Master's thesis

NTNU
Norwegian University of Science and Technology
Faculty of Medicine and Health Sciences
Department of Circulation and Medical Imaging

Lisen Brønmo Næss

The physiological impact of simulated combat training on Norwegian soldiers

A study of stressors and health outcomes

Master's thesis in Physical activity and health - Exercise physiology
Supervisor: Morten Høydal
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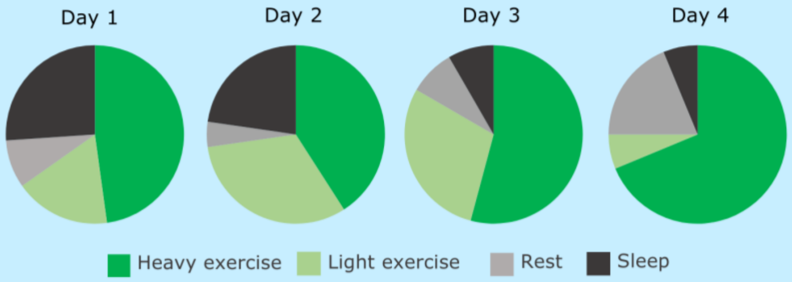
Infographic

THE PHYSIOLOGICAL IMPACT OF SIMULATED COMBAT TRAINING ON NORWEGIAN SOLDIERS: A STUDY OF MULTIPLE STRESSORS AND HEALTH OUTCOMES

BACKGROUND

Recent studies show that multiple stressors during combat training influence both acute and chronic stress response in soldiers, ultimately affecting their performance and health. The aim of this study was to investigate the acute physiological effects of four days of *simulated combat traning (SCT)*, characterised by strenuous physical activity, mental strain and sleep deprivation on soldiers.

SIMULATED COMBAT TRAINING



METHODS

Type of study: Cohort study with a pre- and post-design
Study population: 48 (12 female & 36 male) soldiers at the Royal Norwegian Air Force Academy, 20- 29 years, participated in mandatory SCT.
Data collection: Body composition scan, blood samples and capillary blood gas
Statistical tests: Graphpad prizm 10

RESULTS

↓
- Total body weight
- Total body water
- Muscle mass
- Body fat mass
- Electrolytes & nutrition
- Metabolic effects (pH, pCO2 and BE)
- Creatinine

↑
- Anion gap
- Cardiac- & muscle stress (CK, CK-MB & troponin)
- Stress (Cortisol)
- Organ injury (ASAT, ALAT and bilirubin)

CONCLUSION

Key observations included primary metabolic acidosis, dehydration, skeletal muscle damage, potential rhabdomyolysis (however no significant kidney impairment), cardiac muscle stress and potential overload on the liver. These findings support the necessity for comprehensive monitoring and management of metabolic acidosis, electrolyte imbalances, hydration status and stress levels in soldiers undergoing SCT. Further reseach is needed for more definitive conlusions.

Abbreviations: *ALAT (Alanin-aminotransferase), ASAT (Aspartat-aminotransferase), BE (Base excess of extracellular fluid), CK (Creatine Kinase), CK-MB (Creatine Kinase MB), pCO2 (Partial pressure of carbon dioxide), pH (Potential of hydrogen), SCT (Simulated combat training)*

Sammendrag

Hensikt: Den militære populasjonen er avhengig av å være i god mental og fysisk form for å kunne prestere under militære operasjoner. Nyere studier viser at flere stressorer under kamptrening påvirker både akutt og kronisk stressrespons i soldater, som videre er vist til å påvirke deres prestasjon og helse. Hensikten ved denne studien er å undersøke de akutte fysiologiske effektene av hard fysisk trening, mental utmattelse, søvnmangel og energi utmattelse i soldater under fire-dagers simulert kamptrening. Målet med studien var å undersøke fysiologiske effekter av stressorer hos soldater ved a sammenligne kroppskomposisjon og blod biomarkører før og etter simulert kamptrening. Spesifikt fokuserte vi på metabolske endringer, skade på skjelettmuskulatur, og stress på hjertet.

Metode: I denne kohort studien med et pre- og post- design, er det inkludert 48 (12 kvinner og 36 menn) soldater fra den norske luftkrigsskolen, i aldergruppen 20-29 år, som deltok i obligatorisk simulert kamptrening. Kroppskomposisjon ble målt ved å bruke bioelektrisk impedance (InBody, 770), i tillegg ble blod bio-markører testet gjennom blodprøver og kapillær blodgass ved St.Olavs hospital, Trondheim. Alle statistiske tester ble utført ved å bruke tilgjengelige programvare (Graphpad prizm 10 for macOS, Versjon 10.2.1 (339).

Resultater: Vi observerte en signifikant nedgang i total kroppsvekt som følge av simulertkamptrening (79.3 ± 12.7 to 74.9 ±12.3, $p < 0.001$), inkludert nedgang i total kropps vann, muskel masse og kroppsfett. Alle elektrolytt- og ernærings markører (triglyserider, glukose, natrium (Na^+), kalsium (Ca^{2+}), og klorid (Cl^-)) ble signifikant redusert, utenom kalium (K^+), som viste ingen endring. Disse funnene var like for både kvinner og menn. Metabolske markører slik som pH, pCO2 og BE ble signifikant redusert, med en signifikant økning i anion gap. Laktatnivåer viste ingen signifikant forskjell som følge av simulert kamptrening. Stressmarkører indikerte en signifikant økning i kortisol, CK, troponin T og CK-MB, hvor CK-MB kun var signifikant hos menn. I tillegg var ACTH signifikant redusert hos menn. Markører for lever og organ skade (ASAT, ALAT, og bilirubin) ble signifikant økt, samtidig ble kreatinin signifikant redusert som følge av simulert kamptrening.

Konklusjon: Funnene i dette studiet bekrefter behovet for omfattende monitorering og håndtering av metabolsk acidose, elektrolytt balanse, hydrerings status and stressnivå i soldater som gjennomgår simulertkamp trening. Å sørge for tilstrekkelig med næring, hydrering, og nok restitusjonstid er kritisk for å redusere mulige negative helseeffekter, samt ivareta prestasjon under og etter simulert kamptrening. Denne studien tilføyer ny kunnskap om militær helse og trening, spesielt rundt de fysiologiske effektene som følge av simulert kamptrening. Mer forskning er nødvendig for å kunne si noe mer konkret rundt kjønnsforskjeller i militære populasjoner, i tillegg er viktig å øke bevisstheten rundt fysiologiske effekter av militær trening for å kunne iverksette strategier for å opprettholde soldaters helse og prestasjon under militære øvelser.

Abstract

Introduction: The military population is dependent on being in a mentally and physically healthy state to be able to perform well during military operations. Recent studies show that multiple stressors during combat training influence both acute and chronic stress response in soldiers, ultimately affecting their performance and health. The aim of this study was to investigate the acute physiological effects of strenuous physical activity, mental strain, sleep deprivation and energy depletion during four days of SCT, by analyzing changes in body composition, and blood biomarkers. Furthermore, we chose to focus primarily on metabolic changes, skeletal muscle damage, and myocardial stress.

Method: This cohort study with a pre- and post-design, included 48 (12 female and 36 male) soldiers from the Royal Norwegian Air Force Academy, aged 20- 29 years, who participated in mandatory SCT. Body composition was measured using the bioelectrical impedance (InBody, 770), and blood bio markers collected through blood samples and capillary blood gas at St.Olavs hospital, Trondheim. All statistical tests were applied using available software (Graphpad prizm 10 for macOS, Version 10.2.1 (339).

Result: We observed a significant decrease in total body weight following SCT (79.3 ± 12.7 to 74.9 ±12.3, $p < 0.001$), including reductions in total body water, muscle mass and body fat mass. All electrolyte- and nutritional markers (triglyceride, glucose, natrium (Na^+), calcium (Ca^{2+}) and chloride (Cl^-)) were significantly decreased, except for kalium (K^+) which showed no change. These findings were consistent for both women and men. Metabolic markers such as pH, pCO2 and BE significantly decreased, while anion gap was significantly increased. Lactate levels showed no significant change following SCT. Stress biomarkers indicated a significant increase in cortisol, CK, troponin and CK-MB, with CK-MB only being significant in the male population. Additionally, ACTH levels were significantly decreased in men. Markers of liver and organ injury (ASAT, ALAT and bilirubin) were significantly increased, while creatinine levels significantly decreased following SCT.

Conclusion: These findings support the necessity for comprehensive monitoring and management of metabolic acidosis, electrolyte imbalances, hydration status and stress levels in soldiers undergoing SCT. Ensuring sufficient nutrition, hydration, and recovery time is crucial to reduce negative health effects and maintain optimal performance during and following SCT. This study contributes new knowledge on military health and training, specifically the physiological effects of SCT. More research is needed about differences in sex in the military population, as well as more knowledge on the physiological effects of military training to further incorporate strategies to enhance soldiers' health and performance in military combat situations.

Acknowledgements

This master thesis was carried out at the Department of Circulation and Medical Imaging, Faculty of Medicine and Health Sciences, Norwegian University of Science and Technology (NTNU), Trondheim. The project was funded by the Norwegian Military Academy. My supervisor through this project has been Morten Høydal, Professor and Head of Group of Molecular and Cellular Cardiology. The project responsible has been Morten and Christian Moldjord, associate professor at the Royal Norwegian Air Force. The test preparation, data collection and analysis were performed in close collaboration with the Department for Medical Biochemestry and Biobank 1 at St. Olavs hospital Trondheim.

