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**Testosterone replacement therapy of opioid-induced male hypogonadism improved body composition but not pain perception**

*a double-blind, randomized, and placebo-controlled trial*

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1 **Testosterone replacement therapy of opioid induced male hypogonadism**  
2 **improved body composition but not pain perception.**  
3 **A double-blind, randomized and placebo-controlled trial**

4

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20 **Clinical trial registration number:** [www.clinicaltrials.gov](http://www.clinicaltrials.gov), NCT02433730

21 **Key words:** testosterone, non-cancer pain, relative hypogonadism

22 **Short title:** testosterone substitution for opioid treated men

23 **Word count:** 4500

24

25 **Abstract**

26 **Background:** Hypogonadism is prevalent during opioid treatment, but the effect of testosterone replacement  
27 treatment (TRT) on body composition, pain perception, and adrenal function is unclear.

28 **Purpose:** To measure changes in body composition, pain perception, quality of life and adrenal function  
29 after TRT or placebo in opioid treated men with chronic non-malignant pain.

30 **Methods:** Double-blind, placebo-controlled study in 41 men (>18 years) with total testosterone <12 nmol/L  
31 were randomized to 24 weeks TRT (Testosterone undecanoate injection 3 times/6 months, n=20) or placebo  
32 (placebo-injections, n=21).

33 **Outcomes:** Body composition (lean body mass and fat mass assessed by DXA), clinical pain intensity  
34 (numerical rating scale), and experimental pain perception (quantitative sensory assessment), quality of life  
35 (SF36), and adrenocorticotrophic hormone (ACTH) test. Data were presented as median (quartiles). Mann  
36 Whitney tests were performed on delta values (24-0 weeks) between TRT and placebo.

37 **Results:** The median age was 55 years (46; 59) and total testosterone before intervention was 6.8 (5.0; 9.3)  
38 nmol/L. TRT was associated with change of testosterone levels 12.3 (7.0; 19.9) nmol/L ( $p < 0.001$  vs.  
39 placebo), increased lean body mass 3.6 (2.3; 5.0) kg vs. 0.1 kg (-2.1; 1.5) during TRT vs. placebo and  
40 decreased total fat mass -1.2 (-3.1; 0.7) kg vs. 1.2 kg (-0.9; 2.5) kg, both  $p < 0.003$ . Changed pain perception,  
41 SF36, and ACTH stimulated cortisol levels were non-significantly changed during TRT compared with  
42 placebo.

43 **Conclusions:** Six months TRT improved body composition in men with opioid induced hypogonadism  
44 without significant changes in outcomes of pain perception, quality of life or adrenal function.

45

46

## 47 **Introduction**

48 Opioid medications are widely used to manage chronic non-cancer pain. Opioids are known to suppress  
49 pituitary function by inhibition of the relevant hypothalamic releasing factors via opioid receptors, which are  
50 widely distributed throughout the central nervous system (1). One of the most well described hormonal side  
51 effects of opioid treatment is male hypogonadism. Treatment with opioids increases the risk of secondary  
52 hypogonadism, characterized by low serum levels of testosterone and low levels of luteinizing hormone and  
53 follicle-stimulating hormone (2). Recent reviews estimate that the risk of hypogonadism ranges from 17 to  
54 89% in men treated with opioids (3). The risk magnitude of male hypogonadism depends on treatment  
55 duration, dosage of opioids, and the applied cut-off level for total testosterone to define hypogonadism (3).  
56 Testosterone replacement therapy (TRT) is indicated in men with severe hypogonadism, whereas the  
57 indication for TRT in opioid-induced relative hypogonadism in men is debated.

58  
59 The potential effect of TRT on pain perception remains to be investigated. In animal studies, the opioid and  
60 gonadal systems interact and regulate sensitivity to nociceptive stimuli (3). In castrated male rodents,  
61 testosterone administration improves pain tolerance to mechanical and thermal nociceptive stimuli (3). Few  
62 studies have investigated the effect of TRT on pain outcomes and quality of life in opioid-treated men with  
63 relative hypogonadism. A recent meta-analysis (4) found one eligible randomized controlled trial (n=84,  
64 study duration 3 months) (5) and four observational studies (total n=157) (4). The authors concluded that  
65 very low-quality evidence supported that TRT was associated with improved pain and emotional  
66 functioning, whereas TRT did not affect physical functioning, role functioning, or social functioning (4).  
67 Furthermore, sexual function is impaired during opioid treatment (6) and data regarding sexual function  
68 during TRT in these men are sparse (5). Obesity is inversely associated with testosterone levels (7). Lean  
69 body mass is positively associated with higher endogenous pain modulation (8). TRT increases lean body  
70 mass and decreases abdominal subcutaneous adipose tissue (9-11). In theory, disability may be linked to  
71 inactivity and low lean body mass and therefore, presence of chronic pain could increase the effect of TRT  
72 on lean body mass in opioid treated men compared to other study populations. Whether a positive effect of

73 TRT on pain perception is mediated via improved body composition with higher lean body mass is  
74 undetermined.

75

76 Secondary adrenal insufficiency is a less described side effect in opioid treated individuals (12). The  
77 prevalence of insufficient adrenal response is estimated to range between 9-29% in men and women treated  
78 with long term opioids (1, 12). Adrenal insufficiency will have an adverse effect on for example  
79 inflammatory disease, joint and muscle pain (1). Furthermore, severe adrenal insufficiency should be treated  
80 according to available guidelines. Previous studies have used a wide range of diagnostic criteria for adrenal  
81 insufficiency (12) and more data are needed regarding risk and possible predictors of adrenal insufficiency in  
82 patients treated with opioids. BMI is inversely related to cortisol responsivity (13, 14), but whether improved  
83 body composition during TRT could affect cortisol levels during the ACTH test remains to be tested.

84

85 The aim of the present study was to investigate the effects of TRT on body composition, pain perception,  
86 pain sensitivity, adrenal cortisol response and quality of life in opioid treated men with relative  
87 hypogonadism.

88

## 89 **Methods**

90 **Study design:** The study was conducted during from August 2016 - August 2019 as a single center,  
91 randomized, placebo-controlled, double-blind, study. The study outcomes were evaluated at baseline and  
92 after six month's (24 weeks) intervention. All participants gave written informed consent. The study was  
93 approved by The Regional Scientific Ethical Committees for Southern Denmark (S-20150004) and the  
94 Danish Health and Medicines Agency. Monitoring was performed according to good clinical practice (GCP)  
95 by the GCP unit at Odense University hospital. The trial was declared in [www.clinicaltrials.gov](http://www.clinicaltrials.gov)  
96 (NCT02433730). The study was planned and approved by Danish authorities May 2015. Participants were  
97 recruited from August 2016 and onwards. The authors confirm that all ongoing and related trials for this  
98 drug/intervention are registered. Study data were collected and managed using REDCap electronic data

99 capture tools (15) hosted at University of Southern Denmark. The study design is presented in the appendix  
100 according to the Consort checklist.

