomatic hyperCKemia, given by EFNS in 2010, recommends investigation with muscle biopsy if serum CK ≥3x normal, if myopathic EMG, or if the patient is <25 years of age [33].
There are no studies addressing the clinical impact and risk factors for asymptomatic, moderately hyperCKemia in the general population.

2. AIMS OF THE STUDY

- To study variation of serum CK and prevalence of persistent hyperCKemia in a Norwegian general population.

- To assess whether the currently used reference intervals for CK are reflecting the general population, and to evaluate the need for reference interval adjustments.

- To assess possible risk factors of persistent hyperCKemia in a Norwegian general population, and study clinical impact of persistent hyperCKemia.

3. SUBJECTS AND METHODS

3.1. Recruitment of participants

All participants were recruited from the 6th survey of The Tromsø Study from October 2007 to December 2008.
The Tromsø Study is a single-centre, population-based prospective study with repeated health surveys every 6-7 years, it consists of six surveys (referred to as Tromsø 1–6) that have been conducted in the municipality of Tromsø from 1974 to 2008. The first survey was initiated in an attempt to help combat the high mortality of cardiovascular diseases in Norway [34]. However, during the 37 years since the first examination of the Tromsø Study took place, increasing emphasis has been put on other chronic diseases and conditions [34]. Tromsø 4–6 also included a second visit with a more extensive examination of the participants. To the 6th survey, a total of 10137 women and 9625 men aged 30-87 years were invited (Figure 3).

They were recruited from 4 different groups; 1) all participants from visit 2 of the 4th survey (1994-95), 2) a 10% random sample of persons aged 30-39 years, 3) all persons aged 40-42 and 60-87 years, and 4) a 40% random sample of persons aged 43-59 years.
The ethnic composition was 87.3% Norwegians, 1.6% of Sami ethnicity, 1.3% of Finnish descent, 2.2% of other ethnicities, and 7.6% without information about ethnicity [34].
A questionnaire on demographics, education level, general health, familiar illnesses, muscle pain, psychiatric illnesses and use of healthcare was enclosed with the letter of invitation. Diet, alcohol and smoking habits, use of medication and leisure physical activity were also questioned. All attendants underwent physical examinations which included measurement of height, weight, waist and hip circumference, blood pressure and collection of blood samples. CK was analyzed in 12828 participants, 64.9% of those eligible (Visit 1, Figure 3).
According to the protocol, persons with CK values > 5000 U/L were especially monitored during the study and promptly remitted to the Department of Neurology and Neurophysiology, University Hospital of North Norway for follow up.