I would like to express my deepest gratitude to my supervisor Morten, for his professional guidance and valuable support throughout working with this thesis. I am forever grateful for his time, advice, friendly encouragement, quick feedback and honest opinions. Having a supervisor with such a deep research knowledge and educational skills has given me much comfort throughout this learning process. I would also like to express my gratefulness to Thomas Fremo, Arnt Erik Tjønna, Lisbeth Røe and Victoria Johansen for helping me during the data collection, and for sharing your knowledge and experience. To all members of the Group of Molecular and Cellular Cardiology, thank you for welcoming me to your team, and for being a valuable source of inspiration.

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List of Abbreviations

ACTH	Adrenocorticotropic hormone
ALAT	Alanin-aminotransferase
ASAT	Aspartat-aminotransferase
BE	Base excess of extracellular fluid
Ca^{2+}	Calcium ions
CK	Creatine kinase
CK-MB	Creatine kinase myocardial band
Cl^-	Chloride ions
H^+	Hydrogen ions
HCO_3^-	Bicarbonate ions
K^+	Kalium ions
Na^+	Natrium ions
pCO_2	Partial pressure of carbon dioxide
pH	Potential of hydrogen
SCT	Simulated combat training

1. Introduction

The military population is dependent on being in a mentally and physically healthy state to perform well during military operations. Soldiers in the Norwegian Army Academy undergo various tests, including one week of simulated combat training (SCT), to simulate combat exposure. During military operations, soldiers face multiple stressors such as strenuous physical activity, mental strain, sleep deprivation, energy depletion and extreme environments (1). Previous research on military personnel show that these stressors significantly influence acute and chronic stress response in soldiers, ultimately impacting their performance and overall health (2). Previous research indicates that prolonged physical exercise, food restriction, and sleep deprivation can lead to physiological disturbances (3). This study contributes new knowledge, as few studies emphasize the clinical relevance of these physiological effects in soldiers (3). Particularly in Norwegian military personnel of both sexes, these types of studies still remains relatively limited (1).

Research on military training report metabolic changes in the military population, such as increased metabolism, lipolysis, fatty acid oxidation, and ketogenesis (4). Other studies indicate that undernutrition and hyper catabolism are major causes of military overtraining manifestation (5). Prolonged starvation triggers a variety of physiological responses in the body, which over time can lead to serious complications. Since the brain cannot utilize free fatty acids for energy due to its inability to cross the blood-brain barrier, it primarily relies on blood glucose for fuel (6). During prolonged starvation, the liver is triggered to produce ketone bodies through ketosis from free fatty acids as an alternate energy source (6). Subsequently, during extensive breakdown of fat, the formation of ketone bodies can be more than what the body can metabolize, leading to ketoacidosis in severe cases (7). During SCT, soldiers likely experience limited excess to proper nutrients, suggesting physiological responses such as increased ketone body production.

Hydration is crucial during continuous physical exercise and heat stress. Additionally, sweat loss during continuous physical exercise is the largest potential source of water loss (8). Even though water loss is expected during physical exercise, it is of relevance when analyzing the physiological effects of SCT on soldiers, as sweat loss without replaced electrolytes, commonly result in hypohydration, even though water is available (8).

Another natural response to exercise is skeletal muscle breakdown, followed by muscle repair and physiological adaption. However, prolonged or strenuous exercise, particularly when associated with other risk factors, can cause exertional rhabdomyolysis in some subjects (9). This is of relevance to military populations, as physical stress, heat stress and dehydration are common, and cases of rhabdomyolysis have previously been documented (10). Rhabdomyolysis is defined as ‟muscle fiber breakdown due to mechanical and metabolic injury that result in the release of muscle contents such as myoglobin, creatine kinase (CK), calcium (Ca^{2+}), and kalium (K^+) into the circulation" (11). Although this is relatively uncommon, rhabdomyolysis is a serious condition that should be monitored carefully.

The potential that prolonged exercise can induce cardiomyocyte damage, has received significant attention recently (12). Myocardial injury is marked by elevated plasma levels of creatine kinase myocardial band (CK-MB) and troponin T (13). Although several factors can determine how much serum enzyme activities increase during and after exercise (14), previous research show that both CK and CK-MB levels are increased following endurance training (15). Although in many cases irreversible, this does become of clinical importance due to soldiers frequently undergoing prolonged endurance exercise or strenuous endurance activity.

Previous research show that women tend to have lower maximal upper body strength, and a lower maximal oxygen uptake compared to men (1). Additionally, women tend to have lost only fat mass and no muscle mass during military exercises, whereas men lost both fat and muscle mass (15). Women are also found to be prone to iron deficiency and have a higher risk of muscle and skeletal injuries when subjected to the same training loads as their male colleagues (1). These sex specific differences seem to be higher during maximal strength activities and lower during endurance activities (1), suggesting that there are significant changes in relation to sex during physical exercise and performance in a military context.

This study aims to predict how exposure to multiple stressors during SCT affects the health of young soldiers in the Norwegian Army Academy, providing valuable tools to guide the planning and implementation of future military exercises.

2. Aim and hypothesis

The aim of this study was to investigate the acute physiological effects of four days of SCT with strenuous physical activity, mental strain, sleep deprivation and energy depletion. The present study focuses on changes in body composition and blood biomarkers in soldiers. In the blood biomarkers we focused on metabolic changes, skeletal muscle damage, myocardial stress in the total population, as well as when separating women and men. We hypothesized that following SCT, the soldiers presented increased levels of stress, metabolic and electrolyte disturbances, as well as indication of skeletal and cardiac muscle damage. Furthermore, we hypothesized that there would be significant physiological differences in responses in women and men following SCT.

3. Method

3.1. Study population

Forty-eight (36 male, 12 female) soldiers from the Royal Norwegian Air Force Academy (stationed in Trondheim, Norway) aged 20- 29 years were included in the study.

3.2. Assessment of SCT

The SCT lasted from Monday 22nd of May at 06.00am to Thursday 25th of May at 21.00pm (87 hours in total). During the SCT the subjects were only able to consume what they were given by the military personnel, which was ~ 940kcal in total. They had unlimited access to water. Periods of rest and sleep were controlled by military personnel (Figure 1.). Environmental conditions during the SCT period included humidity ranging from 100 – 30.3%, and ambient temperatures ranging from 19.9 – 3.9°C. Weather conditions ranged from sunny to several periods of rain (up to 5.7mm) (Figure 2). For specific details on the implementation of the SCT, see *Supplement S1*.

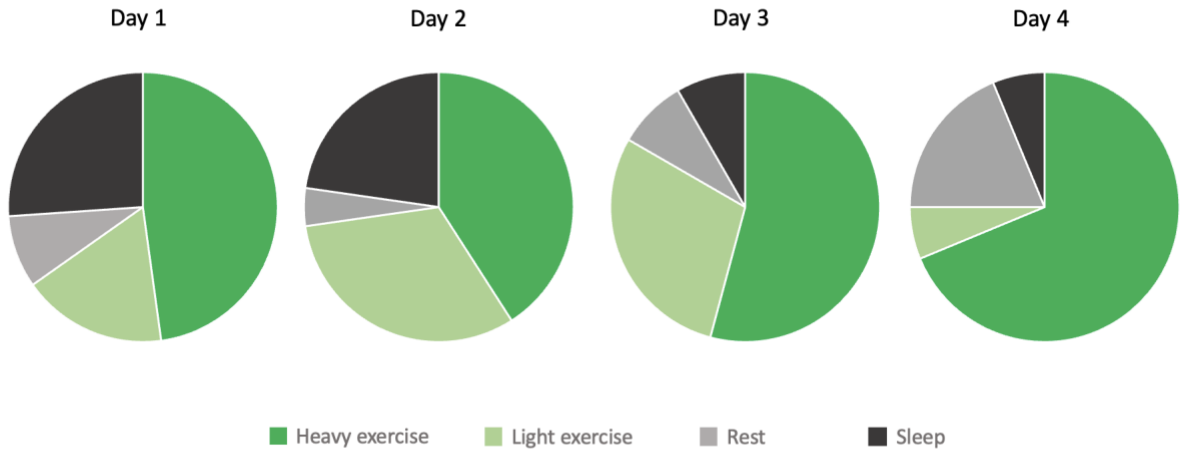


Figure 1. Distributed amount of time during SCT

Figure 2. Weather and temperature during SCT
(Data collected from Meteorological institute, Værnes lufthavn, valid as of 23.04.2024 (CC BY 4.0)).

3.3. Research ethics

This clinical study is conducted according to international standards of ethical medical research set out by The World Medical Association Declaration of Helsinki, and national and institutional research policies and procedures. All participants volunteered and provided a written consent for their participation. The study is performed according to the General Data Protection Regulation and both the institution, NTNU and The Royal Norwegian Air Force Academy, the Principal Investigators has responsibility to ensure that all personal data will be handled on a legal basis. This study is approved by the reginal ethical committee (REK Nord Id: 606073).

3.4. Data collections

All tests were performed at St.Olavs hospital. Pre- tests were conducted two weeks (8th of May) before the SCT. Follow-up testing was completed immediately following the four days of SCT (25th of May).