101

102 **Participants:** The study participants were men aged >18 years with total testosterone (T-testosterone) <12  
103 nmol/L and treated with opioids for non-malignant pain disease. Inclusion criteria were at least two measures  
104 of T-testosterone < 12 nmol/L (measured between 8 and 10 o'clock in the morning), treatment with opioids  
105 for at least 3 months at a dosage corresponding to at least 50 mg morphine/day (for conversion to morphine  
106 equivalent doses, we applied <https://www.oregonpainguidance.org/opioidmedcalculator/>), and LH and  
107 prolactin within reference interval. Exclusion criteria were wish to conceive during the trial period,  
108 hematocrit > 53% , previous or ongoing malignant disease, prostate specific antigen (PSA) > 3 ng/dl,  
109 untreated ischemic heart or respiratory disease, alcohol or drug abuse, abnormal routine blood samples (TSH,  
110 ionized calcium, hemoglobin, liver and kidney function), treatment with 5 $\alpha$ -reductase inhibitors and oral  
111 glucocorticoid steroids, previous or current testosterone treatment.

112

113 **Randomization:** Randomization numbers were assigned to the participants in order of enrollment into the  
114 study. The randomization list, medicine labeling and randomization- and code break envelopes were  
115 generated by the pharmacy at Odense University Hospital to ensure double blinding.

116

117 **Interventions:** Participants were randomly assigned to receive testosterone (TRT, testosterone undecanoate  
118 (Nebido) 1,000 mg) or placebo injections. The pharmacy at Odense University Hospital handled packaging  
119 of medicine for each study participant. Patients then received medicine according to randomization number.  
120 Placebo injections were prepared by the pharmaceutical company to ensure identical packaging of TRT and  
121 placebo. The study participants received injections at time of randomization and at 6 and 18 weeks.

122

123 **Outcomes and assessments**

124 Participants underwent clinical examination, dual X-ray Absorptiometry (DXA) scan, assessment of clinical  
125 and experimental pain, fasting blood samples, ACTH test and OGTT and answered questionnaires by time of  
126 study inclusion and the examination program was repeated after 24-weeks intervention.

127

## 128 **Body composition measures**

129 **Clinical examination** included height, weight, and waist circumference.

130 **DXA:** Trunk fat mass, total fat mass, fat percentage, and lean body mass were measured by DXA using a  
131 Hologic Discovery device (Waltham, MA, USA). The CV was 0.8% for total fat mass and 0.6% for lean  
132 body mass.

133

## 134 **Assessment of clinical pain parameters**

135 Data on clinical pain manifestations were collected the electronic software system PainData used in the Pain  
136 Center at the Odense University Hospital in Denmark. Data was collected on pain duration and intensity of  
137 clinical peak pain, and clinical average pain on a 0-10 numerical rating scale (NRS) with 0 defined as “no  
138 pain” and 10 “as worst imaginable pain” during the previous 24 hours. Absolute difference in NRS pain  
139 intensity was calculated as pain intensity at baseline minus pain intensity at 6 months follow-up with positive  
140 numbers indicating a reduction in pain intensity at follow-up.

141

142 **Assessment of experimental pain sensitivity:** Participants underwent assessments of pressure pain  
143 thresholds (PPTs and cPPT) and pressure pain tolerance (PTT), a protocol for temporal summation of  
144 pressure pain (TSP), a protocol to determine pain wind-up ratio (WUR), a protocol for conditioned pain  
145 modulation (CPM), as well as assessment of heat pain thresholds (HPT) and cold pain thresholds (CPT). All  
146 pain sensitivity assessments were performed with the participant seated on a plinth with both arms resting on  
147 the thighs. The assessment lasted 30 minutes and was performed by the same experienced assessor (HBV).  
148 Pain assessment was undertaken in the same order for all participants (PPTs, cPPT, PTT, TSP, CPM, WUR,  
149 HPT and CPT). Details regarding experimental pain measurement are presented in the appendix.

150

151 **Questionnaires:** The Short-Form 36 (SF-36) is a generic measure of health status comprising 36 items  
152 related to eight dimensions: physical functioning for the limitation in performing all physical activities, role  
153 physical for problems with work or other daily activities, bodily pain, general health, vitality, social  
154 functioning, role emotional, and mental health (16). QualityMetric's Scoring Solutions and license  
155 agreements were obtained from Qualitymetric.com.

156 **Sexual function:** Participants fulfilled a Danish questionnaire regarding sexual function (17). The  
157 questionnaire was developed and validated by Søren Buus Jensen (17) and included questions regarding  
158 partner status, sexual activity, and erectile dysfunction. Erectile dysfunction was defined to be present if the  
159 patient agreed to one the following statements regarding sexual intercourse and/or masturbation: I can get  
160 erection, but it disappears during intercourse/masturbation. I often have problems getting erection. It is not  
161 possible for me to get erection.

162 We had information regarding prescription of phosphodiesterase type 5 (PDE5)-inhibitors, but we did not  
163 have information regarding actual use. Therefore, use of PDE5-inhibitors was not included in results.

164  
165 **Oral glucose tolerance test (OGTT):** A 2 hour OGTT was performed at 8:00am after overnight fasting.  
166 Blood glucose was measured at 0, 30, 60, and 120 minutes after oral ingestion of a glucose load containing  
167 the equivalent of 75 g anhydrous glucose dissolved in water. Area under the curve for glucose was calculated  
168 applying the trapezium rule.

169  
170 **Adrenocorticotrophic hormone (ACTH) test:** The ACTH test was standardized to be performed after the  
171 OGTT. An intravenous bolus of 0.25 mg Synachten (Novartis Healthcare, Copenhagen, Denmark) was  
172 administered and cortisol levels were measured at 0 and 30 minutes. Delta cortisol was defined as cortisol 30  
173 minutes – cortisol 0 minutes. We defined insufficient response to ACTH test as 30 minutes cortisol value <  
174 420 nmol/l according to the reference interval at our laboratory.

