The association between circulating androgens, aerobic capacity and muscular power in female athletes

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PREFACE

This study was a part of a larger research on female athletes titled " The female competiveness study". The project investigates the association between circulating androgen hormones and different physical parameters such as VO_2max and muscular power in addition to sexual preferences, mental health, competitiveness, symptoms of eating disorders, body composition, bone mineral density, and menstrual disturbances. A total of 73 athletes from different sports participated in the project. This masters` thesis will focus on circulating androgen hormones, endurance and power in the three sport groups cross-country skiing, handball and soccer. These were the three largest sport groups in the competitive female athletes study. They were selected to ensure the results to be as reliable and comparable as possible. The excluded groups, which were material arts, estetical, and technical sports, had only two or three subjects in each sport group.

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ABSTRACT

Previous research has focused primary on the effects of testosterone supplementation and circulating androgen influence on physical performance among men. However the relationship between physical performance and natural variations of androgens in female athletes is a relatively unexplored field, thus further research on females and androgens are necessary. The primary aim of this study was to analyze the relationship between baseline androgen hormones, VO_2 max and maximal power in female athletes from three different sports. Due to the physical differences between sports, we also examined these relationships within the three different groups of female athletes; handball players (n=17), soccer players (n=24) and cross-country skiers (n=16). 57 females aged 18-34 years participated in the study. VO_2max was determined while running. Jump power was measured with squat jumps (SQJ) on a force platform, and a standard bench press test was used to measure the upper body power. Standard procedure blood samples were taken to measure the levels of testosterone (T), anti muller hormone (AMH), androstendione, sex hormone binding globulin (SHBG), free testosterone index (FTI), dihydroepiandrostendione (DHEA) and hydroepiandrostendionesulfate (DHEAS). A significant negative association between VO_2max and FTI was found in the cross-country skiers (r=-0.760, p=0.001) and a positive relationship was found between FTI and SQJ power (r=0.620, p=0.014), and SHBG and VO_2max(r=0.459, p=0.034). Moreover, a negative correlation was found between DHEA and maximal power produced in bench press (r=-0.578, p=0.024). No association was found between androgens and physical performance in the handball or soccer players. In conclusion no significant association between androgens and the physical performance was found when the female athletes were analyzed as a group. However, in the endurance-trained athletes, androgens appear to have a complicated relationship with performance, where VO_2max is negatively, and maximal SQJ power is positively correlated with androgens.

Key words: Androgens, female athletes, VO_2max, muscular power

INTRODUCTION

Androgen hormones, also regarded as androgens, are hormones that are often associated with steroids and masculinity. The power of androgens was early discovered in men, and the synthetic version of testosterone was found to be performance enhancing especially in power sports like weightlifting or 100m sprints already in the 1950s (Hartgens & Kuipers, 2004). The synthetic versions are often referred to as Androgenic-anabolic steroids (AAS). The use of AAS became common across sports and proven to be performance enhancing. AAS are now included in the illegal substance list and are forbidden by most sport federations around the world. Later on more research focused on the natural variation of androgens produced in the body and the topic has been of high interest for researches with male athletes.

Androgens are steroid hormones synthesized from cholesterol through steps catalyzed by enzymes. In males the main production is in the gonads, while in females they are synthesized in the adrenals and the ovaries (Vingren et al., 2008). The most important androgen hormone is testosterone, which is mainly produced in the Leydig cells located in men's testicles. Females do not have Leydig cells or any similar cells that can produce testosterone in such large amount and thus men produce approximately 10 times more testosterone than women (Vingren et al., 2008). A major part of the testosterone binds to albumin or sex hormone-binding globulin (SHBG) (Koch et al., 2011). A minor amount of the total testosterone circulates unbound in the blood, and is estimated as free testosterone index (FTI). FTI is often considered the biologically active fraction (Mazer, 2009). This theory has later been challenged by several studies, which have found specific receptors for SHBG in different cells (Hammes et al., 2005; Hilpert et al., 2001).

The amount of androgens in the blood is affected most importantly by genetics, but also by age, physical exercise, the hormonal differences throughout the menstrual cycle, and oral contraceptives (Balogh et al., 2000; Lebrun, 1994). Kumuru et. al (2005) found higher testosterone levels in regularly exercising young women compared to non-exercising ones, but did not detect any difference between groups whom perform different exercises. Exercise might not only affect the resting blood levels, it is also found acute up-regulation of androgens during and soon after exercise. There were not detected a significant difference between endurance and resistance workouts, except for DHEA which only increased significantly in response to resistance exercise (Copeland et al., 2002).

Research has found that higher natural levels of androgens are positively correlated to bone mineral density, body lean mass, speed, power and muscular strength, and lead to lower body fat levels compared with persons with normal values (Miller et al., 2006; Rickenlund et al., 2003). These results are mainly found in male studies (Storer et al., 2003; Crewther et al., 2009), but also in some female studies (Rickenlund et al., 2003). Moreover testosterone can also contribute to better performance trough enhanced recovery after exercise, which may allow the athlete to train harder (Bhasin, Woodhouse, & Storer, 2001).

In addition to this, androgens are found to simulate the production of erythropoietin (EPO) (Naets & Wittek, 1968), and is the main reason men are found to have higher hemoglobin concentrations compared to women (Enea et at., 2011). Hemoglobin levels is one of the limiting factors for VO_2max (Bassett & Howley, 2000) so the androgen stimulation of EPO production may play an important role in endurance performance for athletes. Contrary to this, a large-scale study performed on males 25-85 years did not detect an association between serum testosterone, FTI, SHBG and VO_2max, or maximal power output, even after adjustment for several potential confounding factors (Koch et al., 2011). Johnson et. al (1975) confirmed the lack of association between endurance performance and androgens in a study on young men and anabolic steroid supplementation.

Muscular strength, muscular power, force production, higher lean body mass, higher EPO production, and better recovery are all important factors in the performance levels of both male and female athletes. Of course several other factors such as work economy, cardiorespiratory variables, velocity of muscular movement, technique, and tactics also are important performance contributors in competition.

When it comes to female athletes, the researchers are few and conflicting. The physiological effects of androgens might be important for athletic performance in women as well as in men, in particular in endurance and power sports (Rickenlund et al., 2003). Although some studies have found some positive relationship between hyperandrogenity in females and physical performance (Rickenlund et al., 2003), most of the previous research is done on men and there is little agreement about how strong the relationship between androgens and physical performance are in female athletes. Therefore, to fully understand these relationships, further research is necessary. Additionally, it is interesting to study the androgen association with physical performance within different sport disciplines to see if these relationships vary and thereby may have different importance in different sports.