Blood samples. Venous blood samples were collected from each subject at baseline and at the end of the SCT according to standard operating procedures by approved bioengineers at the Department of Medical Biochemistry at St Olavs Hospital, Trondheim, Norway. Serum samples were collected in VACUETTE® Serum Gel 5 mL tubes (G456073) and plasma were collected in VACUETTE®EDTA-K2 6 mL tubes (G456043). All blood samples were immediately sent to Biobank1 for further processing and allocation to cryotubes for storage in a -80ºC freezer. All analyses of blood were performed at the laboratory of the Department of Medical Biochemistry, St. Olavs Hospital, that has accreditation according to the laboratory standard NS-EN ISO 15189.

Blood gas. Capillary blood gas was collected according to the user manual for medical biochemistry at St. Olavs Hospital, Trondheim, Norway. Blood gas is used to measure oxygen and carbon dioxide in the blood. The subjects were first instructed to put their hands into a bucket of lukewarm water, with intention to increase blood circulation to the fingers. The tip of the finger was then wiped with alcohol. Capillary blood gas was taken from the subject's finger using a safety lancet (Unistick touch, 21G, 2.0mm), and collected using capillary tubes (Safe clinitubes). Capillary blood gas was analysed using the Radiometer 90 Flex Blood gas Analyzer (ABL90 Flex).

Body composition. Body compositions measurements was measured using the bioelectrical impedance (InBody, 770, BIOSPACE, Seoul, Korea) according to the user manual at St. Olavs hospital. InBody scan is a body analysis used to measure the body's composition of body fat, fluid, and muscle mass. It uses four pairs of electrodes attached to handles, and a floor scale connected to the analyser. Participants were instructed to fast for a minimum of two hours before the test, as well as encouraged to go to the toilet right before the test started. During the analysis the subjects stood barefoot on the instrument, in an upright position. Before the test started demographic characteristics such as sex, age and hight were plotted on the display of the scale. After 60 seconds, the scale reported weight (kg), body mass index, muscle mass (kg), visceral fat (cm^2), and fat %. Validation studies have shown that InBody 770 is an appropriate alternative to dual-energy x-ray absorptiometry to measure body composition (16).

3.5. Study design

In this cohort study, we used a pre-post design to examine the outcomes of exposure to strenuous physical and mental stress (17). The study population consisted of first-year cadets in their twenties from the Royal Norwegian air force academy, whom all had been screened for excellent health status. The inclusion criteria required participants to be soldiers at the Royal Norwegian Air Force Academy who were participating in the mandatory SCT from May 22nd to May 25th and were healthy enough to participate in four days of combat training. The exclusion criteria were interrupted participation. All subjects were exposed to the same type of conditions during the SCT. Baseline tests were conducted before exposure, and follow-up tests were conducted at the end of the SCT. Out of 49 participants initially included in the study, one had to drop out due to sickness.

3.6. Statistical analysis

Normal distribution of all variables was confirmed using a Kolmogorov-Smirnov test, and values are reported as mean ± SD. All analysed variables measured at pre- and post SCT were compared using a paired samples t-test. All statistical tests were applied using available software (Graphpad prizm 10 for macOS, Version 10.2.1 (339), February 29th , 2024). Statistical significance with p-value was set to <0.05.

4. Results

4.1. Change in body composition

We found a significant decrease in total body weight following SCT in the total population (79.3 ± 12.7 to 74.9 ±12.3 at pre- and post, respectively, p <0.001) (Table 1). We also found a significant decrease in skeletal muscle in the total population (38.0±6.9 to 37.2±6.8 at pre- to post, respectively, p <0.001), as well as a decrease in body fat mass (12.4±4.6 to 9.7±4.0 at pre- to post, respectively, p<0.001) (Table 1). Additionally, there was a significant reduction in total body water in the total population following SCT (48.9±8.5 to 47.6±8.2 at pre- to post, respectively, p <0.001). Consistent observations were found for women and men (Table 1).

Table 1. Physical characteristics and changes in body composition pre and post SCT.
*Data are presented as mean ± standard deviation. P p-value. P- value: * 0.05, ** 0.01, *** <0.001.*

Variable	**Total**			**Women**			**Men**		
	Pre	**Post**	***P***	**Pre**	**Post**	***P***	**Pre**	**Post**	***P***
Weight (Kg)	79.3±12.7	74.9±12.3	***	67.7±7.3	63.7±7.0	***	83.1±11.8	78.6±11.4	***
Skeletal muscle mass (Kg)	38.0 ±6.9	37.2±6.8	***	29.7±4.1	29.3±4.0	***	40.8±5.3	39.8±5.3	***
Body fat mass (Kg)	12.4±4.6	9.7± 4.0	***	14.4±3.4	11.5±2.6	***	11.7±4.8	9.2±4.2	***
Total body water (L)	48.9±8.5	47.6±8.2	***	39±5.2	38.1±4.8	***	52.2±6.6	50.7±6.5	***

4.2. Nutritional status and electrolyte balance

Triglycerides in adipose tissue, provides the largest and most efficient energy storage in the body (18). Our study found a significant decrease in triglyceride levels in the total population following SCT (1.3 ± 0.8 to 0.7 ± 0.1 at pre- and post, respectively, p <0.001) (Table 2). Consistent observations were found when separating women and men.

Blood glucose have many crucial functions in the body, and it is the brains most important energy source (7). We found that the levels of glucose were significantly decreased following SCT in the total population (5.4 ± 0.6 to 3.7 ± 0.5 at pre- and post, respectively, p <0.001) (Table 2). Consistent observations were found when separating women and men.

Kalium (K^+) is used as a marker for hydration-electrolyte- and acid-base regulation (19). We found no significant changes in kalium levels following SCT (4.3 ± 0.3 to 4.2 ± 0.2 at pre- and post, respectively, p = NS) (Table 2). Consistent observations were found when separating women and men.

Natrium (Na^+) is a marker of electrolyte balance of the body, as well as nerve and muscle function (19). We found that the levels of natrium in the total population were significantly decreased following SCT (142.8 ± 1.7 to 139.0 ± 1.7 at pre- and post, respectively, $p<0.001$) (Table 2). Consistent observations were found when separating women and men.

Calcium (Ca^{2+}) is a marker for functioning of the heart, muscles, and nervous system (19). We found that levels of Ca^{2+} in the total population were significantly decreased following SCT (1.22 ± 0.026 to 1.19 ± 0.03 at pre- and post, respectively, $p<0.001$) (Table 2). Consistent observations were found when separating women and men.

Chloride (Cl^-) is a marker of acid-base regulation of the body (19). We found that levels of Cl^- in the total population were significantly decreased following SCT (106.4 ± 1.7 to 102 ± 2.3 at pre- and post, respectively, $p<0.001$) (Table 2). Consistent observations were found when separating women and men.

Table 2. Blood markers of nutritional status and electrolytes pre and post SCT.

*Data are presented as mean ± standard deviation. P p-value, Ref. reference value (in relation to the subjects age), NS not significant. P- value: * 0.05, ** 0.01, *** <0.001.*

Variable	**Total**			**Women**				**Men**			
	Pre	**Post**	***P***	**Pre**	**Post**	***P***	***Ref.***	**Pre**	**Post**	***P***	***Ref.***
Glucose (mmol/L)	5.4±0.6	3.7±0.5	***	5.3±0.6	3.7±0.5	***	4.0-6.0	5.5±0.6	3.7±0.5	***	4.0-6.0
Triglycerides (mmol/L)	1.3±0.8	0.7±0.1	***	1.0±0.5	0.7±0.1	***	0.45–2.6	1.4±0.8	0.7±0.1	***	0.45–2.60
Kalium (K+)	4.3±0.3	4.2±0.2	NS	4.2±0.3	4.1±0.3	NS	3.4-4.4	4.3±0.3	4.2±0.2	NS	3.5-4.4
Natrium (Na+)	142.8±1.7	139±1.7	***	142.8±1.4	139.7±1.9	***	137-145	142.8±1.8	138.8±1.5	***	137-145
Calcium (Ca^{2+})	1.2±0.03	1.2±0.03	***	1.2±0.02	1.2±0.04	*	1.14-1.28	1.2±0.03	1.2±0.03	***	1.14-1.28
Chloride (Cl-)	106.4±1.7	102±2.3	***	108±0.7	104.2±1.6	***	101-108	105.9±1.6	101.3±2.04	***	101.108

4.3. Metabolic effects

Lactate levels in plasma is used to indicate the acid levels in the blood stream (19). We found no significant changes in lactate levels in the total population following SCT (1.2 ± 0.4 to 1.3 ± 0.3 at pre- and post, respectively, p = NS) (Figure 3A). Consistent observations were found when separating women and men (Figure 3B-C).

pH (potential of hydrogen) is a marker for acid/base balance and oxygenation of blood (19). We found that pH levels in the total population were significantly decreased following SCT (7.4 ± 0.02 to 7.3 ± 0.04 at pre- and post, respectively, $p<0.001$) (Figure

3D). Consistent observations were found when separating women and men (Figure 3E-F).

The partial pressure of carbon dioxide (pCO_2) is a marker of oxygen or pH imbalance in blood (19). Abnormal levels of pCO_2 can indicate signs of a kidney, respiratory, or metabolic disorders. We found that the levels of pCO_2 in the total population were significantly decreased following SCT (5.6 ± 0.4 to 5.2 ± 0.6, at pre- and post, respectively, $p < 0.001$) (Figure 3G). Consistent observations were found when separating women and men (Figure 3H-I).