175  
176 **Biochemical analyses**



177 **Testosterone and SHBG:** Testosterone levels were measured in the morning in the fasting state by liquid  
178 chromatography tandem mass spectrometry. For testosterone measurements the intra-assay coefficient of  
179 variation was 10% for total testosterone >0.2 nmol/l and 30% in the range between 0.1- 0.2 nmol/l. SHBG  
180 was measured by autoDELFI assay and bioavailable testosterone (BioT) was calculated according to the  
181 formulas of Vermeulen (18), <http://www.issam.ch/freetesto.htm>. During calculations, we used the  
182 assumption that albumin concentration in participants was 4.3 g/L. The normal range and 95% confidence  
183 interval for BioT was 7.3 nmol/l (7.0 – 7.5 nmol/l) (7).

184 **Lipids:** Plasma total cholesterol, high-density lipoprotein cholesterol (HDL), and triglycerides (TG) were  
185 analyzed by enzymatic colorimetric reactions (Modular P, Roche), and low density lipoprotein cholesterol  
186 (LDL) was calculated using the Friedewald equation. For fasting lipid parameters, reference intervals were  
187 as follows: total cholesterol: 3.6–6.8 mmol/l, LDL: 1.8–4.5 mmol/l, HDL: 0.76–1.68 mmol/l, and TG: 0.47–  
188 2.31 mmol/l.

189 Serum cortisol was measured using an automated solid-phase, competitive chemiluminescent enzyme  
190 immunoassay using an Immulite 2000 XPi analyzer, Siemens Healthineers. At cortisol level 290 nmol/l, the  
191 precision was CV 7.7% (SD 22.4) and at cortisol 587 nmol/l, the CV was 6.4% (SD 37.8).

192 Blood glucose was measured with a Hemocue device (Hemocue, Ängelholm, Sweden), which converted  
193 blood glucose concentrations to equivalent plasma glucose concentrations (19). The meter was checked with  
194 a control cuvette every day and a hemolysate every 1–2 weeks.

## 196 **Statistical analysis**

197 By the time of study planning, no study had investigated the effect of TRT on pain perception. Therefore, the  
198 sample size of the study was determined by the effect of TRT on lean body mass based on a meta-analysis on  
199 testosterone therapy in aging men by Isidori (20). If the study had sufficient power to detect significant  
200 changes in lean body mass, we hypothesised that the study would be sufficiently powered to detect clinical  
201 relevant changes in pain perception. The inclusion of study participants and number of drop outs is presented  
202 in the attached Consort flow diagram (figure 1).

203 Pre-treatment differences between participants in the testosterone and placebo group were tested using Mann  
204 Whitney U tests. The dataset was of limited size, and therefore the effects of TRT and placebo were analyzed  
205 by comparing delta ( $\Delta$ ) values of hormonal and metabolic variables using Mann-Whitney test as described  
206 by Altman (21).  $\Delta$ -values on clinical and biochemical markers were calculated as post-treatment level minus  
207 pretreatment level.  $\Delta$ -Pain sensation measures were correlated with  $\Delta$ -values of total testosterone and body  
208 composition using Spearman non-parametric correlation tests.  
209 All statistics were performed using SPSS 17.0 (SPSS Inc, Chicago, USA) for calculations and p-values <  
210 0.05 were considered significant. Data are given as median and interquartile range.

211

## 212 **Results**

### 213 **Study population (n=41)**

214 Baseline data are shown in Table 1. The two intervention groups (TRT, n=20 and placebo, n=21) were  
215 comparable at baseline regarding all clinical and biochemical study outcomes. At baseline, insufficient  
216 ACTH test was observed in 6 men (16%, 3 placebo, 3 TRT). In participants with insufficient cortisol  
217 response to the ACTH test, unstimulated cortisol levels ranged from 86-192 nmol/L and stimulated cortisol  
218 levels ranged from 372- 419 nmol/L. The median morphine equivalent dosage (ME) was 163 mg/day (range  
219 86-192 mg/day) in participants with insufficient cortisol response compared to median morphine equivalent  
220 dosage of 90 mg/day (range 45-360 mg/day) in participants with sufficient cortisol response (p=0.22 between  
221 groups).

### 222 **TRT vs. placebo treatment**

223 TRT was associated with higher testosterone levels (total and free testosterone), improved body composition  
224 (increased lean body mass and decreased BMI, fat trunk, total fat mass, and fat percentage), unchanged lipids  
225 (T-cholesterol, LDL, TG, HDL (HDL not shown)), and unchanged glucose levels (fasting blood glucose, 2h  
226 blood glucose and AUC glucose during OGTT) (Table 1).  
227 Changes in cortisol levels during the ACTH test (cortisol 30 min and delta cortisol) were comparable  
228 between TRT and placebo. The number of participants with insufficient cortisol response during ACTH test  
229 was unchanged during medical intervention (3 participants in TRT and 3 participants in placebo) and no

230 participants changed response pattern from sufficient to insufficient response or *vice versa* during the  
231 treatment period.  
232 TRT improved SF36 physical component score (PCS), whereas the remaining dimensions of SF36 and visual  
233 analogue (VAS) scores were unchanged. Changes in experimental pain sensitivity outcomes were not  
234 significantly different between TRT and placebo (Table 2).

### 235 **Bivariate associations between $\Delta$ -pain and $\Delta$ -body composition and $\Delta$ -testosterone levels during TRT**

236  $\Delta$ -peak NRS pain scores showed an inverse association with  $\Delta$ -T-testosterone ( $r= 0.50$ ,  $p=0.04$ ) and  $\Delta$ -Bio T  
237 ( $r= 0.55$ ,  $p=0.02$ ), suggesting that higher change in T-testosterone and Bio T was associated with larger  
238 reductions in peak pain intensity in the TRT group.  $\Delta$ -pain measures were not significantly associated with  
239  $\Delta$ -lean body mass or  $\Delta$ -fat mass (data not shown).

240

### 241 **Sexual function**

242 **Baseline:** A total of 23/36 men participating in the study were married (16/19 TRT vs. 12/17 placebo,  $p=$   
243  $0.43$ ) with median marriage duration of 16 (6; 28) years and fatherhood of 2 (2; 3) children. At baseline,  
244 erectile dysfunction was present in 23/35 (65%) men (12/18 vs. 11/17,  $p=0.9$ ) with no ability to obtain  
245 erection in 13/35 men (8/18 vs. 5/17,  $p=0.6$ ), whereas 12/35 (6/18 vs. 6/17,  $p=0.56$ ) had no erection  
246 problems. At baseline, 27/35 (13/18 vs. 14/17,  $p=0.34$ ) men had coitus at intervals longer than one month  
247 with average duration since last coitus of 150 (10; 1662) days (150 (10; 2007 vs. 257 (13; 1095) days,  
248  $p=0.7$ ).