Aim of the study

This primary aim of this study was to describe the association between androgens, muscular power and VO_2 max in female athletes competing in cross-country skiing, handball and soccer.

Hypothesis

It was hypothesized that increased levels of circulating androgens in female athletes would associate with higher maximal oxygen uptake (VO_2 max) and greater muscular power.

METHOD

Subjects:

Fifty-seven participants, from the three sport groups; handball (n=17), soccer (n=24) and cross-country skiing (n=16) were involved in this study. The athletes included in this study had to be actively competing in their sport and were between the ages of 18 and 34 years old. Women who were breastfeeding, pregnant or unfit to participate for any reason were excluded from the study.

Table 1. Anthropometrics characteristics.

	Cross-country skiers (n=16) Mean ($\pm SD$)	**Handball players (n=17) Mean ($\pm SD$)**	**Soccer players (n=24) Mean ($\pm SD$)**
Age (years)	23 ($\pm$3)	20 ($\pm$3)	23 ($\pm$4)
Weight (kg)	59.3 ($\pm$5.4)	73.3 ($\pm$10.3)	62.7($\pm$6.7)
Height (cm)	170($\pm$5)	171 ($\pm$9)	167 ($\pm$5)
BMI (kg/m^2)	20.6 ($\pm$1.0)	25.6 ($\pm$2.7)	22.3 ($\pm$1.9)

* BMI=body weight(m)/body height$(kg)^2$

The participants were included on their own free will, and they all signed a declaration of consent. They had full access to withdraw from the study whenever they wanted to, without giving any reason for it. This is in accordance to the declaration of Helsinki. The results were anonymously analyzed and stored. The Regional Ethical Committee in Mid-Norway (REK) approved the project.

Study Design

This study had a cross-sectional study design, which began in September 2011 and lasted until November 2012. The data was collected by 5 master students at NTNU, and was conducted for various research aspects involving female athletes from different sports, such as physical performance, psychology, body composition and hormone levels. This MA-thesis concentrates on the physical performance and androgens.

Procedure

The subjects were recruited through direct contact with the teams before training sessions. They were informed about the content of the study both orally and by a written form and signed a written consent for participation in the study. The participants could withdraw from the study at any time.

Three master students conducted all the physical tests in the human movement laboratory at NTNU. The blood-samples were drawn at St.Olavs University Hospital Trondheim, NO. This was to ensure the consistency in the implementation method. The data was stored for offline analyses.

Anthropometric data

Body height was measured with a stadiometer standing without shoes, and calculated to the nearest whole centimeter. Weight was also measured without shoes and with lightweight training clothes, measured by a digital scale to the closest kg. The body mass index (BMI) was calculated as weight divided with height^2 (Kg/m^2.)

Physical performance tests

The subjects performed three different types of physical performance tests: first a squat jump test, followed by a bench press test, and finally a VO_2 max test.

Squat jump

The squat jump test was performed on Amti Biomechanics Force Platform (model bp6001200, Massachusetts, USA). The subjects stood on the platform with their feet approximately shoulder-width apart. They were instructed to go down into the position they found most comfortable to perform a maximal vertical jump, with hands placed on their hips. Then they jumped a maximal vertical jump without countermovement. The subjects completed the test when three correct jumps were registered determined by the force plate. Jump height, maximal force, time to peak power and rate of force development was calculated by the Biojump force plate (Biojump Program version 2.2, Oslo, Norway).

Bench-press

The subjects performed a standard bench-press test were it was used an Olympic barbell with free weights set up in a rack. The subjects were instructed to lay on the bench with both feet flat on the floor, with a 90 degrees knee angle to avoid them to use the legs and arching of the back. The index fingers were placed immediately perpendicular to the acromion process. Each subject performed a series of warm-up presses with just the bar as resistance in order for the subjects to become familiar to the movement. They were instructed to lower the bar to the chest vertically on the end of sternum, and pushed it back up in the original position with maximal voluntary effort. The resistance used in the test was 50% of the subject's body weight. If the subjects were not able to lift 50% of their own body weight, the resistance was reduced to a weight they could lift as close to 50% of the bodyweight as they were able to. The subjects each performed 3 recorded lifts and the average values from these three lifts were used. Power, force and velocity were measured using a linear encoder (Muscle Lab Erog Test Tecnology A/S, Langesund, Norway).

VO_2 max

A VO_2max test was performed in order to measure the athlete's maximal aerobic capacity. The subjects performed a 15 minute warm up at approximately 60% of self-reported maximal heart rate (HR) before the test started. The test was performed on a treadmill at an incline of 10.5% so most athletes will be able to reach their VO_2max. A lower incline can make running economy a limiting factor, and a higher incline could make the ballplayers feel that the steepness inhibits their potential, as they are used to completely horizontal fields. The starting velocity was individually estimated based on the speed that elicited 60% of their maximal HR. The maximal test increased 1 km/h every minute until exhaustion. Test duration was between 3 and 8 minutes, depending on when the subjects reached exhaustion. This is a standardized test procedure used to test VO_2max in cross-country skiers in Norway (Sandbakk et al., 2011). Two out of three criteria's had to be fulfilled for the test to be considered valid; the subjects should reach a plateau in VO_2 despite an increasing exercise intensity, blood lactate levels above 8 mmol*L^{-1}, and/or respiratory exchange ratio above 1.0.

Ventilatory variables were assessed employing open-circuit indirect calorimetry with an Oxygen Pro apparatus (Jaeger GmbH, Hoechberg, Germany). Prior to each test day, the VO_2 and VCO_2 were calibrated against commercial gas with known concentrations (5.0%CO_2 and 16.0%O_2) and ambient air. The expiratory flow meter was calibrated with a 3L volume syringe (SensorMedics, Yorba Linda, CA). The heart rate was monitored continuously by using a Polar Rs800. Lactate samples were taken directly after the participants reached exhaustion and measured by using Lactate Pro (LT-1710, ArkRay Inc, Kyoto, Japan).