Base excess of extracellular fluid (BE) represents the amount of excess or deficit of HCO_3^- in the blood and is used to interpret measurements of acid-base balance (19). We found that levels of BE in the total population were significantly decreased following SCT (1.8 ± 1.3 to -5.5 ± 4.2 at pre- and post, respectively, $p < 0.001$) (Figure 3J). Consistent observations were found when separating women and men (Figure 3K-L).

Anion gap is a marker for acid- base balance in blood (19). We found that levels of anion gap in the total population were significantly increased following SCT (15.0 ±1.2 to 20.9±2.1 at pre- and post, respectively, $p < 0.001$) (Figure 3M). Consistent observations were found when separating women and men (Figure 3N-O).

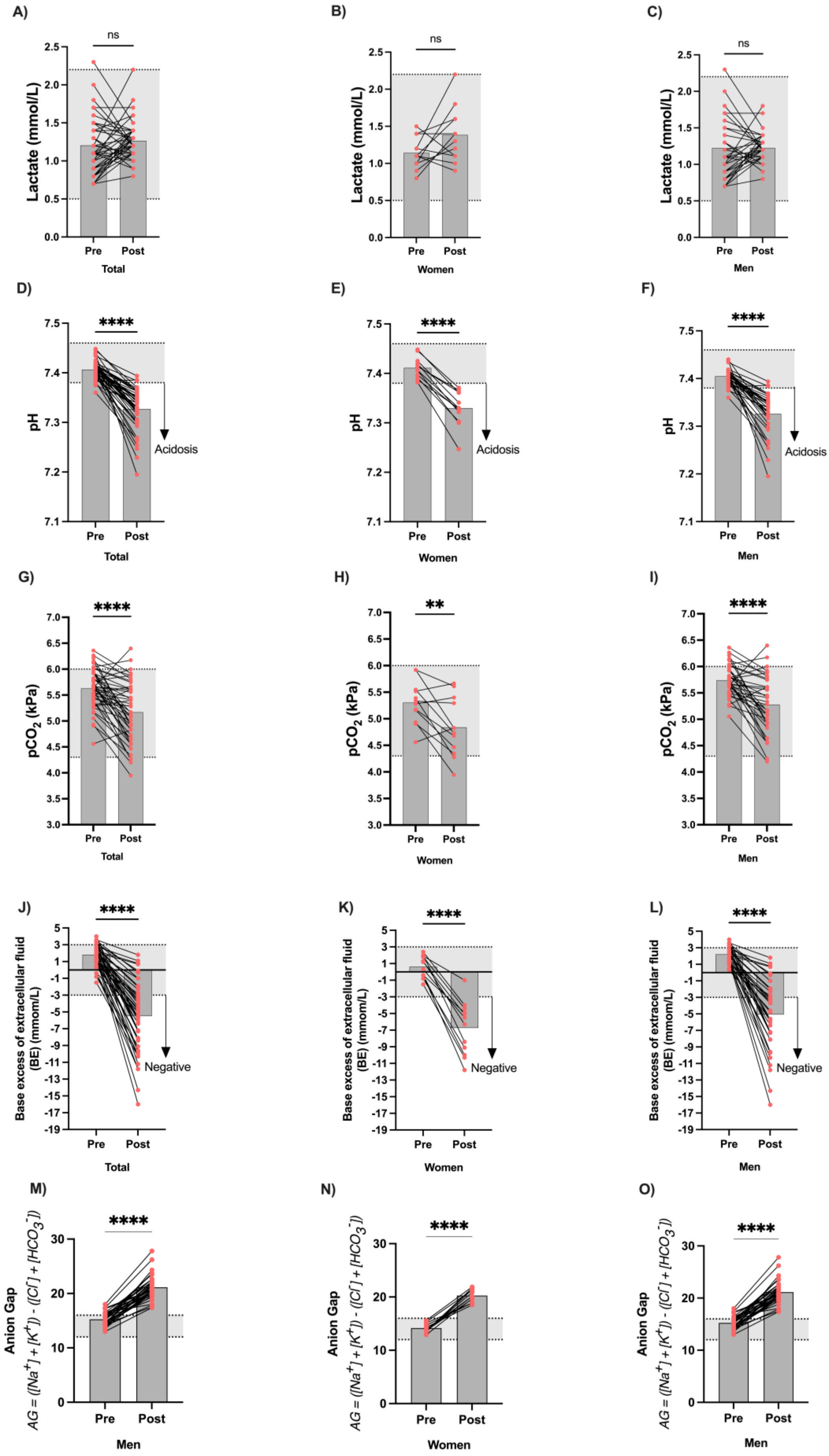

A)
B)
C)
ns
Lactate (mmol/L)
Pre
Post
Total
Women
Men
D)
E)
F)
****
pH
Acidosis
G)
H)
I)
**
pCO$_2$ (kPa)
J)
K)
L)
Base excess of extracellular fluid (BE) (mmom/L)
Negative
M)
N)
O)
Anion Gap
AG = ([Na$^+$] + [K$^+$]) - ([Cl$^-$] + [HCO$_3^-$])

Figure 3. Lactate, pH, pCO2, BE, and anion gap levels pre- and post SCT.
(A) *lactate (mmol/L) in total population;* ***(B)*** *lactate (mmol/L) in women;* ***(C)*** *lactate (mmol/L) in men.* ***(D)*** *pH in total population;* ***(E)*** *pH in women;* ***(F)*** *pH in men.* ***(G)*** *pCO2 (kPa) in total population;* ***(H)*** *pCO2 (kPa) in women;* ***(I)*** *pCO2 (kPa) in men.* ***(J)*** *BE (mmol/L) in total population;* ***(K)*** *BE (mmol/L) in women;* ***(L)*** *BE (mmol/L) in men.* ***(M)*** *Anion gap (mmol/L) in total population;* ***(N)*** *anion gap (mmol/L) in women;* ***(0)*** *anion gap (mmol/L) in men. Data are presented as mean ± SD. Statistical significance indicated in figure. Normal reference values (in relation to the subjects age) for women and men indicated by shaded area. P- value: * 0.05, ** 0.01, *** 0.001, **** < 0.0001.*

4.4. Biomarkers of stress

Adrenocorticotropic hormone (ACTH) is a protein hormone produced by the anterior pituitary and regulates cortisol production (19). We found that the levels of ACTH were significantly decreased following SCT (4.2 ± 1.9 to 2.9 ± 2.2 at pre- and post, respectively, p = 0.004) (Figure 4D). However, the difference was only significant in men when separating women and men (4.3± 1.8 to 3.0± 2.2 at pre- and post, respectively, p = 0.004) (Figure 4E-F).

Cortisol is a glucocorticoid hormone secreted by the adrenal cortex in response to physical or psychological stressors (19). We found that the levels of cortisol in the total population were significantly increased following SCT (254.8 ± 98.1 to 475.6 ± 166.5 at pre- and post, respectively, p <0.001) (Figure 4A). Consistent observations were found when separating women and men (Figure 4B-C).

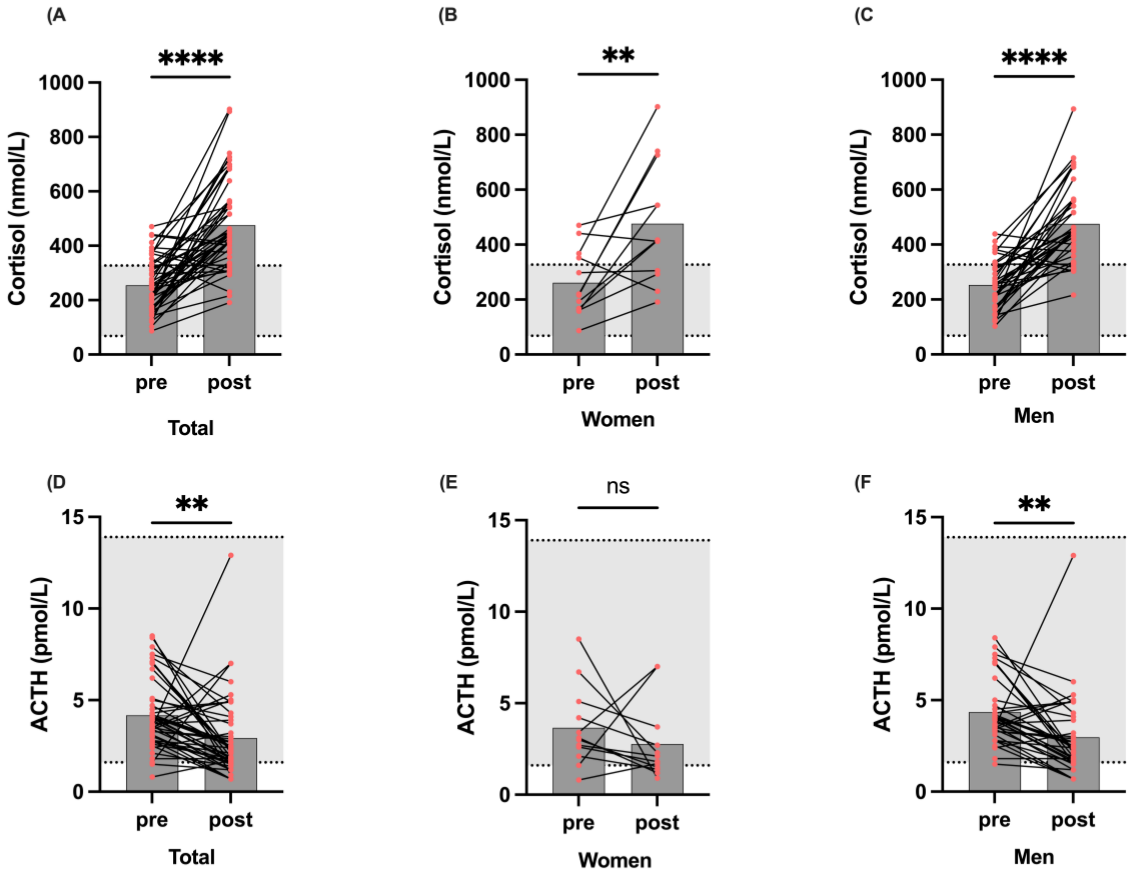


Figure 4. Cortisol and ACTH levels pre- and post SCT.
(A) *Cortisol levels in total population;* ***(B)*** *Cortisol levels in women;* ***(C)*** *Cortisol levels men.* ***(D)*** *ACTH levels in total population (pmol/L);* ***(E)*** *ACTH (pmol/L) levels in women;* ***(F)*** *ACTH (pmol/L) in men. Data are presented*