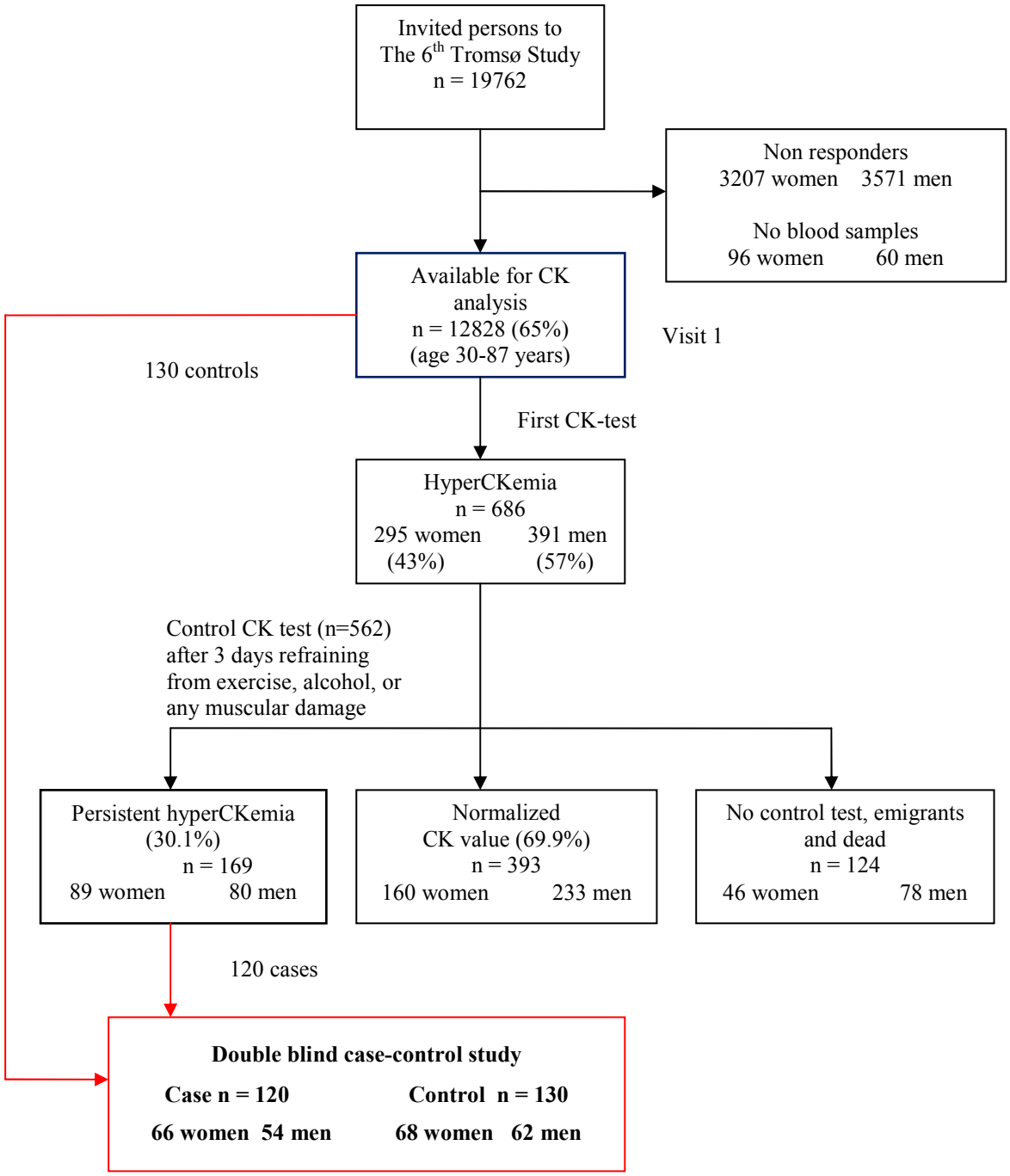


Fig. 3. Flow chart defining the study population

Recruitment of participants, Paper I

All 686 persons with elevated CK values from Visit 1 were offered a standardized control test 3 days after refraining from use of alcohol, leisure physical activity, muscular training, physiotherapy, acupuncture or any muscular damage. The control procedure was described in writing. If the control CK value was above the upper reference limit, the person was classified as having persistent hyperCKemia. In all, 562 persons had a control test and 169 had persistent hyperCKemia.

Recruitment of participants, Paper II

A total of 5924 (46.2%) individuals from Visit 1 with one or more potential causes of hyperCKemia were excluded as reference individuals; statin users (n=1846), hypothyroidism (n=1093), current kidney disease (n=525), hard leisure physical exercise defined as high activity (sweating or out of breath) at least 1 h/week (n=1361), and high alcohol consumption defined as drinking alcohol at least 2 times/week (n=2774).
The remaining 6904 individuals (53.8%) were defined as reference individuals.
Data from the standardized CK controls (562 out of 686 individuals with incidentally elevated CK values, shown in Figure 3) were included after first excluding 143 individuals owing to use of statins, reporting hypothyroidism or kidney disease. Seventy-two individuals performed hard leisure physical exercise and 65 reported high alcohol consumption; these 137 were all included as reference individuals as the standardized CK control analysis adjusted for these conditions.
Visual inspection of the histograms revealed one man with a CK value 7888 U/L. After applying the Dixon range statistics (“one-third” rule) suggested by Reed et al. this person was excluded as an outlier [35].

Recruitment of participants, Paper III

All persons aged 30–80 years with persistent hyperCKemia from visit 1, together with sex-and age-matched controls (randomly within 5 years groups) with CK values in the 30-50 percentile of reference CK (Figure 3), were invited to participate in a case-control study in order to compare clinical characteristics, neurological findings, and distribution of variables associated with hyperCKemia.

Also the controls were instructed to restrain from use of alcohol and muscular load, leisure physical activity, muscular training, physiotherapy, acupuncture or any muscular damage 3 days ahead of a control CK blood sample before they were finally included.
Both the participants and the investigators were blinded to CK values to avoid selection and observation bias. The double blinding procedure was organized by a research consultant (AKK) from the National Neuromuscular Centre (www.unn.no/nmk).

3.2. Methods

In paper III, eligible cases and controls underwent a standardized interview supplied by information from the hospital medical record. A complete clinical neurological examination supplied by a neurophysiological examination were performed by HL and co-workers (SIB, SHJ, ES, KA and SL), all experienced neurologists at the Department of Neurology and Neurophysiology. Demographic variables included age, sex, occupation and ethnicity ("Caucasian", "African origin" or "Other").
Duration of current medication was noted. All participants were asked if they or their first or second degree relatives had a known muscle disease diagnosis. Previous or current peripheral nerve disease or other diseases known to cause hyperCKemia (eg. heart disease, cancer illness, systemic metabolic disease, haematological disease, thyroid and parathyroid diseases, liver and kidney diseases) were recorded [13,14,33].
Any history of malignant hyperthermia or malignant neuroleptic syndrome and myoglobulinuria was registered [13,14,33].
Presence of muscle pain, stiffness or cramps last two weeks, and any muscle trauma, seizures, fever cramps or any muscular injections last four weeks were noted.
The participants were asked to stipulate their leisure physical activity the last year and the last month. "Mild activity" was defined as activity without sweating or breathlessness ≥ 3 hours per week last month, and "Strenuous activity" was defined as activity with presence of sweating or breathlessness ≥3 hours per week last month.
The alcohol use disorders identification test (AUDIT) was used to evaluate alcohol consumption [36]. High consumption was defined as AUDIT ≥11.
Blood pressure was measured in supine position with a digital blood pressure monitor (A&D Model UA-779; A&D Instruments Ltd, Abingdon, Oxon, UK).