249 **TRT vs. placebo:** Partner status was unchanged in all men during the study. At 6 months, erectile  
250 dysfunction was present in 14/32 men (4/16 vs. 10/16,  $p=0.03$ ) with no ability to obtain erection in 10/32  
251 (3/16 vs. 7/16,  $p=0.10$ ), whereas 18/32 (12/16 vs. 6/16,  $p=0.03$ ) reported no erection problems. At 6 months,  
252 20/31 (9/16 vs. 11/15,  $p=0.32$ ) had coitus at intervals longer than a month with average duration since last  
253 coitus of 18 (6; 1825) days in TRT vs. 365 (30; 1450) days in the placebo group ( $p=0.04$   $\Delta$ -TRT vs.  $\Delta$ -  
254 placebo).

255

### 256 **Discussion**

257 In the present study, body composition improved during 6 months randomized intervention with TRT  
258 without significant changes in clinical and experimental pain perception and adrenal function in men treated  
259 with opioids for non-malignant chronic pain disease. The present study is the first long term randomized  
260 term study that investigated possible associations between improved body composition (higher lean body  
261 mass), quality of life and pain perception during TRT in a male study population treated with opioids.

## 262 **Body composition**

263 A median increase in lean body mass of 3.6 kg was observed during TRT, whereas fat mass decreased with  
264 median 1.2 kg. A positive effect of TRT treatment on body composition has previously been reported in  
265 other study populations (20) and change in lean body mass was our primary study outcome. According to the  
266 meta-analysis by Isidori *et al* (20), our power calculation was based on an expected increase in lean body  
267 mass of 1.6 kg. The meta-analysis (20) was based on randomized placebo controlled studies performed in  
268 diverse study populations and of different study duration, whereas limited data regarding change in body  
269 composition during TRT are available in opioid-induced male hypogonadism (5). Basaria *et al* described on  
270 average 1.0 kg increase in lean body mass during 3 month intervention with transdermal testosterone gel in  
271 opioid treated men (5). Importantly, men included in the study by Basaria *et al* (5) were comparable to our  
272 study population regarding inclusion criteria (both studies included opioid treated men with testosterone <12  
273 nmol/l), mean age (49 vs. 54 years), mean BMI (32 vs. 29 kg/m<sup>2</sup>) and mean daily opioid dosage (114 vs. 100  
274 mg). The findings by Basaria *et al* (5) supported that men with hypogonadism due to opioid treatment had  
275 similar improvement in lean body mass during TRT compared to men with hypogonadism due to other  
276 diseases (20). Post treatment total testosterone was 27.2 nmol/L in the study by Basaria *et al* (5) compared to  
277 19.3 nmol/L in the present study, which could support that our finding of higher gain of lean body mass  
278 could be explained by longer treatment duration of TRT and not average serum testosterone concentration.  
279 We found that fat mass decreased median 1.2 kg during TRT, which was comparable to results reported by  
280 Basaria *et al* (average loss in fat mass 0.8 kg) (5) and by Isidori *et al* (average loss of fat mass 1.6 kg) (20).  
281 The present data therefore add to the evidence that body composition is improved during TRT and the  
282 positive effect of TRT on body composition is not dependent on patient cohort but is dependent of  
283 testosterone dosage and treatment duration.

284

285 **Pain perception**

286 Measures of clinical pain intensity, experimental pain sensitivity, and measures of quality of life were  
287 unchanged during TRT in the present study, which contrasted our study hypothesis. By time of study  
288 planning, animal studies (3) and human uncontrolled studies (4) had shown improvements in pain perception  
289 and quality of life during TRT (4). Although the study size was limited, all outcomes of pain sensitivity  
290 measures were unchanged during study intervention, which did not support a placebo effect. The SF36 PCS  
291 domain was significantly increased during TRT, but all other SF36 domains, numerical rating scale (NRS) of  
292 pain and VAS score were unchanged during TRT, which did not support an overall benefit of TRT on quality  
293 of life. It is possible that the significant change in the SF36 domain PCS should be considered to be caused  
294 by chance as no other SF36 outcomes showed a trend towards significant changes. At present, the only  
295 available placebo controlled study regarding changes in pain and QoL during TRT is the study by Basaria *et*  
296 *al* (5), whereas remaining studies were uncontrolled (4). In contrast to the present study, Basaria *et al*  
297 reported changes in some but not all pain sensitivity measures with significantly higher changes in pressure  
298 pain threshold at the thumb and lower pain intensity after 10 pinprick stimulations at the hand after TRT,  
299 whereas changes in pressure pain threshold at the trapezius muscle, pain intensity after 1 pinprick  
300 stimulation, cold pain tolerance, self-reported clinical pain and SF-36 outcomes were not significantly  
301 different between TRT and placebo (5). As already mentioned, study participants in the study by Basaria *et*  
302 *al* (5) were comparable to the present study, but participants obtained higher serum levels of total  
303 testosterone during TRT, and the effect of TRT on lean body mass was more modest than in the present  
304 study. In accordance, in the present study the reduction in NRS peak pain was associated with  $\Delta$ -  
305 testosterone, whereas changes in pain measures were not associated with changes in body composition.  
306 The findings by Basaria (5) and the present study supported that possible effects of TRT on pain modulation  
307 and QoL was mediated primarily by higher testosterone levels instead of increased lean body mass.  
308 Furthermore, it could be important to note that the study duration was 3 months in the study by Basaria *et al*  
309 (5) in contrast to the study duration of 6 months in the present study. It is possible that a modest positive  
310 effect of TRT on pain modulation could wear off during prolonged treatment. In accordance, 6 months

311 androgen deprivation therapy in men with prostate cancer resulted in worsening of depression scores and  
312 QoL, whereas pain sensitivity and clinical pain were unchanged (22). Available data do not support any  
313 clinical significant effect of long term TRT on clinical and experimental pain, but future studies should  
314 discriminate between short and long term effects of TRT on pain modulation.