*as mean ± SD. Statistical significance indicated in figure. Normal reference values (in relation to the subjects age) for women and men indicated by shaded area. P- value: * 0.05, ** 0.01, *** 0.001, **** < 0.0001.*

4.5. Biomarkers of skeletal and cardiac muscle stress

CK is an intra cellular enzyme that is found in skeletal muscle, myocardium, and brain, as well as in smaller amounts in visceral tissue (19). CK is used to indicate skeletal muscle damage. We found that the levels of CK were significantly increased following SCT (484.3 ± 936.7 to 1597 ± 1664 at pre- and post, respectively, p=0.001) (Figure 5A). However, the difference was only significant in men (445± 810.9 to 1733± 1865 at pre- and post, respectively, p= 0.001) (Figure 5B-C).

CK-MB is an isoenzyme found almost exclusively in the myocardium (19). The appearance of elevated CK–MB levels in serum is highly specific and sensitive for myocardial cell wall injury (19). We found that the levels of CK-MB were significantly increased in the total population following SCT (1.1 ± 1.2 to 19.1 ± 30. 1 at pre- and post, respectively, p= 0.001) (Figure 5D). The same observation was found when separating women and men (Figure 5E-F).

Troponin T is a protein used as a biomarker for detection of cardiac injury (19, 20). We found that the levels of troponin in the total population were significantly increased following SCT (4.8 ± 2.2 to 8.8 ± 3.3 at pre- and post, respectively, p<0.001) (Figure 5G). The same observation was found when separating women and men (Figure 5H-I).

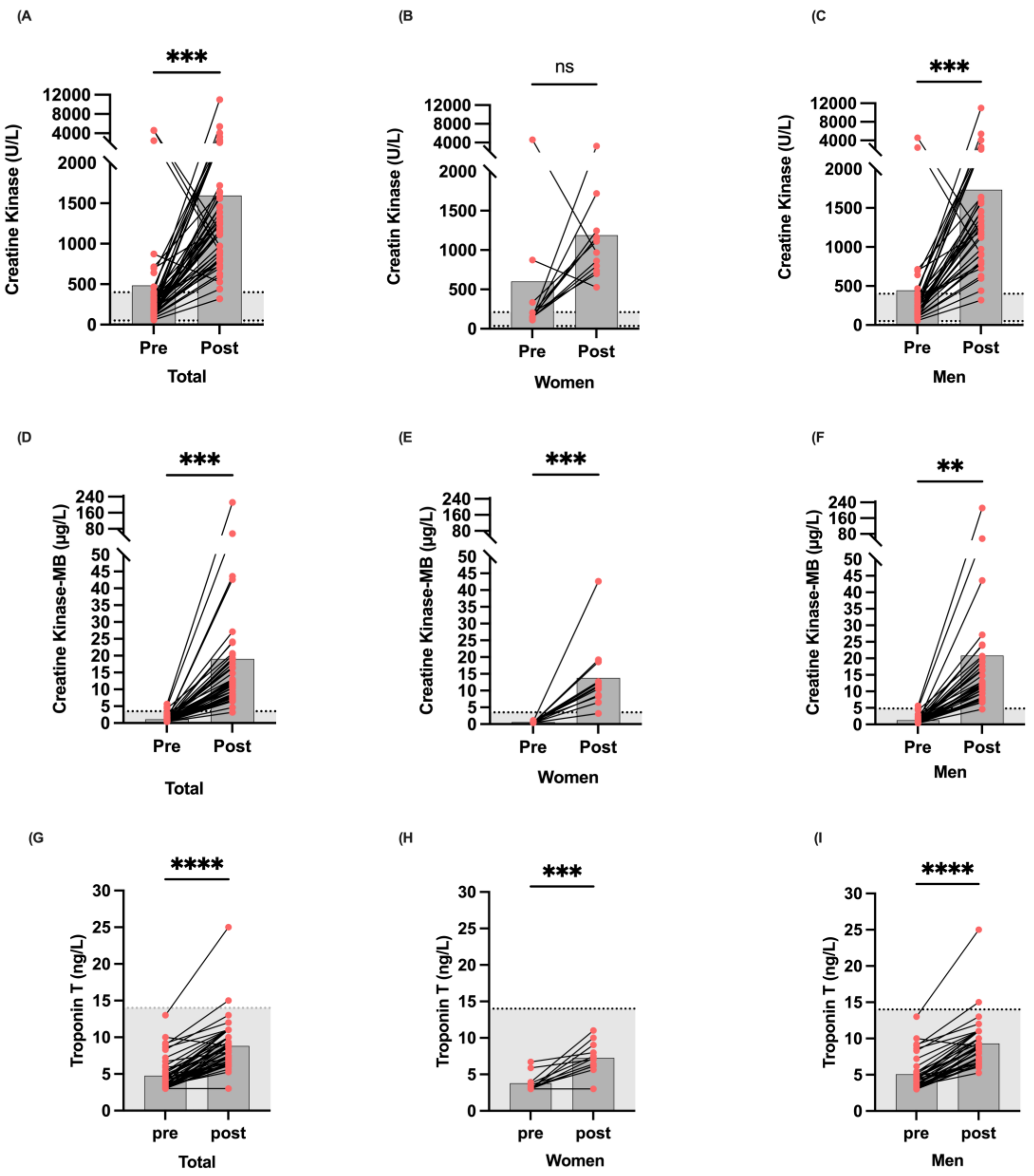


Figure 5. CK, CK- MB and troponin T levels pre- and post SCT.
(A)** CK (U/L) in total population; **(B)** CK (U/L) in women; **(C)** CK (U/L) in men. **(D)** CK-MB (µg/L) in total population; **(E)** CK-MB (µg/L) in women; **(F)** CK-MB (µg/L) in men. **(G)** troponin T (ng/L) in total population; **(H)** troponin T (ng/L) in women; **(I)** troponin T (ng/L) in men. Data are presented as mean ± SD. Statistical significance indicated in figure. Normal reference values (in relation to the subjects age) indicated by shaded area. P- value: * 0.05, ** 0.01, *** 0.001, * < 0.0001.*

4.6. Liver and other organs

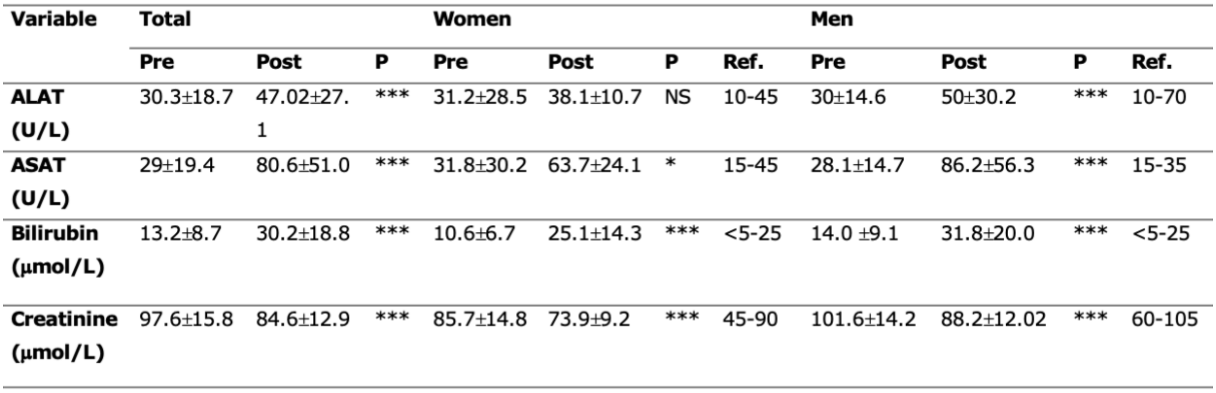
ASAT (aspartat-aminotransferase) is an enzyme fount in the heart, liver, and skeletal muscles (19). Abnormal ASAT activity is detectable in several tissues, especially in heart, liver and skeletal muscles. We found that the levels of ASAT in the total population were significantly increased following SCT (29.0 ± 19.4 to 80.6 ± 51.0 at pre- and post, respectively, p <0.001) (Table 3). The same observation was found when separating women and men.