Hormones

Hormones were measured in blood samples taken within 3 days after the physical tests. The blood samples were taken in the morning hours after fasting since the previous evening. The samples were analyzed by the laboratory at St. Olav`s Hospital, and the hormones used in this thesis were testosterone, androstendione, sex hormone binding globulin (SHBG), free testosterone index=T/SHBG *10 (FTI), dihydroepiandrostendione (DHEA), dihydroepiandrostendionesulfat (DHEAS) and anti muller hormone (AMH). These hormones were chosen because they were believed to be important in the androgenic processes in the human body, and thereby considered to be relevant for the study.

Statistics

All statistical analyzes was performed using IBM SPSS version 21. A correlation analyzes was performed separately in the three different activity groups between all the hormone and physical variables. T-tests were used to compare the groups.

The results viewed as Person correlation Coefficient (r) and statistically significance levels ($p<0,05$). Variables of particular interest were presented in scatterplots with regression lines. Further on regression analyzes were performed and presented in one table for each sport with β-value, p-value ($p<0,05$ regarded as statistical significant) and a 95% confidence interval (CI).

RESULTS

Subject characteristics

Cross-country skiers had a significantly higher VO_2max compared with soccer and handball players (all $p<0.000$). Soccer players had a higher VO_2max than handball players ($p=0.003$). However, during squat jumps both handball and soccer players produced higher maximal force (N) than the cross-country skiers ($p<0.000$ and $p=0.013$ respectively). There were no statistical differences in jump height between the three groups, and no difference in the force output in the bench press test. These parameters are presented in Table 2. The BMI was lower in cross-country skiers than soccer players ($p=0.02$) and handball players ($p<0.000$). Handball players had higher BMI than the two other groups ($p<0.000$) (Table 1.). The three groups did not differ significantly in any of the hormone levels. Hormone values are presented in Table 3.

Table 2. Results for VO_2max, end speed on the VO_2max test, maximal force produced in Squat jumps, Squatjump height, average force produced in benchpress, weight resistance used in benchpress presented as mean and standard derivation (SD)

	Cross country skiers (n=16) Mean ($\pm SD$)	Handball players (n=17) mean($\pm SD$)	Soccer players (n=24) mean ($\pm SD$)
VO_2max ($mL \bullet Kg^{-1} \bullet min^{-1}$)	64.2 (±3.9)	49.3 (±6.3)	54.7 (±4.6)
End speed (km/h)	13.0 (±0.8)	10.6 (±1.2)	11.2 (±0.9)**
Squat jump force (N)	1158.1 (±175.0)	1465.0 (±231.8)	1305.3 (±163.2)
Squat jump height (cm)	25.1 (±4.5)	25.5 (±4.9)	28.6 (±4.9)
Bench press average force (N)	394.6 (±138.6)	407.6 (±49.0)*	463.1 (±187.2)
Bench press (kg)	29.9 (±2.7)	38.2 (±4.9)*	31.6 (±3.6)
Benchpress (W)	210.9 (±98.4)	205.3 (±74.9)*	206.3 (99.5)
		* n=11	**n=19

Table 3. Hormone levels presented as mean and standard derivation (SD)

	Cross country skiers (n=16) Mean ($\pm SD$)	**Handball players (n=17) mean($\pm SD$)**	**Soccer players (n=24) mean ($\pm SD$)**
SHBG (nmol/L)	88.82 ($\pm$38.73)	147.10 ($\pm$121.01)	100.26 ($\pm$59.45)
DHEAS (nmol/L)	2.92 ($\pm$0.88)	3.71 ($\pm$1.19)	3.81 ($\pm$1.60)
AMH (nmol/L)	22.01 ($\pm$13.35)	24.46 ($\pm$15.75)	24.62 ($\pm$24.47)
DHEA (nmol/L)	46.85 ($\pm$27.72)	42.89 ($\pm$16.94)	43.19 ($\pm$20.31)
Testosterone (nmol/L)	1.71 ($\pm$0.51)	1.88 ($\pm$0.86)	2.00 ($\pm$0.74)
FTI ((testosterone nmol/L)/(SHBG nmol/L)*10)	0.26 ($\pm$0.22)	0.24 ($\pm$0.86)	0.28 ($\pm$0.21)
Androsentione (nmol/L)	9.96 ($\pm$3.42)	11.53 ($\pm$6.34)	9.61 ($\pm$2.63)

There were no significant differences in hormone levels between the groups

Correlations analysis

Correlation analyzes between the six different androgens and the different physical test results (force in squat jump, VO_2max and average force in bench press, BMI and weight,) were performed in all three-sport groups individually and in total.

No association between the tested hormones and the physical performance tests were found in the groups of handball players or soccer players. Handball players´ VO_2max was negatively associated with BMI (r=-0.704, p<0.000), but no hormones showed any association with weight and/or BMI in either of the two groups.

Cross-country skiers' VO_2max and FTI have a negative association (r=-0.760, p=0.001), In the analyzes of the cross-country skiers a positive relationship was found between FTI and mean bodyweight(r=0.588,p=0.031), and also with FTI and maximal power produced in the squat jump(r=0.620, p=0.014). VO_2max and SHBG had a positive association (r=0.459, p=0.034). Finally, DHEA and force produced in bench press were found to have a negative relationship (r=-0.578, p=0.024).

There were no significant association between any of the androgens and physical parameters when the group was analyzed as one. However, when analyzed as a total, VO_2max had a negative association with BMI (r=-0.733, p<0.000). Furthermore BMI had a positive correlation with max power in the squat jump test.

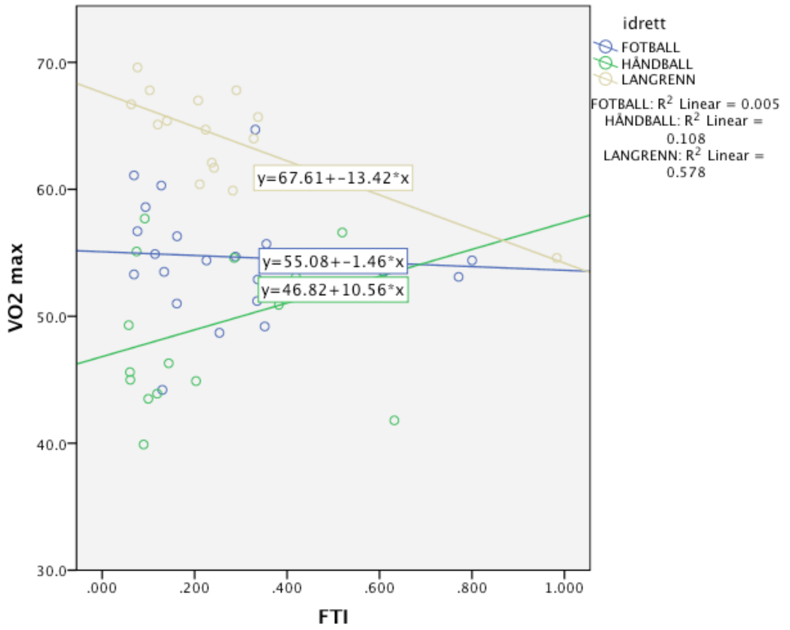

Fig 1. The association between FTI against VO_2max in the three different groups, The solid green line represents the association between VO_2max against FTI in handball players and the solid blue line represents the same relationship for soccer players. Neither of these were significant. The solid yellow line represents the FTI against VO_2max in cross-country skiers, and this relationship was significant. The dots represent the individuals, where green dots are handball players, blue dots are soccer players, and yellow dots are cross-country skiers.