315

316 **Adrenal insufficiency** was found in 16% men in the present study, and presence of adrenal insufficiency  
317 was associated with higher median morphine equivalent dosage. Interestingly, 30 minutes cortisol levels  
318 during the ACTH test in men with adrenal insufficiency were in all cases only moderately decreased and we  
319 found no participants with severe adrenal insufficiency. Increased risk of adrenal insufficiency in opioid  
320 treated patients have been reported in previous studies (1, 12), however, different tests were applied for  
321 adrenal function assessment (1, 12). The adrenal sensitivity for morphine treatment differs between  
322 individuals and could be due to opioid receptor polymorphisms resulting in altered opioid receptor affinity or  
323 genetic variations in interleukin 1b, a stimulator of corticotropin and corticotrophin releasing hormone  
324 (CRH) (12). We found no significant change of adrenal activity after TRT, despite significant improvements  
325 of body composition. Previous studies found an inverse association between BMI and cortisol responsivity  
326 (13, 14), but it is possible that the change of body composition in the present study was too modest to affect  
327 adrenal function. Furthermore, adrenal drive (23) and CBG levels (24) could decrease after weight loss,  
328 which could be an alternate explanation of unchanged levels of total cortisol during TRT. Insufficient  
329 adrenal function could affect quality of life and low cortisol levels could have an adverse effect on for  
330 example inflammatory disease, joint and muscle pain (1). Severe adrenal insufficiency should be treated  
331 according to available guidelines, but the possible benefits of treating more mild adrenal insufficiency with  
332 hydrocortisone regarding quality of life and pain perception remains to be evaluated in future studies.

333

334 **Sexual function:** Sexual function was significantly improved during TRT as erectile dysfunction improved  
335 and sexual activity increased. These data supported the findings by Basaria (5) who reported significantly  
336 greater increase in sexual desire during TRT, whereas they found no differences in orgasmic domain and  
337 erectile function domain between TRT and placebo. More than 50% men in the present study reported

338 erectile dysfunction, and 37% of participants were not able to have erection. Impaired sexual function in  
339 opioid treated men is well described (6). Present data support that sexual function is improved but not fully  
340 restored during TRT, which could be due to unchanged pain perception during TRT.

341

342 **Strengths and limitations** may apply in the present study. The trial was double blind and placebo controlled  
343 with only few drop outs. The primary study outcome was lean body mass, whereas the study could be  
344 underpowered to detect minor changes in secondary study outcomes such as pain perception. However, the  
345 use of multiple standardized assessments for pain, pain threshold and tolerance is an important strength of  
346 the study as the use of a single assessment can lead to questions of reliability. The study was planned during  
347 2014-2015 and by this time, the placebo controlled study by Basaria *et al* was not published (5). Therefore,  
348 the power calculation could not be based on their results. Our study was sufficiently powered regarding our  
349 primary study outcome, however the study could be underpowered to detect minor changes in pain and  
350 quality of life outcomes. We did not include information about smoking, alcohol and other lifestyle factors,  
351 which could have changed during study intervention and could have affected our study results. Several study  
352 outcomes were addressed and therefore the issue of multiple testing needs to be discussed. We could  
353 categorize the study outcomes into a limited number of aspects including body composition (weight, lean  
354 body mass, total and regional fat mass), measures of pain perception and quality of life (objective  
355 measurements and questionnaires) and adrenal function (ACTH test). The use of several screening  
356 modalities for each study outcome strengthened the study conclusion.

357

### 358 **Conclusion**

359 Six month TRT improved body composition without significant changes in pain perception, pain sensitivity  
360 and quality of life in men with relative opioid induced hypogonadism.

361

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368

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447 Figure 1: Consort flowchart of included study subjects.

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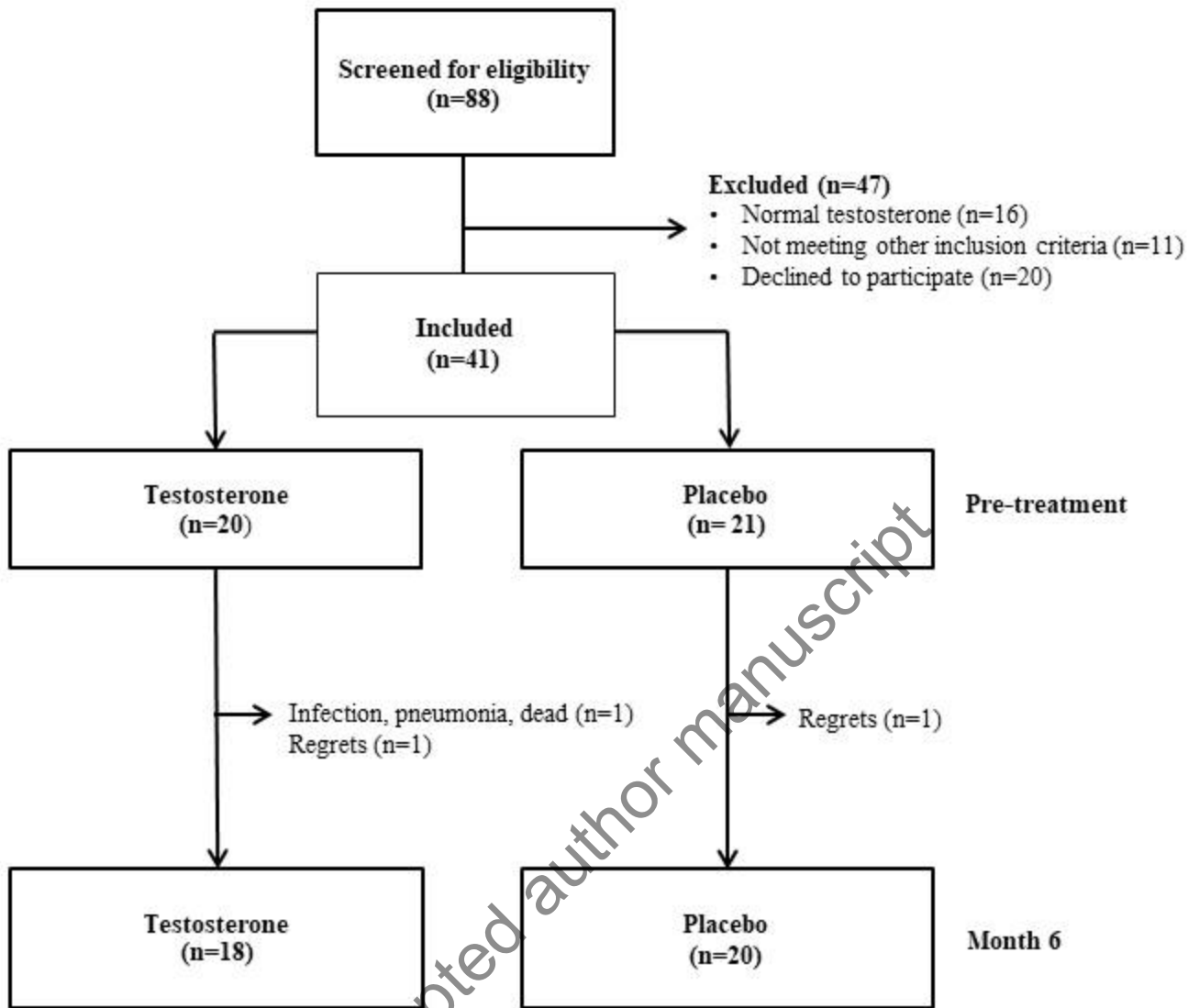