ALAT (alanin-aminotransferase) is an enzyme found in the liver. ALAT is a marker used in evaluation of potential liver disease. We found that the levels of ALAT in the total population was significantly increased following SCT (30.3 ± 18.7 to 47.02 ± 27.1 at pre- and post, respectively, p <0.001) (Table 3). The same observation was found when separating women and men.

Creatinine is a biproduct of from skeletal muscles and is used to evaluate the liver function (19). We found that the levels of creatinine in the total population were significantly decreased following SCT (97.6 ± 15.8 to 84.6 ± 12.9 at pre- and post, respectively, p <0.001) (Table 3). The same observation was found when separating women and men.

Bilirubin is used to indicate the level of breakdown of old red blood cells and sign of possible liver damage (19, 21). We found that the levels of bilirubin in the total population were significantly increased following SCT (13.2 ± 8.7 to 30.2 ± 18.8 at pre- and post, respectively, p <0.001). The same observation was found when separating women and men (Table 3).

Table 3. Blood markers of liver and organs pre and post SCT.
*Data are presented as mean ± standard deviation. P p-value, Ref. reference value (in relation to the subjects age), ALAT Alanine transaminase, ASAT Aspartate aminotransferase. P- value: * 0.05, ** 0.01, *** <0.001.*



Variable	**Total**			**Women**				**Men**			
	Pre	**Post**	**P**	**Pre**	**Post**	**P**	**Ref.**	**Pre**	**Post**	**P**	**Ref.**
ALAT (U/L)	30.3±18.7	47.02±27.1	***	31.2±28.5	38.1±10.7	NS	10-45	30±14.6	50±30.2	***	10-70
ASAT (U/L)	29±19.4	80.6±51.0	***	31.8±30.2	63.7±24.1	*	15-45	28.1±14.7	86.2±56.3	***	15-35
Bilirubin (μmol/L)	13.2±8.7	30.2±18.8	***	10.6±6.7	25.1±14.3	***	<5-25	14.0 ±9.1	31.8±20.0	***	<5-25
Creatinine (μmol/L)	97.6±15.8	84.6±12.9	***	85.7±14.8	73.9±9.2	***	45-90	101.6±14.2	88.2±12.02	***	60-105

5. Discussion

In this cohort study with a pre-post design, we included 48 healthy soldiers aged 20-29 years who participated in mandatory four days of SCT. Blood biomarkers were compared before and after exposure to multiple stressors. Recent studies show that multiple stressors during combat exposure influences both acute and chronic stress response in soldiers, ultimately affecting their performance and health (2). Examples of these stressors include strenuous physical activity, mental strain, sleep deprivation and energy depletion (1). The purpose of this study was to investigate the physiological effects of these stressors on soldiers participating in mandatory SCT. The main findings in this study indicate significant metabolic changes causing metabolic acidosis, increased markers of skeletal muscle damage, and elevated bio markers of myocardial stress.

5.1. Metabolic effects

Following SCT, we observed a significant decrease in pH in the total population, and in women and men, indicating acidosis with a pH below normal reference values. Acidosis, defined as blood pH below 7.35, is typically due to an imbalance between the supply and elimination of hydrogen ions (H^+) in the blood (7). Depending on pCO_2 levels in the blood, the observed acidosis can either be respiratory or metabolic acidosis. Our data showed a significant decrease in pCO2 within normal reference values, ruling out that the observed acidosis is explained by respiratory factors (7). A pCO_2 above normal reference values, is characterized by insufficient CO_2 elimination of the lungs (7). Given that the acid-base disturbance occurred due to an increase in H^+ ions, unrelated to CO_2, indicates metabolic acidosis. This type of acidosis occurs when there is an increase in H^+ from another source than CO_2 (7).

To further support this, we measured BE, which is an indicator of excess or deficit of HCO_3^- levels in the blood. The BE value indicates the content of anions that can bind to H^+ (7). We found a significant decrease in BE in the total population, below normal reference values, confirming a HCO_3^- deficit in the blood. Taken together, our data points to primary metabolic acidosis (7), a condition characterized by a reduction in the serum concentration of HCO_3^-, normal pCO_2, and a decrease in blood pH (Figure 6)(22).

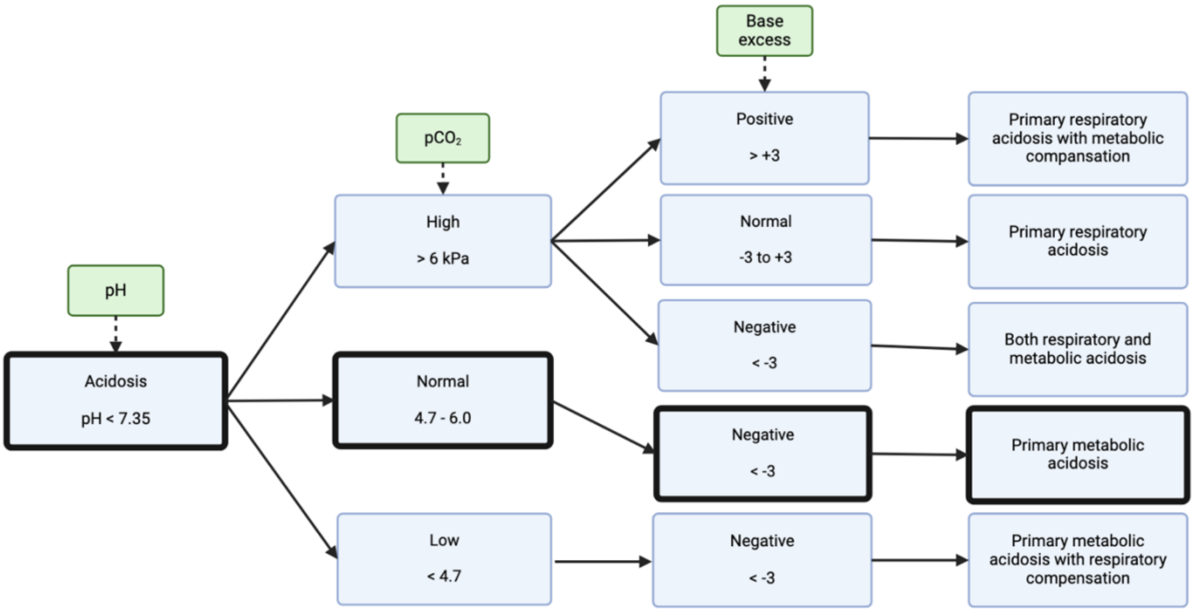


Figure 6. Pathway to the diagnosis of primary metabolic acidosis.
(created in BioRender)

Various factors could contribute to primary metabolic acidosis, including lactacidosis, ketosis, and HCO_3^- loss through diarrhea. Strenuous physical exercise can lead to production of lactic acid through anaerobe muscle work, leading to lactacidosis in severe cases (7). However, our study did not find a significant change in lactate levels. Diarrhea could also cause metabolic acidosis due to HCO_3^- loss through feces because of rapid passage through the intestines (7). However, if diarrhea was the cause, we would not have seen a significant increase in the anion gap, because there is a significant loss of Na^+ along with the loss of HCO_3^-, which would lead to a normal or reduced anion gap (7).

In clinical practice the anion gap is a marker used to evaluate the cause of primary metabolic acidosis. Anion gap represents the difference, or gap, between negatively and positively charged electrolytes in the blood (7). In normal physiological conditions the anion gap remains relatively small, due to a balance between positively and negatively charged ions. The anion gap, calculated as ($[Na^+] + [K^+]) - ([Cl^-] + [HCO_3^-]$), helps evaluate primary metabolic acidosis (22). Our study found a significant increase in the anion gap, measured above normal reference values, indicating metabolic acidosis with a high anion gap. In this context, this is likely due to ketoacidosis from prolonged starvation and exercise with high energy demands during the SCT (22).

In ketoacidosis, the body produces excess ketone bodies as an alternative energy source (6, 7). Since the brain cannot utilize free fatty acids for energy due to its inability to cross the blood-brain barrier, it primarily relies on blood glucose for fuel. When glucose levels are low, the liver is trigged to produce ketone bodies as an alternate energy source (6, 7). The acidic ketone bodies increase the H^+ concentration in the blood, consequently depleting HCO_3^- through the buffering of H^+, leading to an increased anion gap due to reductions in HCO_3^- (7). Ketoacidosis is induced by extensive breakdown of fat, leading to the formation of more ketones than what the body can metabolize, and subsequently ketoacidosis (7). Since ketones are organic acids, their accumulation results in acidosis, causing primary metabolic acidosis.

Our findings are supported by significant decreases in glucose and triglyceride levels, and notable losses in body weight, fat mass, and muscle mass, indicating a state of catabolism. Acute metabolic acidosis can cause decreased cardiac output, arterial dilatation with hypotension, altered oxygen delivery, decreased ATP production, predisposition to arrhythmias, and impairment of the immune response (22). Therefore, early diagnosis and treatment of metabolic acidosis are crucial to reduce these risks.