Linear regression analyzes were performed to see if the androgen hormone levels could predict the physical fitness values VO_2max, max force in SQJ and average force in bench press. The regression analyzes was performed separately in the three different groups.

Table 4. Linear regression in cross-country skiers compares VO2max, maximum force (N) produced in squat jump (SQJ) and average force (N) produced in bench press and androgens presented as β, p-value and 95% confidence interval

Variables	β	p-value	CI interval
VO2max			
FTI	-0.760	0.001*	(-20.28−-6.55)
SHBG	0.549	0.039*	(0.01−0.11)
DHEAS	-0.151	0.592	(-3.29−1.95)
AMH	0.107	0.704	(-0.14− 0.20)
DHEA	-0.040	0.886	(-0.09−0.08)
Testosterone	-0.395	0.145	(-7.24−1.19)
Androstendione	-0.506	0.054	(-1.16−0.01)
Max force SQJ			
FTI	0.620	0.014*	(119.33−870.05)
SHBG	-0.318	0.248	(-4.01−1.13)
DHEAS	0.121	0.669	(-94.85−143.07)
AMH	-0.111	0.693	(-9.27−6.35)
DHEA	-0.209	0.456	(-5.02−2.38)
Testosterone	0.220	0.430	(-126.14−278.61)
Androstendione	0.374	0.170	(-9.34−47.64)
AVG force benchpress			
FTI	-0.188	0.502	(-490.67−253.12)
SHBG	0.094	0.739	(-1.80−2.47)
DHEAS	-0.499	0.058	(-161.19−3.26)
AMH	-0.202	0.471	(-8.19−3.99)
DHEA	-0.578	0.024[a]	(-5.33−0.44)
Testosterone	-0.227	0.416	(-22.11−97.82)
Androstendione	-0.458	0.086	(-40.22−3.00)

*: P-value is significant p<0.05 level

Table5. Linear regression in Soccer players between VO2max, maximum force(N) produced in squat jump(SQJ) and average force(N) produced in bench press and circulating androgens presented as β, p-value and 95% confidence interval

Variables	β	p-value	CI interval
VO2max			
FTI	-0.068	0.763	(-11.43−8.51)
SHBG	-0.102	0.652	(-0.03−0.04)
DHEAS	-0.070	0.755	(-1.53−1.13)
AMH	-0.081	0.719	(0.10−0.07)
DHEA	0.156	0.289	(-0.07−0.14)
testosterone	-0.162	0.427	(-3.87−1.86)
androstendione	-0.062	0.785	(-0.92−0.70)
Max force SQJ			
FTI	0.010	0.964	(-348.47−363.89)
SHBG	-0.016	0.943	(-1.33−1.24)
DHEAS	0.220	0.324	(-23.94−68.91)
AMH	-0.252	0.259	(-4.69−1.33)
DHEA	0.163	0.469	(-2.39−5.01)
testosterone	0.195	0.384	(-58.11−144.58)
androstendione	-0.074	0.743	(-33.40−24.23)
AVG force benchpress			
FTI	0.266	0.231	(-160.79−626.98)
SHBG	-0.158	0.481	(-1.95−0.95)
DHEAS	0.151	0.503	(-36.34−71.62)
AMH	-0.162	0.473	(-4.755−2.29)
DHEA	0.090	0.689	(-3.45−5.11)
Testosterone	0.217	0.332	(-60.62−170.83)
Androstendion	0.200	0.373	(-18.29−46.67)

Table 6. Linear regression in handball players between VO2max, maximum force (N) produced in Squat jump (SQJ) and average force (N) produced in bench press and circulating androgens presented as β, p-value and 95% confidence interval

Variables	β	p-value	CI interval
VO2max			
FTI	0.329	0.213	(-6.79−27.91)
SHBG	-0.188	0.486	(-0.04−0.02)
DHEAS	-0.084	0.757	(-3.46−2.57)
AMH	-0.138	0.609	(-0.28−0.17)
DHEA	-0.115	0.673	(-0.26−0.17)
Testosterone	0.228	0.369	(-2.43−5.79)
Androstendione	0.294	0.270	(-0.26−0.84)
Max force SQJ			
FTI	-0.301	0.257	(-993.60−287.80)
SHBG	0.227	0.398	(-0.63−1.50)
DHEAS	-0.193	0.475	(-145.85−71.46)
AMH	-0.027	0.920	(-8.83−8.03)
DHEA	-0.237	0.376	(-10.87−4.37)
Testosterone	-0.214	0.426	(-208.48−93.25)
Androstendione	-0.338	0.200	(-32.12−7.35)
AVG force benchpress			
FTI	-0.274	0.414	(-273.34−123.32)
SHBG	0.018	0.958	(-0.28−0.29)
DHEAS	-0.175	0.608	(-36.25−22.44)
AMH	0.203	0.549	(-1.46−2.58)
DHEA	0.195	0.565	(-1.74−2.98)
Testosterone	-0.077	0.821	(-77.35−62.94)
Androstendione	-0.134	0.695	(-11.62−8.09)

DISCUSSION

We hypothesized that a positive association between the performance tests and the androgens would be observed. However, no statistical significant relationships were found when the group was analyzed in one pool, or in the handball player or soccer players analyzed separately. The only group with any significant relationships was found in the cross-country skiers, were a negative correlation between FTI and VO_2max, and between DHEA and upper body power. Furthermore a positive association was found between SHBG and VO_2max, and the relationship between the squat jump power and FTI. Hormone levels did not differ among the groups even though the androgen relationships with physical performance did.