Table 1: Clinical characteristics, quality of life and adrenal function at baseline and 6 months follow-up in the TRT and placebo groups.

|  | All                   |                       | TRT                    |                        | Placebo                |                      | Delta change        |  | P value          |
|--|-----------------------|-----------------------|------------------------|------------------------|------------------------|----------------------|---------------------|--|------------------|
|  | Baseline (n=41)       | Baseline (n=20)       | 6 months (n=18)        | Baseline (n=21)        | 6 months (n=20)        | TRT (n=18)           | Placebo (n=20)      |  |                  |
| Age (years)                                | 55<br>(46; 59)        | 54<br>(49; 59)        |                        | 55<br>(45; 61)         |                        |                      |                     |  |                  |
| Median morphine equivalent dosage (mg/day) | 100<br>(75; 171)      | 98<br>(75; 200)       | 98<br>(75; 180)        | 100<br>(75; 156)       | 95<br>(76; 173)        | 0<br>(0; 3.8)        | 0<br>(0; 0)         |  | 0.94             |
| Weight (kg)                                | 97.8<br>(88.7; 108.8) | 97.6<br>(88.6; 107.8) | 100.5<br>(95.4; 108.4) | 100.9<br>(87.8; 112.2) | 100.6<br>(88.0; 108.0) | 2.7<br>(1.0; 4.6)    | 0.9<br>(-2.0; 3.0)  |  | <b>0.024</b>     |
| BMI (kg/m <sup>2</sup> )                   | 30.7<br>(28.8; 34.0)  | 29.1<br>(28.8; 33.6)  | 30.7<br>(29.3; 34.9)   | 32.3<br>(28.7; 34.8)   | 32.5<br>(30.0; 34.3)   | 0.9<br>(0.3; 1.5)    | 0.0<br>(-0.9; 0.9)  |  | <b>0.020</b>     |
| Waist (cm)                                 | 114<br>(107; 120)     | 111<br>(106; 119)     | 111<br>(107; 118)      | 117<br>(108; 121)      | 116<br>(108; 122)      | 0<br>(-4; 4)         | 0<br>(-2; 3)        |  | 0.82             |
| Hip (cm)                                   | 111<br>(106; 116)     | 110<br>(104; 114)     | 111<br>(107; 114)      | 111<br>(107; 119)      | 114<br>(108; 118)      | 1<br>(-1; 4)         | 2<br>(-2; 4)        |  | 0.87             |
| Waist-hip-ratio                            | 1.00<br>(0.97; 1.06)  | 1.00<br>(0.98; 1.07)  | 1.00<br>(0.98; 1.06)   | 1.01<br>(0.96; 1.07)   | 1.02<br>(0.96; 1.06)   | 0<br>(-0.02; 0)      | 0<br>(-0.03; 0.01)  |  | 0.87             |
| Fat Trunk (kg)                             | 18.4<br>(14.3; 19.7)  | 17.8<br>(13.6; 19.3)  | 15.8<br>(12.7; 20.4)   | 19.1<br>(14.8; 19.8)   | 19.6<br>(15.2; 21.6)   | -0.3<br>(-1.8; 0.3)  | 0.7<br>(0; 1.1)     |  | <b>0.006</b>     |
| Total fat mass (kg)                        | 32.9<br>(26.7; 37.8)  | 32.6<br>(26.4; 36.4)  | 29.6<br>(25.3; 34.7)   | 33.7<br>(28.3; 38.7)   | 34.2<br>(28.4; 38.6)   | -1.2<br>(-3.1; 0.1)  | 1.2<br>(-0.9; 2.5)  |  | <b>0.003</b>     |
| Fat percentage (%)                         | 35.4<br>(32.7; 36.8)  | 36.4<br>(31.2; 37.8)  | 31.9<br>(28.5; 36.0)   | 35.3<br>(33.4; 36.6)   | 34.8<br>(33.2; 37.6)   | -2.3<br>(-3.7; -0.8) | 0.9<br>(-0.9; 1.9)  |  | <b>&lt;0.001</b> |
| Lean body mass (kg)                        | 59.7<br>(53.7; 66.2)  | 60.4<br>(53.7; 66.3)  | 63.4<br>(59.6; 67.8)   | 58.6<br>(53.9; 63.7)   | 57.3<br>(54.1; 63.3)   | 3.6<br>(2.3; 5.0)    | 0.1<br>(-2.1; 1.4)  |  | <b>&lt;0.001</b> |
| Total testosterone (nmol/L)                | 6.8<br>(5.0; 9.5)     | 6.6<br>(5.0; 9.2)     | 19.3<br>(13.8; 26.6)   | 7.1<br>(4.9; 9.5)      | 7.7<br>(5.2; 8.5)      | 12.3<br>(7.0; 19.9)  | -0.4<br>(-2.5; 1.1) |  | <b>&lt;0.001</b> |
| Sex hormone binding globulin (nmol/L)      | 38<br>(25; 48)        | 41<br>(24; 51)        | 37<br>(23; 45)         | 34<br>(25; 46)         | 32<br>(21; 51)         | -3<br>(-12; 0)       | -1<br>(3; 7)        |  | <b>0.016</b>     |
| Bio testosterone (nmol/L)                  | 2.9<br>(2.1; 4.0)     | 2.7<br>(1.9; 3.8)     | 8.8<br>(8.1; 11.9)     | 3.4<br>(2.1; 4.3)      | 3.0<br>(1.9; 4.1)      | 6.2<br>(4.2; 9.9)    | -0.5<br>(-1.3; 0.5) |  | <b>&lt;0.001</b> |
| T-cholesterol (mmol/L)                     | 4.6<br>(3.9; 5.4)     | 4.6<br>(3.8; 5.3)     | 4.3<br>(3.6; 4.8)      | 4.9<br>(4.0; 5.4)      | 4.7<br>(4.1; 5.3)      | -0.5<br>(-0.8; 0.2)  | -0.1<br>(-0.6; 0.4) |  | 0.19             |
| LDL (mmol/L)                               | 2.8<br>(2.0; 3.4)     | 2.9<br>(1.7; 3.3)     | 2.5<br>(2.0; 3.0)      | 2.7<br>(2.2; 3.6)      | 2.6<br>(2.0; 3.3)      | -0.3<br>(-0.6; 0.4)  | -0.1<br>(-0.6; 0.3) |  | 0.61             |
| Triglycerides (mmol/L)                     | 1.6<br>(1.1; 2.4)     | 1.5<br>(1.1; 2.2)     | 1.8<br>(1.0; 2.3)      | 1.7<br>(1.1; 2.8)      | 1.8<br>(1.2; 3.2)      | -0.1<br>(-0.3; 0.4)  | 0.1<br>(-0.3; 0.3)  |  | 0.57             |
| Fasting glucose (mmol/L)                   | 5.6<br>(5.4; 6.0)     | 5.6<br>(5.3; 6.0)     | 5.8<br>(5.5; 6.2)      | 5.7<br>(5.4; 6.3)      | 5.9<br>(5.5; 6.3)      | 0.1<br>(-0.4; 0.3)   | -0.1<br>(-0.4; 0.1) |  | 0.40             |
| 2h glucose (mmol/L)                        | 6.4<br>(5.6; 7.6)     | 6.4<br>(5.5; 7.6)     | 6.6<br>(5.4; 8.1)      | 6.5<br>(5.8; 7.5)      | 6.1<br>(5.1; 7.3)      | 0.2<br>(-1.0; 1.5)   | -0.3<br>(-1.6; 0.5) |  | 0.28             |