5.2. Electrolyte and hydration

As previously mentioned, we found a significant decrease in body weight. The same observation was found in total body water in women and men. The sequence of physiologic events affecting the loss of water is via breathing, sweating, feces, and urine output (8). Exercise induced sweat loss can lead to substantial quantities of electrolytes being lost (8). In this study we analyzed markers of K^+, Na^+, Ca^{2+} and Cl^- to evaluate possible electrolyte imbalances.

We found a significant decrease in Na^+ following SCT in the total population, as well as in women and men, indicating a population in a state of dehydration (19). Na^+ is the primary electrolytes involved in maintaining extracellular fluid volume and balance. Changes in in specially Na^+, but also Cl^- can affect fluid- and the acid-base balance (23) (19). Cl^- levels were also decreased following SCT in both women and men, although within normal reference values. A reduction in electrolytes due to sweat loss was expected in this study, however sweat loss in combination with a lack of supply of new electrolytes through diet, could lead to severe dehydration (8). Although Na^+ levels were found to be within normal reference values in this study, electrolytes need to be replaced to be able to restore fluid balance and total body water (8).

Other electrolytes analyzed was K^+, which is also important in cellular metabolism and acid-base balance (19, 23), however we found no significant changes in K^+ in this study. We found a significant decrease in Ca^{2+} levels in the total population following SCT, with reference values being within normal ranges. Our findings emphasize the importance of electrolyte replacement, as unlimited access to water alone may not be enough to maintain hydration.

5.3. Stress

During SCT, soldiers are exposed to multiple stressors shown to affect their performance and health (2). Biomarkers of stress, such as cortisol and ACTH, are valuable for measuring physiological stress, and evaluating psychophysiological response to military training (3). We found a significant decrease in ACTH levels in the total population, with the difference only being significant in men, likely due to the negative feedback loop from cortisol, which could explain the reduced ACTH levels following SCT (7). Arguably, post SCT levels could also indicate that the level of stress is going down due to breaks before collecting the tests, and the soldiers thinking the SCT being over.

We found a significant increase in cortisol levels above normal ranges in the total population. Upon exercise, the hypothalamus secretes corticotropin releasing hormone, stimulating the release of ACTH, subsequently stimulating the adrenal cortex to secrete cortisol (24). Cortisol plays a crucial role many physiological processes, which include proteins in the skeletal muscle to be broken down into amino-acids, and hydrolysis of triglycerides in adipose tissue into free fatty acids and glycerol. High circulating levels of cortisol may induce gluconeogenesis in the liver, generating additional carbohydrates for energy production (24). Furthermore, cortisol promotes fat breakdown via lipolysis in adipose tissue, leading to the release of free fatty acids. Cortisol provides a vital source for ketogenesis in the liver to form ketone bodies for energy production (24). Abnormally high cortisol levels may indicate a dysregulated glucose metabolic, potentially resulting in ketoacidosis and liver overload.

Recent studies show that multiples stressors impact sleep quality in military personnel. In addition, even with the opportunity to sleep, additional environmental disruptors in the operational context hinders restorative sleep (25). Previous research has shown that sleep deprivation increases serum cortisol levels (26), negatively affecting mental health. Furthermore, one study reports that serum cortisol levels at 500 nmol/L during sleep deprivation, is the optimal cut-off value to predict manic attacks (26). In our study, mean cortisol levels were 475 nmol/L in the total population, with some soldiers exceeding this, indicating severe sleep deprivation and potential negative psychological effects.

Previous research on military populations also indicate a significant association between sleep duration and musculoskeletal injuries (25). Studies on the US Army Special Operations Command soldiers show that sleep deprivation impairs reaction time, cognitive function, fatigue and sleep duration (27), which could contribute to a higher risk of injury in young soldiers. These findings highlight the importance of adequate sleep for maintaining physical performance and reducing injury risk in military personnel.

5.4. Skeletal and cardiac muscle damage

CK, troponin T and ASAT are key serum markers of skeletal muscle injury (14). We also analyzed CK-MB and cardiac specific troponin T to evaluate potential cardiomyocyte damage (13). We found a significant increase in total CK levels following SCT, above the normal range. Significant observations were only found in men, indicating skeletal muscle damage and potential rhabdomyolysis. Previous research show that strenuous exercise damages skeletal muscle cell structures at the level of sarcolemma and Z-disks, elevating CK levels (14). Clinically, CK levels must be more than five times the upper limit of normal reference values, to be able to diagnose rhabdomyolysis (10). Although most often caused by direct traumatic injury, rhabdomyolysis can also result from other factors, such as electrolyte and metabolic disorders (28). Early treatment of rhabdomyolysis is crucial to prevent acute kidney failure and cardiac arrest if not properly addressed (10).

The potential that prolonged exercise can induce cardiomyocyte damage, has received significant attention recently (12). In this study we found a significant increase in CK-MB levels in the total population following SCT, indicating a population in myocardial stress. Although occurring less frequently, elevated plasma CK-MB has also been reported in athletes most often following acute strenuous exercise or during chronic intensive exercise (13). Some studies suggest elevated CK-MB levels to come from noncardiac sources (13), and that abnormalities found is due to normal adaption after exercise (29). However, due to contradicting findings, more research is crucial to be able to evaluate the cause and severity of elevated levels of CK-MB in young and healthy populations, specifically after strenuous and prolonged exercise.

Troponin T is a more specific marker for myocardial injury compared to CK-MB. CK-MB is mainly found in the heart but is also expressed at lower levels in highly oxidative skeletal muscle fibres (20). We found a significant increase in troponin T levels in the total population following SCT. Several factors determine how much serum enzyme activities increase during and after exercise (14). Exercise of different types, durations, and intensities is commonly shown to increase troponin T levels, sometimes exceeding the clinical threshold for myocardial injury (30). This may support the debate about whether exercise-induced increases indicate myocardial damage or temporary myocardial fatigue (29). Further research is needed to clarify these findings.

5.5. Liver and other organs

Evaluating the impact of physical and physiological stressors on different organs is crucial to understanding their effects on the body. ASAT, found in multiple tissues, and ALAT, primarily found in the liver, are biomarkers for liver injury (19). In this study we observed significant increases in ASAT and ALAT levels, indicating possible liver or skeletal muscle overload (19). Strenuous physical exercise is shown to elevate ASAT levels, suggesting that the observed abnormalities may result from normal adaption after exercise (29). When the liver is injured, its ability to process and excrete bilirubin is impaired, leading to elevated bilirubin levels in the blood (19). We found a significant increase in bilirubin levels in the total population following SCT, above normal reference values for women and men. This indicates possible impaired bilirubin processing or excretion (31). These markers, along with ASAT and ALAT, help diagnose and monitor liver diseases and conditions (19).

ASAT and ALAT can also indicate ketosis, as ketosis increases liver stress (32). In addition, during starvation, the body shifts to using ketone bodies for fuel, straining muscle tissue and elevating ALAT levels found in the blood (7). In relation to rhabdomyolysis, an isolated rise in levels of ASAT and ALAT should be considered in the diagnosis. However, current diagnostic tools have been reported to not have adequate specificity to differentiate liver injury from isolated muscle injury (32). These results indicate that levels of ASAT and ALAT measured in patients who have participated in exercise should be interpreted with caution.

Furthermore, rhabdomyolysis releases myoglobin from damaged muscle cells, increasing serum CK and myoglobin, which serve as primary indicators for laboratory diagnosis (33). If the concentration of myoglobin in the blood exceeds a certain threshold, it is excreted in the urine and could be interpreted though urine samples (33). However, urine samples were not analyzed or conducted during this study. Excessive myoglobin from damaged muscle cells can be harmful, particularly to the kidneys, potentially leading to acute kidney injury or acute renal failure (9, 32-36). Previous research show that serum myoglobin tends to increase as creatinine clearance decreases, and that the high level of myoglobin in the blood usually leads to the appearance of myoglobin in the urine (34). In this study, creatinine levels were significantly decreased in the total population following SCT. This indicates no severe damage or impairment in kidney function. Furthermore, if the soldiers experienced severe rhabdomyolysis, accompanied by kidney failure, we would probably have seen increased levels of creatinine (19). This suggests that the observed muscle damage, and potential rhabdomyolysis, was not significant enough to leave a chronic strain on the kidneys.

5.6. Physiological differences in men and women

The women in this study were measured to weigh less, have less muscle mass and more fat mass, compared to the men in this study. Previous research show that women tend to have lower maximal upper body strength, and a lower maximal oxygen uptake compared to men (1). Additionally, in a performance context, these sex specific differences seem to be higher during maximal strength activities and lower during endurance activities (1). This suggests that there will be differences in performance during SCT between the sexes, however we did not include any specific measurements for this in this study. Furthermore, previous research has also observed that women lost only fat mass and no muscle mass whereas the men lost both fat and muscle mass during military training (15). Even though this finding has not been fully explained, it is suggested that a possible reason could be due to women oxidizing more fat, and less carbohydrates and amino acids, as energy substrates during physical exercise, compared to men (1, 15). This can be advantageous in the military for long-duration exercises and operations. Previous research also show that women are more often prone to iron deficiency and have a higher risk of muscle and skeletal injuries if subjected to the same training loads as their male colleagues (1). In this study however, we found no significant differences in men and women in any of our results when analyzing changes from pre- to post SCT. These data suggest that the physiological differences in men and women in this study, does not directly influence the effect of SCT on the total population divided by sex.