Androgens and VO_2max

The results showed that serum FTI in the blood is negatively correlated to the VO_2max in cross-country skiers. This is interesting, considering that androgens are found to increase the EPO production, and thereby lead to higher hemoglobin levels. (Hartgens & Kuipers, 2004) Hemoglobin levels are an important limiting factor for the VO_2max (Bassett & Howley, 2000). However, many of the studies that have found a relationship between androgens and Erythropoietin production, focus on testosterone and men (Krabbe et al., 1978; Naets & Wittek, 1968). As mentioned previously, the natural levels of testosterone is ten times lower in females compared to men, thus this mechanism may not be as present in females. A study performed by Ferrucci et. al (2006) on the total and bioavailable testosterone levels on older men and woman in the relation to hemoglobin levels found that in men there seemed to be a linear relationship between both total testosterone levels, bioavailable levels of testosterone, and the hemoglobin levels (Ferrucci et al., 2006). However, in women, higher levels of bioavailable testosterone (Free and albumin bound, but not SHBG bound) were found to be linearly associated to higher hemoglobin levels. It was concluded that testosterone affects the risk of developing anemia, however it was also shown that both women with high and low levels of androgens do not have anemia. This may indicate that androgens and hemoglobin in fact have a positive relationship, but also that the relationship is very individual. Moreover, the relation between androgens and lower risk of anemia found by Ferrucci et. al (2006) may not translate into that high levels of androgens directly lead to high hemoglobin levels even though they seem to be positively related.

High hemoglobin levels are important for the O_2 binding affinity of the blood, and the importance of this factor is shown for example by the endurance enhanced effect of blood doping. Mind you, this is not the only limiting factor for VO_2max (Bassett & Howley, 2000). VO_2max is limited by the ability of the cardiorespiratory system to provide the skeletal muscles with oxygen. In addition to EPO production and hemoglobin levels, other factors as cardiac output, pulmonary diffusing capacity and in some extent peripheral factors as capillary density and mitochondrial enzyme levels are important factors that influence the VO_2max capacity (Bassett & Howley, 2000). Training is known to increase VO_2max primarily due to increased maximal cardiac output, and also some of the peripheral limiting factors can be improved by training. This implies that training can lead to a higher VO_2max even if the hormone status and EPO production is unchanged (Blomqvist & Saltin, 1983).

The results found by Bassett & Howley (2000) may indicate that even if testosterone levels have a positive relationship to hemoglobin, other parameters for VO_2max might play a greater role for endurance capacity, and mask the effect of testosterone on hemoglobin levels in this study. Moreover it could be that FTI has a negative association with some of the other parameters that determinate VO_2max. Unfortunately we did not have access to the hemoglobin levels of the subjects in this project. The lack of association between androgens and endurance performance in the group as a total may due that the physiological variations of androgens was not big enough between the sport groups to be able to separate the different performance levels. This explanation was earlier used by Koch et. al (2011) in a study on VO_2max and androgens in a large scale populations on men. They found no association between total testosterone and VO_2max or SHBG and VO_2max and suggested that a possible explanation to the lack of association could be that levels of testosterone did not vary enough across the different groups to clearly mark the difference in exercise capacity (Koch et al., 2011).

In the present study, there was no significant correlation between total testosterone and endurance performance. However, in the group of cross-country skiers a moderate linear association between the SHBG and VO2max was seen. Earlier studies claim that SHBG is not considered as biologically active (Damassa et al., 1991), however recent research have uncovered endocytic pathways for cell-specific uptake of protein, such as albumin and SHBG (Hammes et al., 2005), which could be an explanation for the positive relationship between SHBG and endurance performance in the cross-country skiers. Since SHBG is a testosterone carrier (Koch et al., 2011), this relationship might also due because SHBG may be an important contributor to the acute elevation of testosterone seen during physical exercise.

The negative relationship between FTI and VO_2max is an interesting and somewhat surprising finding. Since this is a cross sectional study it is impossible to draw a concrete conclusion about the causal relationships thus this relationship should be investigated further to understand the true mechanisms behind this result.

Androgens and muscular power

Further on, the present study found that FTI related positively to squat jump power in cross-country skiers. This was also found by Cardinale & Stone (2006) when they did a very similar study on testosterone and vertical jumping performance. They studied male and female athletes participating on national teams and in the European cup in handball, soccer, volleyball, and sprints. The athletes were tested on a resistive platform and blood samples were collected to find the serum testosterone levels. They found a significant positive relationship between basal testosterone levels and vertical jump in both genders; concluding that testosterone plays a role not only in muscle remodeling, but also in the neuromuscular function. When comparing males and females, men had a higher correlation between testosterone and vertical jump compared with the women, which may be caused by gender differences in skeletal muscle sensibility to testosterone. It might also be because other androgens and hormones may play an important role in woman, as they only had 9,5% of the testosterone found in the male athletes (Cardinale & Stone, 2006). Indications that testosterone influences power and speed was also found in a project on testosterone levels and vertical jumping performance (Bosco et al., 1996), and serum levels and sprinting in soccer players (Bosco et al., 1996). The previous research on this area seems to confirm the findings in the present study.

DHEA did not have any relationship with physical performance when the group was analyzed all together. Nor had it any significant association in the handball or soccer players. But when analyzing cross-country skiers, a slight negative correlation to the bench press test was found. The results in earlier research considering DHEA and physical performance vary a lot. Some studies have found no relationships directly linking DHEA and muscular strength in females (Morales et al., 1998). However, others have found an enhanced effect of muscular strength training on elderly men and woman when training with DHEA supplementation (Villareal & Holloszy, 2006). Though such inconsistencies are found in the literature, this study result may indicate that DHEA is negatively associated with power in the upper body in endurance-trained athletes.

Training status and performance level

The differences between sports disciplines considering VO_2max and measured jump force might be due to specific training within the three groups, where jumping is a more central part in handball and soccer than in cross-country skiing. Handball- and soccer players do most of their training on flat floor or soccer fields while cross-country skiers would also naturally focus more on endurance training in tough terrain. The differences in the physical parameters between the three groups are most likely due to different training status. The effects of chronic exercise are inconclusive regarding the circulating androgen concentrations in females (Enea et al., 2011). Kumru et. al (2005) studied 94 young women where the subjects where divided into three groups with different training status. The study was designed to examine, among other things, how physical exercise affects androgen hormone concentrations. They found significantly higher testosterone levels in the exercising groups. However they did not find any significant differences between the different types of training. Since this is a cross-sectional study, it is impossible to comment if the training status may influence the hormone levels in these athletes. To find the cause effect, another study design is needed.