Weight was measured by a validated digital weight (Weighingblock VB3-200-EC, Class III; Vetek, Sweden). Medical Research Council sumscore (MRC-sumscore) was used for manual muscle strength testing [37].

To measure muscle strength we examined knee extension of the dominant leg (peak torque, Nm) using a Cybex NORM dynamometer (CSMI, Norwood, MA, USA). After a short, standardized warm up, the participants had an introductional test- examine of 3 subsequent knee extension and flexions. Then the formal 3 tests were performed, and the average Nm recorded.

The handgrip strength (kPa) was measured on the dominant hand using a Martin vigorimeter, (Elmed Inc., Addison, IL, USA) [38]. The participants could choose between 3 different sizes of handcuffs, i.e. finding the one fitting each person's hand size. The test was done recording the highest of 3 attempts.

Nerve conduction studies (NCS) and electromyography (EMG) were performed unilaterally (dominant side) in all participants using Keypoint equipment (Medtronic, Copenhagen). The protocol included motor NCS of the median, ulnar and tibial nerves and sensory NCS of the median, ulnar and sural nerves. EMG investigation was performed in the extensor digitorum communis, deltoid, vastus lateralis and tibialis anterior muscles.

Motor- or sensorimotor axonal neuropathy was considered a possible cause of hyperCKemia if EMG showed a neurogenic pattern with denervation activity in two or more muscles (of which one was the tibialis anterior muscle), with or without NCS abnormalities in two or more nerves (not including carpal tunnel syndrome).

3.3. Assay

All blood samples were analyzed at the University Hospital of North Norway. CK was measured by photometry using an enzymatic method (CK-NAC, Roche Diagnostics®, Mannheim, Germany). The samples were consecutively analyzed in an automated clinical chemistry analyzer (Modular P, Roche) within 6 hours from withdrawal. The analytical coefficient of variation (CV_a) was ≤1.6%, and the reference interval for this method was that elaborated by the Nordic Reference Interval Project (NORIP); women aged ≥18 years, 35 – 210 U/L, men aged 18 – 50 years, 50 – 400 U/L, and men ≥ 50 years, 40 – 280 U/L [31].

When the CK level in a sample was beyond the upper limit, the sample was diluted according to recommendation given by the manufacturer.

The laboratory participates in LABQUALITY, a quality control system including about one hundred laboratories, mainly in the Nordic countries. The Department of Laboratory Medicine

receives control samples four times annually, and has always had results within the analytical target limits of CK (personal announcement).

3.4. Statistics

SPSS version 16.0 for Windows (Inc., Chicago, IL) was used for statistical analyses in all papers, in addition the SAS System version 9.2 (SAS Institute, Inc., Cary, North Carolina) was used in paper II. Descriptive data were presented as median (range) or mean (standard deviation), and a two-tailed p value <0.05 was considered statistically significant.

Paper I

Proportions of hyperCKemia were calculated in 10-year age groups for women and men. Wilcoxons signed rank test was used to compare differences in CK levels between the initial CK test and the control test (within group difference), and Mann-Whitney test to compare the CK levels between statin and non-statin users (between group differences). Chi square test was used to compare the proportion of CK-normalization between statin- and non-statin users.

Paper II

In order to reduce the skewness and kurtosis of the data, CK were raised to the power of -0.1 as the most optimal power transformation (using Box-Cox transformations). Fractional polynomials were fitted in a linear regression model to find the best relationship between the transformed CK and age. The powers for fractional polynomials were chosen from a set $\varphi = (-2, -1, -1/2, 0, 1/2, 1, 2, 3)$ [39]. The best fitting fractional polynomials was of degree 2 with powers -2 and 3 in men and -0.5 and 1 in women.
Age-related reference centiles were constructed using the absolute residuals approach proposed by Altman [40]. Figure 4 shows back-transformed centiles to the original CK scale.