|                                       |                    |                    |                    |                    |                    |                  |                 |      |
|---------------------------------------|--------------------|--------------------|--------------------|--------------------|--------------------|------------------|-----------------|------|
| AUC glucose                           | 936<br>(852; 1017) | 936<br>(840; 1021) | 894<br>(862; 1047) | 938<br>(861; 1016) | 954<br>(807; 1028) | 26<br>(-65; 94)  | 5<br>(-119; 90) | 0.54 |
| ACTH                                  | 3<br>(2; 4)        | 3<br>(2; 4)        | 4<br>(3; 6)        | 3<br>(2; 4)        | 2<br>(2; 3)        | 1<br>(-1; 2)     | 0<br>(-2; 1)    | 0.20 |
| Cortisol 30 min                       | 566<br>(506; 655)  | 580<br>(531; 652)  | 550<br>(493; 589)  | 541<br>(494; 680)  | 569<br>(521; 636)  | -36<br>(-90; 18) | 2<br>(-93; 95)  | 0.38 |
| delta cortisol<br>(30-0 min)          | 329<br>(234; 397)  | 303<br>(244; 380)  | 286<br>(228; 367)  | 350<br>(218; 419)  | 347<br>(278; 406)  | -3<br>(-68; 27)  | 21<br>(-27; 80) | 0.10 |
| Visual analogue score<br>(VAS) score  | 40<br>(24; 50)     | 40<br>(21; 50)     | 40<br>(23; 62)     | 38<br>(30; 50)     | 40<br>(30; 50)     | 0<br>(-7; 0)     | 0<br>(-10; 9)   | 1.00 |
| SF36,<br>physical functioning<br>(PF) | 40<br>(28; 63)     | 43<br>(10; 65)     | 58<br>(21; 70)     | 33<br>(24; 57)     | 40<br>(30; 50)     | 6<br>(-5; 20)    | 0<br>(-15; 9)   | 0.14 |
| Role functioning<br>Physical (RP)     | 0<br>(0; 0)        | 0<br>(0; 25)       | 0<br>(0; 18)       | 0<br>(0; 25)       | 0<br>(0; 0)        | 0<br>(-25; 0)    | 0<br>(-25; 0)   | 0.75 |
| Bodily pain (BP)                      | 25<br>(10; 31)     | 22<br>(0; 29)      | 10<br>(0; 31)      | 22<br>(12; 34)     | 31<br>(22; 37)     | 0<br>(-17; 10)   | 0<br>(-5; 10)   | 0.84 |
| General health (GH)                   | 40<br>(25; 55)     | 44<br>(23; 49)     | 35<br>(25; 61)     | 43<br>(24; 62)     | 45<br>(28; 55)     | 0<br>(-12; 10)   | -5<br>(-15; 5)  | 0.69 |
| Vitality (VT)                         | 28<br>(15; 46)     | 35<br>(16; 40)     | 25<br>(13; 43)     | 20<br>(10; 43)     | 35<br>(15; 50)     | 0<br>(-9; 5)     | 0<br>(0; 10)    | 0.26 |
| Social functioning (SF)               | 63<br>(38; 81)     | 44<br>(25; 75)     | 56<br>(28; 75)     | 50<br>(38; 75)     | 75<br>(38; 100)    | -6<br>(-13; 22)  | 13<br>(-19; 25) | 0.36 |
| Role functioning<br>emotional (RE)    | 66<br>(33; 100)    | 33<br>(0; 100)     | 33<br>(0; 100)     | 67<br>(33; 100)    | 67<br>(33; 100)    | 0<br>(-67; 0)    | 0<br>(0; 33)    | 0.18 |
| Mental health (MH)                    | 66<br>(52; 77)     | 64<br>(45; 83)     | 60<br>(52; 76)     | 60<br>(50; 74)     | 72<br>(48; 82)     | -4<br>(-8; 7)    | 0<br>(-8; 8)    | 0.45 |
| Physical component<br>score (PCS)     | 29<br>(26; 35)     | 29<br>(25; 33)     | 31<br>(28; 37)     | 31<br>(26; 33)     | 28<br>(21; 34)     | 3<br>(-1; 5)     | -2<br>(-8; 1)   | 0.03 |
| Mental component<br>score (MCS)       | 45<br>(37; 57)     | 58<br>(21; 70)     | 43<br>(37; 48)     | 45<br>(36; 54)     | 54<br>(37; 60)     | -4<br>(-11; 4)   | 2<br>(-5; 5)    | 0.16 |

Data presented as median (quartiles)

P-value represents unpaired Mann Whitney test on changes (delta values) during intervention with testosterone vs. placebo.