None of the correlation analyzes where found to be significant for the handball players or soccer players. A possible reason for this may be because the physical performance level within these groups varied more in comparison to the cross-country skiers. In the handball and soccer groups, some of the athletes might have their strengths in factors like technique with the ball, speed and having a good mind for the game, which makes the physical performance tested done in the laboratory less predicting for the game performance. This mean that the athletes in these two sports can differ more within the groups in the physical test although they can perform at the same level in games. The cross-country skiers were perhaps representative of a more homogenous trained group of athletes considering physical parameters measured in the laboratory. As training has very high influence on physical capacity, the athletes should be trained similar to be able to compare the androgen impact on their performance. The more homogenous training might be the reason relationships were found in the cross-country skiers. The athletes represented a pretty high athletic level in all three groups. The handball players belonged to a 1.st division team, with a few players from 3rd division. They were included because most of them were aspiring to play for the 1. division team the coming season and were considered to be on a high level. The soccer players represented a team from 1.st division in Norway. The cross-country skiing group consisted of athletes who all performed in the top 15 national, and at least top 50 international.

The results in this study are likely representative for athletes on these levels in all three groups. They imply that androgens have a more important role for cross-country skiers than for handball and soccer players.

Acute up regulation during and after physical activity and baseline levels

Researchers discuss if higher resting levels of androgens in female athletes are due to prolonged endurance and resistance exercise in addition to individual genetics, or decided by genetics alone (Keizer et al., 1989; Kraemer et al., 2001). Some have found no difference in hormone levels after resistance or endurance exercise (Hickson et al., 1994; Alen et al., 1988). Others have observed that resistance training leads to a greater increase in androgen levels than endurance exercise. This indicates that the resting level of androgens may be higher in resistance-trained females compared with endurance-trained females (Hakkinen et al., 1988; Staron et al., 1994). The results in this study did not find a clear difference in baseline hormone levels between the three groups we tested. Handball, soccer and cross-country skiing are all complex sports that require various types of training to succeed. In that way it is natural that the androgen hormone levels between the groups did not differ significantly even if different types of exercise can mediate androgen production differently.

Hickson et. al (1994) found that the resting levels of steroids did not elevate after a training period of 8 weeks in women but that the acute levels of androgens were increased during and immediately after exercise prior the intervention. Moreover plasma concentrations of total testosterone, free testosterone and androstenedione have been observed to increase after prolonged exercise both in laboratory conditions and field contexts. A acute increase of DHEA and DHEAS concentrations are also observed during exercise in females (Baker et al., 1982). This may indicate that acute up regulation of androgens can be important when studying the androgen relationships with exercise and physical performance. Because the hormone samples used in this study are taken at least 12 hours after the physical test where performed, the difference in acute up regulations would not be registered in the present study. The relationship between the acute up regulation of androgens and physical performance may be stronger than the associations between circulating androgen hormones and performance we found in this study. Further research is needed to be able to make some conclusions in this area.

Androgens, menstrual cycle and oral contraceptives

Different factors, such as age, physical exercise, the hormonal differences throughout the menstrual cycle, and oral contraceptives may affect the amount of androgens in the blood (Balogh et al., 2000; Lebrun, 1994). Researchers disagree about how much the menstrual cycle affects physical performance (Oosthuyse & Bosch, 2010), and the impact oral contraceptives has on physical performance is also widely discussed (Balogh et al., 2000; Lebrun, 1994; Devries et al., 2006). But they agree that these factors have influence on the hormonal status, and may further also affect the relationships between physical performance and circulating androgen hormones in this study. Unfortunately it was not possible to time the testing with all the athletes so that they would be in the same place in the menstrual cycle. It was neither registered if the participants used oral contraceptives. On the other hand, if the hormonal status has a direct effect on the performance, this relationship would occur regardless of whether it is because of the menstrual cycle or hormone contraceptives, or if the hormonal status is naturally higher in some individuals all the time.

Summary

When the athletes were analyzed together in one pool, none of the relationships between androgens and the measured physical parameters were significant. When the groups were analyzed divided in groups, no significant associations were found in the handball players or soccer players either. Further it was found some associations between androgens and physical performance in cross-country skiers. FTI seems to have a positive association with muscular power production, and a negative association with VO_2max. Moreover, DHEA is negatively related with power in bench press. This means that androgens not necessarily have a positive impact on all types of physical performance in female athletes. The negative associations were surprising considering that several earlier studies have found positive relationships between androgens and physical performance (Aizawa et al., 2011; Rickenlund et al., 2003). The reasons behind these interesting findings need further research. These future studies should contain acute up regulation of androgens, look into how training status affects the androgen levels, and try to examine what mechanisms lies behind the relationships found in the present study.

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APPENDICES

Appendix 1. Approval form the Regional Ethics Committee

Below is a copy of the approval of the project from Regional Ethics Committee, Mid-Norway, received October 2011.

2011/1460 Konkurranseinstinkt hos toppidrettsutøvere

Prosjektleder: Professor Sven Magnus Carlsen

Forskningsansvarlig: St. Olavs Hospital, Medisinsk klinikk v/klinikksjef Eiliv Brenna

Med hjemmel i lov om behandling av etikk og redelighet i forskning § 4 og helseforskningsloven (hfl.) § 10 har Regional komité for medisinsk og helsefaglig forskningsetikk Midt-Norge vurdert prosjektet i sitt møte 21. oktober 2011. Komiteen viser til prosjektprotokoll, målsetting og plan for gjennomføring, og finner at prosjektet har et forsvarlig opplegg som kan gjennomføres under henvisning til evt. merknader og vilkår for godkjenning, jf. hfl. § 5.

Merknader og vilkår:

- -Komiteen ber om at grunnlagsdata ikke blir anonymisert, slettet eller destruert, men blir oppbevart på en betryggende måte i minimum 5 år etter prosjektslutt av kontrollhensyn. Instanser som kan tenkes å kontrollere grunnlagsmaterialet er f.eks. forskningsansvarlige, Uredelighetsutvalget for forskning og Helsetilsynet.
- -Komiteen minner om at de aller fleste kliniske studier skal registreres i det offentlig tilgjengelige registeret www.clinicaltrials.gov. Prosjektleder er ansvarlig for å avgjøre om forskningsstudien omfattes av kravet til registrering.
- -Prosjektleder skal sende sluttmelding til den regionale komiteen for medisinsk og helsefaglig forskningsetikk når forskningsprosjektet avsluttes. I sluttmeldingen skal resultatene presenteres på en objektiv og etterrettelig måte, som sikrer at både positive og negative funn fremgår, jf. hfl. § 12.