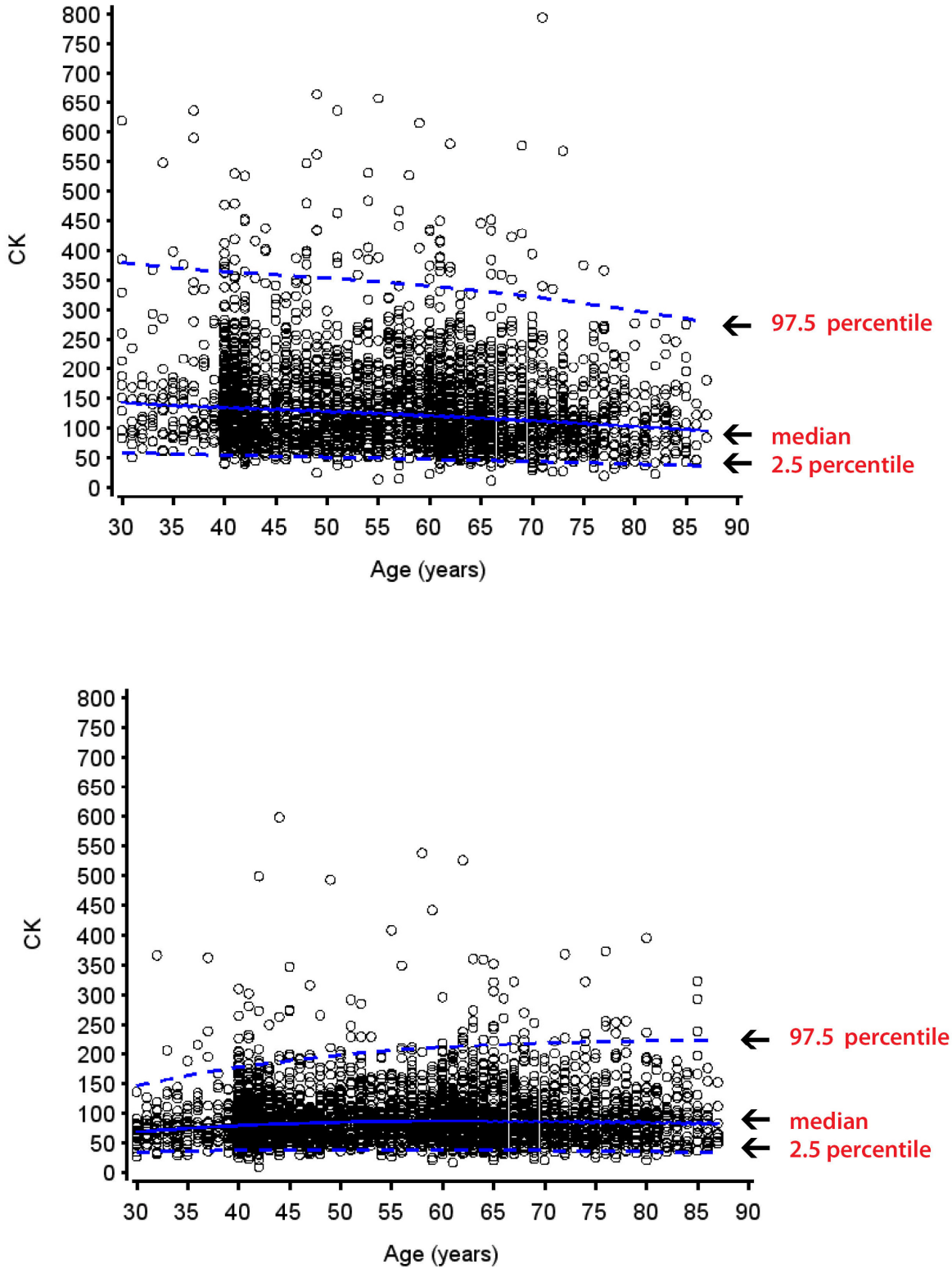


Figure 4 Distribution of CK in men (upper) and women (lower) by age (CK values > 800 U/L are not shown)

Chi square test for cross tables was used to test differences between CK-quartiles, and to test for linear trend in normalization after control analysis (Figure 5).