**Table 2:** Experimental pain sensitivity at baseline and 6 months follow-up in the TRT and placebo groups.

|  | TRT                |                    | Placebo            |                    | Delta change     |                   | P value |
|--|--------------------|--------------------|--------------------|--------------------|------------------|-------------------|---------|
|  | Baseline<br>(n=20) | 6 months<br>(n=18) | Baseline<br>(n=21) | 6 months<br>(n=20) | TRT<br>(n=18)    | Placebo<br>(n=20) |         |
| Peak Pain numerical rating scale                         | 8.0 (7.0; 8.8)     | 9.0 (7.0; 10.0)    | 7.0 (6.0; 8.5)     | 8.0 (6.3; 8.0)     | -0.5 (-1.0; 0.0) | 0.3 (-0.8; 1.8)   | 0.13    |
| Average Pain numerical rating scale                      | 6.0 (4.3; 7.0)     | 6.0 (5.0; 8.0)     | 5.0 (4.0; 6.5)     | 5.0 (3.3; 6.0)     | -0.2 (-0.5; 0.0) | 0.45 (0.0; 1.8)   | 0.18    |
| Heat pain threshold (°C)                                 | 44.5 (40.2; 46.5)  | 43.3 (39.2; 47.3)  | 41.1 (38.3; 45.7)  | 42.6 (39.4; 46.5)  | -0.3 (-2.5; 1.8) | 0.8 (-2.3;3.4)    | 0.45    |
| Cold pain threshold (°C)                                 | 11.9 (5.1; 20.6)   | 13.8 (5.7; 20.8)   | 10.0 (5.0; 17.9)   | 12.3 (6.6; 15.3)   | 1.7 (-2.9;6.3)   | 0.4 (-1.2;2.5)    | 0.80    |
| Pressure pain thresholds (kPa)                           | 372 (292; 530)     | 355 (211; 387)     | 314 (233; 443)     | 310 (252; 430)     | -58 (-163;53)    | -2 (-71;59)       | 0.25    |
| Cuff pressure pain threshold (kPa)                       | 20.5 (16.0; 28.2)  | 20.5 (15.2; 29.1)  | 22.2 (14.0; 26.0)  | 21.4 (16.6; 25.8)  | -1.3 (-6.5;4.3)  | 2.1 (-2.3;5.6)    | 0.12    |
| Cuff pressure pain tolerance (kPa)                       | 45.0 (30.9; 59.7)  | 42.9 (30.5; 80.0)  | 44.0 (31.8; 52.9)  | 47.1 (30.9; 52.9)  | -3.6 (-14.3;2.6) | 0.1 (-5.3;4.7)    | 0.29    |
| Cuff-induced temporal summation of pressure pain (ratio) | 2.2 (1.5; 2.6)     | 1.9 (1.4; 2.5)     | 1.9 (1.2; 2.9)     | 1.7 (1.3; 3.5)     | 0.5 (-0.9;1.2)   | -0.1 (-1.5;1.5)   | 0.75    |
| Wind-up ratio  | 2.0 (0.5; 3.0)     | 3.0 (1.0; 5.3)     | 1.0 (0.0; 2.0)     | 1.5 (0.0; 2.3)     | 0.6 (-1.0;1.0)   | 0.9 (0.0;2.0)     | 0.46    |
| Conditioned pain modulation (kPa)                        | 6.8 (1.0; 16.1)    | 4.2 (-1.4; 14.3)   | 7.5 (3.8; 11.9)    | 6.9 (1.5; 10.3)    | -2.5 (-6.7;5.1)  | -1.2 (-8.8;5.0)   | 0.52    |

Data presented as median (quartiles)

P-value represents unpaired Mann Whitney test on changes (delta values) during intervention with testosterone vs. placebo

**Table 3:** Experimental pain sensitivity at baseline and 6 months follow-up in the TRT and placebo groups.

|  | TRT                |                    | Placebo            |                    | Delta change     |                   | P value |
|--|--------------------|--------------------|--------------------|--------------------|------------------|-------------------|---------|
|  | Baseline<br>(n=20) | 6 months<br>(n=18) | Baseline<br>(n=21) | 6 months<br>(n=20) | TRT<br>(n=18)    | Placebo<br>(n=20) |         |
| Peak Pain numerical rating scale                         | 8.0 (7.0; 8.8)     | 9.0 (7.0; 10.0)    | 7.0 (6.0; 8.5)     | 8.0 (6.3; 8.0)     | -0.5 (-1.0; 0.0) | 0.3 (-0.8; 1.8)   | 0.13    |
| Average Pain numerical rating scale                      | 6.0 (4.3; 7.0)     | 6.0 (5.0; 8.0)     | 5.0 (4.0; 6.5)     | 5.0 (3.3; 6.0)     | -0.2 (-0.5; 0.0) | 0.45 (0.0; 1.8)   | 0.18    |
| Heat pain threshold (°C)                                 | 44.5 (40.2; 46.5)  | 43.3 (39.2; 47.3)  | 41.1 (38.3; 45.7)  | 42.6 (39.4; 46.5)  | -0.3 (-2.5; 1.8) | 0.8 (-2.3;3.4)    | 0.45    |
| Cold pain threshold (°C)                                 | 11.9 (5.1; 20.6)   | 13.8 (5.7; 20.8)   | 10.0 (5.0; 17.9)   | 12.3 (6.6; 15.3)   | 1.7 (-2.9;6.3)   | 0.4 (-1.2;2.5)    | 0.80    |
| Pressure pain thresholds (kPa)                           | 372 (292; 530)     | 355 (211; 387)     | 314 (233; 443)     | 310 (252; 430)     | -58 (-163;53)    | -2 (-71;59)       | 0.25    |
| Cuff pressure pain threshold (kPa)                       | 20.5 (16.0; 28.2)  | 20.5 (15.2; 29.1)  | 22.2 (14.0; 26.0)  | 21.4 (16.6; 25.8)  | -1.3 (-6.5;4.3)  | 2.1 (-2.3;5.6)    | 0.12    |
| Cuff pressure pain tolerance (kPa)                       | 45.0 (30.9; 59.7)  | 42.9 (30.5; 80.0)  | 44.0 (31.8; 52.9)  | 47.1 (30.9; 52.9)  | -3.6 (-14.3;2.6) | 0.1 (-5.3;4.7)    | 0.29    |
| Cuff-induced temporal summation of pressure pain (ratio) | 2.2 (1.5; 2.6)     | 1.9 (1.4; 2.5)     | 1.9 (1.2; 2.9)     | 1.7 (1.3; 3.5)     | 0.5 (-0.9;1.2)   | -0.1 (-1.5;1.5)   | 0.75    |
| Wind-up ratio  | 2.0 (0.5; 3.0)     | 3.0 (1.0; 5.3)     | 1.0 (0.0; 2.0)     | 1.5 (0.0; 2.3)     | 0.6 (-1.0;1.0)   | 0.9 (0.0;2.0)     | 0.46    |
| Conditioned pain modulation (kPa)                        | 6.8 (1.0; 16.1)    | 4.2 (-1.4; 14.3)   | 7.5 (3.8; 11.9)    | 6.9 (1.5; 10.3)    | -2.5 (-6.7;5.1)  | -1.2 (-8.8;5.0)   | 0.52    |

Data presented as median (quartiles)

P-value represents unpaired Mann Whitney test on changes (delta values) during intervention with testosterone vs. placebo