Vedtak

"Regional komité for medisinsk og helsefaglig forskningsetikk, Midt-Norge godkjenner at prosjektet gjennomføres med de vilkår som er gitt."

Vennlig hilsen

Sven Erik Gisvold

Professor, dr.med.

Leder, REK midt

Arild Hals

Appendix 2. Information letter

Forespørsel om deltagelse i forskningsprosjekt:

"The Female Competitiveness Study" – er det sammenheng mellom hormonnivåer og konkurranseinstinkt hos kvinnelige toppidrettsutøvere?

Bakgrunn og hensikt

Toppidrettsutøvere presser sin fysiske og psykiske kapasitet til det ytterste. De fysiske og psykiske aspektene og sammenhengen med hormonnivåer har vært studert tidligere, spesielt hos menn. Sammenhengen mellom hormonnivåer, fysisk kapasitet og mentale faktorer hos kvinner er derimot lite studert.

Nivået av androgene hormoner (hormoner med testosteronvirkning) og spesielt testosteron er noe forhøyet hos kvinner med polycystisk ovarialsyndrom (PCOS). Kvinner med PCOS utgjør 10-15 % av kvinner i fruktbar alder, de synes å ha høyere konkurranseinstinkt og delta mer i idrett enn kvinner uten PCOS. Det synes også å være en svak sammenheng mellom PCOS, humørsvingninger og spiseadferd. Vi tror mye av dette kan ha sammenheng med de noe økte nivåene av hormoner med testosteronvirkning og at kvinner med økte testosteronnivå i spesiell grad trekkes mot idrett generelt og toppidrett spesielt.

Vi henvender oss til deg fordi du er en kvinnelig toppidrettsutøver for å be om ditt samtykke til deltakelse i dette forskningsprosjektet. Formålet med studien er å få økt kunnskap om sammenhengen mellom hormonnivåer hos kvinnelige toppidrettsutøvere og konkurranseinstinkt, muskelmasse, fysisk kapasitet, beintetthet, mentale forhold inkludert seksuell orientering og forekomsten av PCOS. Studien er et samarbeidsprosjekt mellom Avdeling for endokrinologi, St. Olavs hospital, Institutt for Bevegelsesvitenskap og Psykologisk Institutt, Norges teknisk- naturvitenskaplige universitet (NTNU) og Olympiatoppen Midt-Norge. Denne forespørselen går til toppidrettskvinner mellom 18 og 40.

Hva innebærer deltagelse i studien?

Den enkelte deltager vil få utført en DEXA-scan og taking av fastende blodprøve ved Avdeling for endokrinologi, St. Olavs hospital. Ved DEXA-scan ligger man stille i truse på ryggen i 10 minutter og det hele er helt smertefritt. Ved denne undersøkelsen bestemmes fettmasse, muskelmasse, beinmasse og beintetthet. I tillegg besvares spørreskjema om konkurranseinstinkt, spenningssøking, spiseadferd, mental helse og seksuell orientering. Vi vil også registrere resultatene fra fysiske tester (VO_2 max, laktat, maksimal muskelkraft ved spensthopp og benkpress). Medisinbruk, spesielt hormonpreparater (p-pille, p-stav, p-sprøyte, hormonspiral etc.) vil bli registrert.

I tillegg håper vi å kunne tilby en gynekologisk undersøkelse for deltagerne i studien.

Mulige fordeler og ulemper

Som deltager har du mulighet til å bidra til ny kunnskap om sammenhengen mellom forhold knyttet til idrettsprestasjoner og toppidrettskvinners helse. Deltagelse i prosjektet medfører testing og analyse av blant annet beintetthet og muskelmasse. Lav beintetthet og menstruasjonsforstyrrelser kan være et problem hos kvinner som trener mye. Dersom dette påvises kan fagpersonene i prosjektgruppen vurdere spesielle tiltak dersom du ønsker det.

Opplever du noen av spørsmålene som ubehagelige er det greit å unnlate å besvare dem. Data vil uansett ikke kunne spores tilbake til enkeltpersoner etter at de er registrert i en database. Vi har dessverre ikke anledning til å gi deltagerne økonomisk kompensasjon så deltagere i prosjektet må selv dekke eventuelle reiseutgifter.

Den fysiske testingen vil bli gjennomført etter standard prosedyrer for slik fysiologisk testing og risikoen for at noe kan skje er minimal. VO_2 max kan oppleves som anstrengende, men gi nyttig informasjon som kan brukes til videre treningsplanlegging. Prestasjonstester vil også være standard og gjennomføres i tråd med trening du som utøver gjennomfører til daglig. Den eneste reelle forskjellen fra den daglige trening og testing er at dataene fra testene vil bli systematisert og lagret for brukt i prosjektet.

Viktig! Dine forberedelser

Blodprøvene vil bli tatt om morgenen, og du må faste 8 timer (dvs. fra midnatt) i forkant (inkludert røyk/snus-avhold). Dersom du er veldig tørst kan du evt. drikke et halvt glass vann morgenen før prøvene tas.

Hva skjer med testene og informasjonen om deg?

Dine resultater fra undersøkelsene vil bli behandlet i ikke identifiserbar form, dvs. uten navn, fødselsnummer eller andre identifiserende opplysninger. En kode knytter deg til dine opplysninger og prøver, gjennom en navneliste. Denne koden oppbevares uavhengig av selve databasen med alle opplysningene fra studien. Det er kun autorisert helsepersonell knyttet til prosjektet som har adgang til navnelisten og som eventuelt kan finne tilbake til deg. Det vil ikke være mulig å identifisere deg i resultatene av studien når disse publiseres. Når prosjektet er avsluttet vil også koden som knytter deg til enkeltdata i databasen slettes.