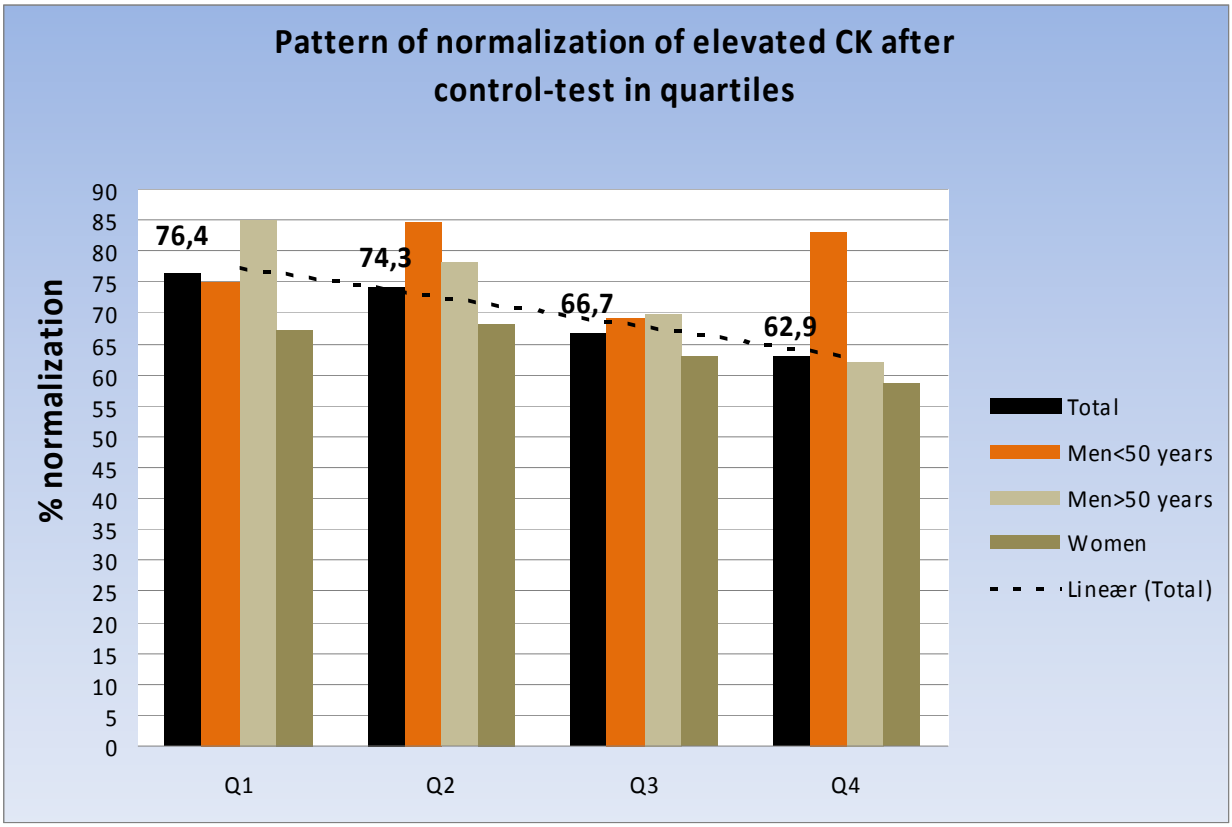


Figure 5 Pattern of normalization of elevated CK after control analysis in quartiles (Q1 – Q4)

Q1; Women: 210-230 U/L, men ≥ 50 years: 280-312 U/L and men < 50 years: 408-458 U/L

Q2; Women: 231-262 U/L, men ≥ 50 years: 315-358 U/L and men < 50 years: 466-628 U/L

Q3; Women: 264 -324 U/L, men ≥ 50 years: 361-448 U/L and men < 50 years: 668-1028 U/L

Q4; Women: 327-3046 U/L, men ≥ 50 years: 448-2452 U/L and men < 50 years: 1050-15941U/L

After deletion of individuals with potential causes of hyperCKemia and one outlier and including the data from individuals with completed standardized control CK analysis, we non-parametrically calculated CK reference intervals according to the 2.5 – 97.5 percentile [41], and compared these with CK reference values elaborated by NORIP [31].

Paper III

Unpaired t-test and Chi square test for cross tables was used to test differences between cases and controls.

3.5. Approvals

The project was approved by The Regional Ethical Committee for research and the Norwegian Data Inspectorate. (Approval number: REK NORD 11/2008). Written consent was obtained from all the participants.

4. SUMMARY OF PAPERS

Paper I

In paper I we assessed the prevalence of hyperCKemia, defined as persistent CK values ≥ 210 U/L in women, ≥ 400 U/L in men < 50 years and ≥ 280 U/L in men ≥ 50 years (reference values according to the Nordic Reference Interval Project).
Blood samples were obtained from 12 828 participants. We identified 686 (5.3%) individuals with incidentally elevated CK. After a standardized control test, 169 persons (1.3%) had persistent hyperCKemia, i.e. 69.9% normalization. Use of statins or other risk factors of hyperCKemia were detected in 78 individuals (46.2%), giving a prevalence of "idiopathic hyperCKemia" of 0.71%. CK variation was highest in younger men, and in females between 60-69 years.
In conclusion, this study identified persistent hyperCKemia in 1.3% of the normal population, and demonstrates the importance of performing controlled CK analyses, also in those with identified risk factors.

Paper II

This study compared creatine kinase (CK) reference intervals calculated from the Tromsø Study population with those elaborated by the Nordic Reference Interval Project (NORIP). It also assessed the pattern of CK-normalization after standardized control analyses. New upper reference limits (URL) CK values were calculated after exclusion of individuals with risk of hyperCKemia and including individuals with incidentally detected hyperCKemia after they had completed a standardized control analysis. After exclusion of 5924 individuals with possible causes of hyperCKemia, CK samples were analyzed in 6904 individuals. URL was defined as the 97.5 percentile.
In the reference sample population the 2.5 – 97.5 CK percentile interval (90 % confidence interval, CI) in women was 37 (36 – 38) U/L – 207 (197 – 212) U/L, compared to NORIP reference interval 35 (31 – 35) U/L – 210 (180 – 233) U/L.
The corresponding finding in men < 50 years of age was 57 (54 – 61) U/L – 395 (362 – 416) U/L, compared to NORIP reference interval 50 (45 – 54) U/L – 400 (351 – 487) U/L. In men ≥ 50 years the 2.5 – 97.5 CK percentile interval was 47 (44 – 51) U/L – 340 (287 – 372) U/L, compared to NORIP reference interval 40 (36 – 46) U/L – 280 (252 – 415) U/L.
In individuals with elevated CK, normalization grade after control analysis was inversely

correlated to the CK level ($p < 0.04$ for linear trend).
In conclusion, URL CK values in women and in men < 50 years of age were in accordance with URL CK values given by the NORIP. In men ≥ 50 years, we found a higher URL suggesting an upward adjustment of URL in this age group.