5.7. Clinical perspective and implications for military personnel

Monitoring electrolyte levels, hydration status, and metabolic markers is crucial in individuals at risk for conditions such as metabolic acidosis and rhabdomyolysis, especially during prolonged periods of stress or extreme physical activity, such as during SCT. Treating acute metabolic acidosis should focus on eliminating the underlying cause.

Theoretically, administration of Na^+ - HCO_3^- could improve acid-base parameters, however this strategy is not always beneficial, as it can result in potential complications (22). When rhabdomyolysis is suspected, early detection and treatment is crucial to prevent acute kidney injury, and acute renal failure (9, 28). Fluid management and resuscitation is critical to prevent further complications (28).

Research indicates that supplements modifying the blood buffering system can significantly enhance high-intensity exercise performance (37). HCO_3^- accounts for over 90% of plasma buffering capacity, and its supplementation can increase HCO_3^- concentration and blood pH (37). Studies suggest that consuming alkaline water post-exercise accelerates and enhances rehydration compared to regular water (37). Additionally, "overdrinking" (relative to sweating) is found to be the primary factor in the development of hyponatremia. However, consumption of sports beverages or water containing Na^+ is shown to delay the development (8). Current recommendations is to consume Na^+ along with water if the exercise duration exceeds two hours, or when significant Na^+ losses are likely, or when the volume of the consumed drink may significantly lead to reduction in plasma Na^+ concentration (8).

6. Limitations

In this study, we measured physiological responses in soldiers undergoing SCT, analyzing biomarkers in capillary blood gas and venous blood, as well as measuring body composition. Although this study offers crucial insights into the physiological demands of SCT, we acknowledge limitations that may influence our results.

A cohort study introduces limitations such as sample size, sex imbalance, and generalizability. Our sample size, particularly the female subgroup, is relatively small, affecting the generalizability of the findings and reducing statistical power. The unequal distribution of male and female participants hinders the ability to draw sex-specific conclusions. Additionally, our findings may not be applicable to populations with different demographic characteristics outside of the Norwegian military, due to the specific stressors and environmental conditions differing in military groups or civilian populations.

We analysed blood markers from capillary blood gas and blood samples, reflecting dynamic physiological changes throughout the day or in response to specific stressors. However, single-time-point sampling risks getting misinterpreted or overlooked, proper handling and processing crucial to maintain integrity of the biomarkers. Confounding variables, such as differences in baseline characteristics or unmeasured factors, may also influence the outcomes and validity of the findings.

The InBody scan measures body composition, however its accuracy can be influenced by variation in hydration status, potentially affecting the results. We did not account for women`s menstrual cycles, which can affect fluid levels in the body. Daily fluctuations in hydration due to exercise and diet can impact measurements of body mass and body fat percentage. To reduce measurement error and improve reproducibility, participants were instructed to use the toilet before the InBody scan. However, factors such as diet and exercise were not standardized due to the military setting. Cortisol levels, which vary throughout the day and between sexes, could be influenced by reversed sleep cycles and sleep deprivation, despite samples being taken at the same time each day.

7. Future perspectives

Future research should explore the use of biomarkers for early detection of overtraining and metabolic disturbances in military personnel. Identifying early signs of overtraining can facilitate interventions that can help prevent severe health issues and ensure optimal performance in military operations. Implementing $VO2_{max}$ testing SCT can provide valuable insights into the fitness levels of the subjects. Wearing pulse sensors can also be a helpful tool in monitoring physiological responses to different intensities during SCT. Additionally, measuring ketone bodies in the blood can offer valuable data on metabolic disturbances. The pre- to- post design could be enhanced by including an additional follow-up in the days or weeks following the post- measurements. This would strengthen the study`s validity and provide additional insight into recovery following SCT.

8. Conclusion

In this study, young, healthy soldiers underwent four days of strenuous physical exercise, sleep deprivation, and food restriction during SCT. Key observations in this study included primary metabolic acidosis, indicated by decreased pH and BE and an elevated anion gap, primarily caused by ketoacidosis from prolonged starvation and high energy demands. Significant dehydration was also observed, marked by reductions in Na^+, Cl^-, and total body water, emphasizing the need for proper electrolyte replacement and hydration to maintain fluid balance and avoid dehydration. Elevated cortisol levels post-SCT reflected an increased stress response, likely due to sleep deprivation and strenuous physical exercise, reducing cognitive and physical performance. This highlights the importance of managing stress and ensuring sleep quality in military training. Skeletal muscle damage, indicated by elevated total CK, suggested potential rhabdomyolysis, particularly among male participants. However, no significant kidney impairment was observed. Increased CK-MB and troponin T suggested cardiac muscle stress, and elevated liver enzymes (ASAT and ALAT) and bilirubin levels indicated potential overload on the liver. These findings should be interpreted with caution due to marker specificity and overlap. Notable, no significant sex differences in physiological responses were found, suggesting that the SCT is equally adaptable across both sexes. Further research on sex-specific responses to SCT is necessary to draw more definitive conclusions. The main findings in this study confirm our initial hypothesis. However, our subsequent hypothesis regarding differences in physiological responses between the sexes, was disproved.

Our findings support the need for comprehensive monitoring and management of metabolic acidosis, electrolyte imbalances, hydration status, and stress levels in soldiers undergoing SCT. Ensuring adequate nutrition, hydration, and recovery is crucial to reduce negative health effects and maintain optimal performance during and following SCT. This study contributes to military health and training knowledge, specifically the physiological effects of SCT. Further research is needed on sex differences, and strategies to enhance soldier health and performance during SCT.

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10. Supplements

10.1. Supplement S1

	Man 22 mai: kl 00 - 24	Tir 23 mai: kl 00 - 24	Ons 24 mai: kl 00 - 24	Tor 25 mai: kl 00 - 24
Fysisk tung belast ant timer pr døgn	7 t. 09-16. Marsj med tung belastning 4 t. 16-20 Klatring i trær (ulik belastning avh av rolle og egeninnsats) 1 t, 22-23. Marsj til Frigården **Tot 11 t.**	4 t. 12-16 Praktisk stridsteknikk, rullering av ulik strids-trening. 3 t. 18-21. Marsj m stridsvest, våpen og lett pakning. Hente tungt objekt. Marsj 2x4 km. 1 t. 22-23. Hente matposer (noen utvalgte per lag henter mat) 1 t. 23-24. Test av beredskap i leir **Tot 9 t**	1 t. 00-01. Stand to. Test av beredskap I leir 3 t. 01-04. Økende trusselnivå rettet mot leir. Intensivering av vakthold. Personell må ut av sove/hvile og på vakt 1 t. 04-05. Alarm. Alle på post 6 t. 09-15. Objektsikring i kom med fysisk krevende oppdrag 1 t. 18-20. Angrep mot leir. Intensiv beredskap 2 t. 22-24. Marsj hente mat, alle **Tot 13 t**	11 t. 03-14. Mestringsløp. Mange ulike caser med fysisk og mental utfordring. Ingen pause underveis. Etter tester St.Olavs 1 t. 20-21. Jaktstart. Lagsvis marsj/løp tilbake til LKSK Mat ved ankomst LKSK. **Tot 11 t** (før tester St. Olavs)
Fysisk lett belastning ant timer pr døgn	3 t. 06-09. Forberedelse før avreise. / stridsdrill. En del stress for mange. 1 t. 23-24. Etablering hvileområde **Tot 4 t**	1t. 00-01. Stående, brief sj HV 1t. 07-08. Oppstilling, klargjøring til skyteøvelser 4t. 08-12. Skytetrening, teori – rullering ulike poster 1t. 21-22. Etablering i leir. Oppsett vakt-rullering **Tot 7 t**	3 t. 05-08. Mindre intensiv, men alle holdes våkne 3 t. 15-18. Vakthold. Mindre intensiv belastning 1 t. 20-21. Oppstilling. Brief **Tot 7 t**	1 t. 01-03. Vekking. Oppstilling. Info ny case **Tot 1 t**
Hvile ant timer pr døgn	1 t x 2. Hvile i form av refleksjonstid/samtale i laget **Tot 2 t**	1t. 17-18. Refleksjon i lag **Tot 1 t**	1 t. 08-09. Refleksjon i lag 1 t. 21-22. Refleksjon i lag **Tot 2 t**	1 t. 14-15. Refleksjon i lag. 1 t. 15-16. Buss til St.Olav **3 t. 16-19. Testing St Olavs**
Søvn ant timer pr døgn	6 t. 00-06 (natt til mandag i egen seng) **Tot 6 t**	5 t. 01-06. Trange forhold/ubekvemt, mange sov dårlig **Tot 5 t**	1 t. Svært begrenset søvn pga intensitet gjennom natten 1 t. 23-24 Søvn/hvile **Tot 2 t**	1 t. 00-01. Søvn lagsvis. **Tot 1 t** (søvn før tester St. Olavs
Mat/kalorier pr døgn	Normal Frokost før 07:30 (ikke matpakke) 1 pk knekkebrød pr lag (8 pax i laget)	2 knekkebrød pr person fra formiddag. Matpose pr lag hentes ved midnatt. 450 kcal pr pers	Matpose pr lag, hentes før midnatt. 320 kcal pr pers.	Ingen mat før endex LKSK etter tester St. Olavs
Vann gj.sn liter pr døgn	Ubegrenset tilgang på vann	Ubegrenset tilgang	Ubegrenset tilgang	Ubegrenset tilgang

Complete script of the SCT.
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