Dersom du ønsker det kan vi gi deg tilbakemelding på testresultatene (som for eksempel kroppssammensetning, VO_2 max, styrketestene, og evt. gynekologisk undersøkelse). Vi kan eventuelt også informere om eventuelle andre helseproblemer vi måtte påvise ved de undersøkelsene du gjennomgår. Dette vil foregå ved studiemedarbeiderne (kroppssammensetning, VO_2 max, styrketestene) eller lege (gynekologisk undersøkelse, beintetthet, evt. andre forhold). Olympiatoppen, trenere eller andre vil ikke på noe tidspunkt få tilgang til informasjon om enkeltpersoner utover resultater fra de fysiske testene Olympiatoppen får tilgang på slik de gjør ved tilsvarende rutinetesting av utøvere.

Studien er vurdert og godkjent av Regional komité for medisinsk forskningsetikk, Midt-Norge og vil bli gjennomført etter de regler og retningslinjer som er nedfelt i Helsinkideklarasjonen. Når studien er avsluttet vil resultatene bli publisert i et engelskspråklig internasjonalt medisinsk tidsskrift.

Frivillig deltakelse

Studien er frivillig, du kan på hvilken som helst tidspunkt trekke deg uten nærmere begrunnelse eller uten at det får noen negative konsekvenser for deg. Dette gjelder selvfølgelig også videre oppfølging fra Olympiatoppen. Dersom du trekker deg fra studien har du rett til innsyn i data registrert om deg. Du kan også trekke tilbake samtykket. Da vil alle innsamlede opplysninger om deg bli slettet og frosne blodprøver vil bli destruert med mindre opplysningene allerede er inngått i analyser eller brukt i vitenskaplige publikasjoner.

Vi ber også om tillatelse til eventuelt å kontakte deg senere for oppfølgning. Dette er kun en forespørsel om vi får lov å ta kontakt med deg senere og ikke noe løfte fra deg om at du vil stille opp. Dette er ingen forutsetning for å delta i studien. Hvis du samtykker i å delta i studien må du undertegne en samtykkeerklæring lik den som er vedlagt før du deltar. Personopplysninger som knytter deg til data vil bli oppbevart til utgangen av 2014 og deretter slettet.

Personvern

Opplysninger som registreres om deg er:

- €Helseopplysninger som du selv gir oss
- €Opplysninger om din aktuelle medisinbruk
- €Resultater av blodprøveanalyser som tas
- €Opplysninger om de tester og undersøkelser du gjennomgår
- €Svar på de spørreskjema du besvarer
- €Enkle kliniske data (høyde, vekt, blodtrykk etc.) Opplysningene legges inn i en database ved Enhet for anvendt klinisk forskning, NTNU i avidentifisert form, dvs. ikke med ditt navn eller fødselsnummer men kun med ditt deltagernummer. Alt personell som er involvert i studien og behandlingen av innsamlede data har taushetsplikt. Representanter for kontrollmyndigheter kan få utlevert studieopplysninger og gis innsyn i relevante deler av din journal. Dette er lovpålagt. Formålet er å kontrollere at studieopplysningene stemmer overens med tilsvarende opplysninger i din journal. Alle som får innsyn i informasjon om deg har taushetsplikt.

Forskningsbiobank

Blodprøvene som blir tatt og informasjonen utledet av dette materialet vil bli lagret i en forskningsbiobank som professor Sven M. Carlsen er ansvarlig for. De vil bli lagret i ikke personidentifiserbar stand, dvs. bare identifisert med deltagernummer.

Utlevering av materiale og opplysninger til andre

Hvis du sier ja til å delta i studien, gir du også ditt samtykke til at prøver og avidentifiserte opplysninger kan utleveres til våre samarbeidspartnere i forskning.

Innsynsrett og oppbevaring av materiale

Hvis du sier ja til å delta i studien, har du rett til å få innsyn i hvilke opplysninger som er registrert om deg. Du har videre rett til å få korrigert eventuelle feil i de opplysningene vi har registrert. Dersom du trekker deg fra studien, vil det ikke samles inn flere opplysninger eller mer materiale. Opplysninger som allerede er innsamlet fra deg vil ikke bli slettet.

Finansiering

Studien og biobanken er søkt finansiert av forskningsmidler fra Olympiatoppen og forskningsmidler som professor Sven M. Carlsen har innestående ved Unimed Innovation. Sponsor (ansvarlig myndighet for studien) er Institutt for kreftforskning og molekylærmedisin, NTNU.

Forsikring

Du er forsikret gjennom Pasientskadeerstatningsordningen.

Med vennlig hilsen ___________________________

Sven M. Carlsen Professor dr. med. Prosjektleder

Prosjektgruppe:

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Appendix 3. Declaration of consent

SAMTYKKEERKLÆRING

For deltakeren:

Jeg bekrefter med dette at jeg har fått den informasjon jeg ønsker om og er villig til å delta i"The Female Competitiveness Study". Jeg vet at jeg uten nærmere begrunnelse kan trekkemed fra studien på et hvert tidspunkt dersom jeg skulle ønske det uten at det vil hakonsekvenser for meg. Jeg er klar over at de innsamlede data brukes utelukkende til forskningog eventuell egen nytte ved økt kunnskap om meg selv. Jeg samtykker i å delta i prosjektet som innebærer følgende:

- Testing av fysisk kapasitet (maksimal muskelkraft og VO2max)

- Dexa-scan av kroppssammensetning

- Blodprøvetaking for hormonanalyser

- Spørreskjema angående konkurranseinstinkt, spenningssøking, spisevaner, mental

helse og seksuell orientering

- Gynekologisk undersøkelse (ingen betingelse for å delta i resten av studien)

- Enkel klinisk undersøkelse (høyde, vekt, blodtrykk etc.)

- Fotokopi av hendene

Dersom du godkjenner at vi kontakter deg for eventuell oppfølgende forskning på et seneretidspunkt, vennligst kryss av her: ☐

Dersom du ønsker å bli kontaktet dersom blodprøver eller andre av undersøkelsene gir mistanke om spesielle medisinske problemer hos deg, vennligst kryss av her: ☐

Sted:____________________________ Dato:___/___- 2012

Navn:____

(Deltakers fulle navn med BLOKKBOKSTAVER)

____

Deltagers underskrift

Jeg bekrefter med dette at deltageren har fått muntlig og skriftlig informasjon om studien, harfått svar på de muntlige spørsmål hun hadde og har underskrevet på dennedeltagerformasjonen:

Sted: ___________________________ Dato:___/___- 2012

Studiemedarbeider: ____

Ansvarlige lege for undersøkelsen: Sven M. Carlsen, Professor, Enhet for anvendt klinisk forskning, NTNU, Overlege, Avdeling for endokrinologi, St. Olavs hospital.