Paper III

In this case-control study we assessed the clinical impact of persistent hyperCKemia in a Norwegian general population. HyperCKemia was defined according to the NORIP- references. We compared the frequency of muscular symptoms and function, neuromuscular diseases and risk factors between 120 cases with persistent hyperCKemia and 130 age- and sex-matched controls with normal CK values, all recruited from the single-centre, population-based prospective Tromsø Study.
The participants underwent a standardized interview assessing muscle symptoms, physical activity, use of statins and presence of other CK risk factors, prior to clinical neurological and neurophysiological examination. Knee extensor muscle strength (Cybex NORM dynamometer) and dominant hand grip strength (Martin Vigorimeter) were assessed.
A total of 85 cases (71%) reported either muscle pain, muscle stiffness or cramps, compared to 70 controls (54%) ($p = 0.017$). There were no differences in muscle strength between the groups.
In men, weight, Body Mass Index and muscle symptoms were significantly higher in the group with persistent hyperCKemia. In women, no differences between the groups were detected. Frequency of statin users was similar in cases and controls. We diagnosed 3 women with previously unknown myopathy, all in the group with persistent hyperCKemia.
This study support that CK may be used as a marker of muscular symptoms in the general population, and support the view of recent publicised EFNS guidelines on diagnostic approach to pauci-or asymptomatic hyperCKemia.

5. DISCUSSION

5.1. Methodological considerations

5.1.1 Study design

The data in this project is derived from Tromsø 6, a repeated epidemiological, cross sectional study, organized by Institute of Community Medicine, University of Tromsø.
A cross-sectional study involve data collected at a defined time or over a short period, and is suitable when assessing prevalence and outcome, identifying risk factors and generating hypotheses for future research.
A limitation is that it may be difficult to make causal inferences, i.e. it is not possible to distinguish whether the exposure preceded or followed the disease. The observations should therefore not be overextended. Another limitation is that the situation may provide differing results if another time-frame had been chosen.
The major strengths of this study are the large sample size, high participation rate and the double blind design in the case control part.

One of the most important drawbacks in case-control studies in general relates to recall bias, i.e. the difficulty of obtaining reliable information about an individual's exposure status over time.
In our study, variables such as self-reported leisure physical activity the recent month and year, and presence of muscular symptoms (pain, stiffness and cramps), etc. might be biased.

Physical activity is known to elevate serum CK. We had no information of the participant's physical activity prior to the first CK blood test (Visit 1), and could therefore not be certain of the validity of these data. A standardized control test 3 days after restraining from use of alcohol and muscular load, leisure physical activity, muscular training, physiotherapy, acupuncture or any muscular damage provided better data quality.
Three days has been used in a prior analogous study [27], and was chosen since increased CK levels after eccentric exercise are associated with muscle injury, with a pronounced increase between 2 and 7 days after exercise [42]. Additionally, we assumed that 3 days instead of 7 days would increase participants' compliance, i.e. reduce the risk of drop-outs not completing the control test. However, 3 days may be too short an interval since the half-life of CK is

considered to be more than 7 days [42,43]. The degree of normalization can therefore be higher than observed.
We clinically assessed muscle symptoms and muscle strength, and used EMG to diagnose neuropathy and myopathy. MRI imaging of affected muscles, muscle biopsy and genetic testing could have added valuable information about the correlation between hyperCKemia and muscle function and muscle diseases.

5.1.2 Internal validity (selection- and observation bias, information bias, confounding)

Selection- and observation bias

A participations rate of 65% is an acceptable response-rate in a large epidemiological study; in addition, there were no predefined selection criteria for invitation other than birth year and being a resident of the municipality of Tromsø. Except from age and gender, we have no information about the 35% non-responders, which may cause selection bias. As Tromsø 6 only invited participants in the age group 30–87 years, we have no data from participants younger than 30 years.
Furthermore, the participation rate was highest in the age group 60–69 years, and lowest among the youngest and the elderly. This is especially of concern for the youngest men, where the CK variation was greatest and the attendance lowest.

By keeping participants unaware of their CK values throughout the study period, selection bias is further minimized. To reduce the risk of different clinical approach to cases and controls (observation bias), the investigators also were unaware of the participants CK values ("double blinded design"). The blinding procedure was performed without exceptions throughout the study.

The 49 non responders (29%) with persistent hyperCKemia not included in the case control study may cause selection bias. A separate analysis of the non-responder's questionnaires from Tromsø 6 (Visit 1, Figure1) revealed however no significant differences regarding sex, age, median CK, use of statins and use of thyroxine, compared to the 120 cases included.
Approximately 75% of the participants were clinical investigated by HL, the remaining 25% were examined by co-workers (SIB, SHJ and ES). A great variation in clinical judgement could cause observation bias, but we have though not done an interobserver reliability test. This is

especially relevant for the tests where clinical judgement is prominent; muscle strength testing, MRC sum score and clinical neurological investigation.
Another limitation is the lack of neurophysiological consensus concerning definition of neuropathy and myopathy. There is neither consensus regarding which subtypes of neuropathy most inclined to cause CK elevation.
In this study motor- or sensorimotor axonal neuropathy was considered a possible cause of hyperCKemia if EMG showed a neurogenic pattern with denervation activity in two or more muscles (of which one was the tibialis anterior muscle), with or without NCS abnormalities in two or more nerves (not including carpal tunnel syndrome).

Information bias

Information bias occurs when measurement of either the exposure or the outcome variables is systematically inaccurate. This problem has been addressed in the Tromsø study in general by having test personnel that are not directly involved in the scientific project and thereby not biased by scientific hypothesis in their measurement. In addition, standard operational procedures and standard protocols contributed to minimize errors.
In paper I information on use of medication and risk factors for hyperCKemia was obtained from self-administrated questionnaires. Such information is likely to be inaccurate and a source of information bias [44,45].

Confounding

A confounder is an independent risk factor for the outcome variable that is also associated with one or more of the exposure variables of interest. Confounding could lead to under- or overestimation of the association studied.

We tried to reduce the effect of confounding by doing age- and gender specific analyses. In addition, as use of statins is considered a potential cause of hyperCKemia [46-49], we also did separate analysis in statin user and none users.
Except from more muscle symptoms, the only differences we could detect were higher weight and BMI in male cases with persistent hyperCKemia. This could theoretically be explained by less leisure physical activity, but there were no significant difference in reported physical activity among the male study participants. A bigger muscle mass in men with persistent hyperCKemia may also explain this difference. Although we lack a distinct clinical measure of

muscle mass, Waist- Hip ratio (WHR) could help differentiate abdominal (central) adiposity from muscle mass, though a crude and non- evidence based estimation. There was no difference in WHR or difference in muscle strength for dominant handgrip or knee extension in males. This may indicate that muscle mass probably was relatively equal.
Nevertheless, weight and BMI may be considered as potential confounders in future CK-studies.

5.1.3 External validity

This refers to the generalizability of results and applicability to other populations. Selection criteria for participation in Tromsø 6 were age and residency in Tromsø, and the Population Registry of Norway was the source for the invitations. The risk factor levels of the Tromsø population are comparable to other Western populations; however, generalizability could be restricted by ethnicity. In Tromsø 6, >90% reported to be Caucasians, and in the case control study all individuals were Caucasians.

5.2. Discussion of main results and clinical implications

In clinical practice a relative frequent challenge is how to deal with individuals with elevated CK, with or without muscular symptoms. Incidentally detected elevated CK values are common, the present study has demonstrated the prevalence to be 5.3% in the general population. In 2011 the Department of Medical Biochemistry, University Hospital of North Norway in Tromsø analyzed 26.198 CK blood samples (personal announcement), illustrating the extent of the problem.
The importance of doing control CK tests was underlined in paper I as we calculated the prevalence of persistent hyperCKemia after a standardized control test to be 1.3% in both genders. An approximately 70% normalization rate was seen also in individuals using statins, emphasizing the importance of doing control test also in individuals with an apparently known risk factors or explanation of elevated CK. This could have both clinical and scientific implications for statin users, i.e. avoiding unnecessary withdrawal of statins in patients with moderately elevated CK, preventing potentially misclassification and exclusion of patients with elevated CK before randomization into statin trials [27].

The prevalence of hyperCKemia is highly dependent of which level of URL CK that is used. Our estimations of hyperCKemia prevalence's were based on URL given by the NORIP [31]. The importance of properly established URL CK values was demonstrated by Brewster et al as the variation in CK activity within the population is wide, and relatively high values occur frequently [27]. In our study population, we identified 4 men with CK values above 5000 U/L (15941, 12022, 8079 and 5660 U/L). All 4 normalized after standardized control tests, and clinical neurological follow up examinations were normal.
In paper II we compared URL given by NORIP to CK values calculated in our general population [41]. URL CK values in women and in men <50 years of age were in accordance with the NORIP criteria. In men ≥50 years we found a higher URL, suggesting an upward adjustment in this age group. This URL found in men ≥50 years is probably more clinical representative compared to the NORIP result, as our calculation is based on five times more reference individuals, all recruited from a general population.

The effect on neuromuscular diagnosis after raising the URL from 174 U/L to 322 U/L in men, and from 140 U/L to 201 U/L in women were evaluated by Nardin et al [50]. The higher URL resulted in a false negative CK of clinical significance in 7 of 94 subjects (7.4%). The clinical impact of loss in sensitivity was considered small, and reduced unnecessary referrals and invasive testing in patients with asymptomatic CK elevations [50].
In paper III we identified only 3 women with previously unknown myopathy among 120 persons with persistent hyperCKemia, recruited from a background population of 12 828 people. Men with hyperCKemia had more muscle symptoms; otherwise we could not demonstrate other clinical relevant differences between individuals with persistent hyperCKemia and the control group. A total of 116 individuals (97%) with persistent hyperCKemia had CK <3x normal. Our findings indicate that the potential loss of sensitivity for the diagnosis of myopathy is small when applying the CK ≥3x normal as a diagnostic limit for further investigation, and these results are in accordance with guidelines on the diagnostic approach to pauci- or asymptomatic hyperCKemia given by EFNS in 2010 [33].

In paper I, based on questionnaires, we found a possible cause or risk factor for persistent hyperCKemia in 46% of the subjects. Use of statins was the most common reported risk factor. The precise mechanisms underlying statin-induced myotoxicity remain unclear. Hypothesized explanations to the increased cytotoxicity are deficiency in chloride channel activation and increased intracellular calcium concentration causing destabilization of the cell membrane, as well as interruption of the glycoprotein synthesis [51].

However, in the case-control study (paper III), we were not able to demonstrate any difference in prevalence or duration of statin treatment between cases and controls. We could neither demonstrate more muscle symptoms nor reduced muscle power among statin users. Neither was the CK level in individuals with persistent hyperCKemia related to statin use. However, as the overall numbers included in the case-control study were small, the statistical power to do subgroup analyses was reduced, and this may explain the negative findings.

Based on observations from black and multiethnic populations, it has been hypothesized that a genetically high tissue CK activity may be an independent factor responsible for primary hypertension [52-54]. It has further been demonstrated that low CK levels are associated with lower blood pressure and an increased prevalence of fainting [55].
In a recent paper from our study group we found that CK was associated with blood pressure [56]. The association was independent of antihypertensive medication, and no difference in CK level was found between those with controlled and uncontrolled hypertension. The effect of CK was present after adjustment for age, sex, BMI, s-glucose, s-creatinine, physical activity and alcohol consumption [56-59]. We do not know whether CK activity is a causal factor involved in the pathogenesis of hypertension or a secondary metabolite caused by impaired renal function or differences in muscle fiber properties. An important assumption for the hypothesis is that CK should be increased, not only in striated muscle but also in smooth muscle and cardiac muscle in hypertensive persons. At present, there is no evidence of this in humans. It would also be of great interest to know whether CK falls when blood pressure is lowered and whether high CK is related to a more therapy-resistant hypertension.
Future studies should focus on these questions.
In the case-control study (paper III) we found higher systolic and diastolic blood pressure in both men and women with persistent hyperCKemia compared to controls, but the differences did not reach statistical significance. This is most likely due to the limited numbers included in the study.

6. CONCLUSION AND FUTURE ASPECTS

6.1. Conclusion

This study identified incidentally elevated CK in 5.3% of the normal population, and a prevalence of persistent hyperCKemia of 1.3% in both genders, based on NORIP –reference intervals. Use of statins or other risk factors of hyperCKemia were detected in 46%, giving a prevalence of "idiopathic hyperCKemia" of 0.7%.

Nearly 70% of all cases with incidentally elevated CK normalized after refraining from use of alcohol and muscular load, leisure physical activity, muscular training, physiotherapy, acupuncture or any muscular damage 3 days ahead of a control CK blood sample.

The normalization grade after control analysis was inversely correlated to the CK level. This emphasizes the clinical importance of doing a standardized control blood test before diagnosing persistent hyperCKemia, also in those with identified risk factors.

After comparing CK- reference intervals calculated from the Tromsø Study population with those elaborated by the NORIP, we have demonstrated that URL CK values in women and in men <50 years of age were in accordance with URL CK values given by the NORIP.

In men ≥50 years, we found a higher URL, suggesting an upward adjustment of URL in this age group.

The frequency of muscular symptoms and function, neuromuscular diseases and risk factors in 120 cases with persistent hyperCKemia compared to 130 age- and sex-matched controls with normal CK values revealed that men with hyperCKemia had more muscle symptoms, higher weight and higher BMI.

This support that CK may be used as a marker of muscular symptoms in the general population. Otherwise we could not demonstrate other clinical relevant differences between cases and controls, indicating that our findings imply little clinical impact of incidentally detected moderate CK elevation.

6.2. Future aspects

Muscle biopsy

The double blind case-control study identified only 3 women with previously unknown myopathy, all in the group with persistent hyperCKemia. The myopathy diagnoses were based entirely on EMG investigation, although this assessment ideally should have been completed with muscle biopsy [60,61]. Our next step is to do muscle biopsy in all participants, to assess potential biological/morphological alterations in the skeletal muscles, and thereby increase the sensitivity of the myopathy diagnosis.

In addition, to examine the correlation between morphological changes in skeletal muscles and muscle function (muscle power), as well as the correlation between morphological changes and muscular symptoms.

Use of statins is considered a potential cause of hyperCKemia [46]. Statin induced myopathy occurs in 0.1–0.2% of patients receiving statins in clinical trials, and in 5–10% in clinical practice [47-49].

In our study, we were not able to demonstrate any difference in prevalence of statin use or duration of treatment between cases and controls. Neither was the CK level in individuals with persistent hyperCKemia related to statin use. We could neither demonstrate more muscle symptoms nor reduced muscle power among statin users.

Doing muscle biopsy in all individuals with persistent hyperCKemia using statins or not could add valuable information.

Genetic screening

A genetic blood sample screening of individuals with persistent hyperCKemia recruited from a normal population is to our knowledge not previously done.

Muscle biopsy and a complementary genetic screening are considered the most comprehensive tools in present diagnostic work up of muscular diseases.

We have planned to do a simplified genetic testing (preferably a commercially available "chip") covering the most frequent hereditary muscular diseases in our region.

Blood pressure

In the case-control study participants with persistent hyperCKemia had higher systolic and diastolic blood pressure compared to controls, but the differences did not reach statistical significance. This is most likely due to the limited numbers included in the study, and a future CK trial should try to include more participants.
Investigation of the effect on CK after lowering the blood pressure is another problem to be addressed.

Follow up

There are a limited number of follow up studies of individuals with persistent hyperCKemia [62], and present knowledge of long term effects of hyperCKemia is sparse. Our study cohort is suitable to reinvestigate after 5-10 years. Such a follow up study could add valuable data about the impact of long term effects of moderately elevated CK on muscular function and muscular diagnoses.

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Paper I

Paper II

Paper III

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