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HORMONES, MUSCLES AND ONCOLOGICAL OUTCOME IN MEN WITH RECTAL CANCER

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“It's a dangerous business, Frodo, going out your door. You step onto the road, and if you don't keep your feet, there's no knowing where you might be swept off to.”

— J.R.R. Tolkien, The Lord of the Rings

To Sofia, Tuva, Alva & Iselinn

Abstract

Paper I. The aim was to elucidate if testosterone (T) dose-dependently increase muscle size in abdomen and pelvis, analogous to the known anabolic influence on appendicular muscles. Participants were young (age 18-50) healthy men participating in the 5α-reductase trail, a double blinded RCT. Endogenous T production was supressed and replaced with four dosages (50, 125, 300, or 600 mg) of T enanthate. Magnetic Resonance Imaging scans from baseline and end of study was used to analyse change in muscle areas of the lower trunk and pelvis. The estimated change (95% CI) of muscle area increase per 100 mg of T enanthate dosage increase was 0.622 cm^2 (0.394, 0.850) for psoas; 1.789 cm^2 (1.317, 2.261) for paraspinal muscles; 2.530 cm^2 (1.627, 3.434) for total abdominal muscles; 0.455 cm^2 (0.233, 0.678) for obturator internus; 0.082 cm^2 (0.003, 0.045) for ischiocavernosus. Areas were also associated on-treatment T and free T levels. In conclusion, the abdominal and pelvic muscle are responsive to T administration, opening up for future studies regarding T treatment in frail men with risk for falls and men with pelvic dysfunction.

Paper II. Preoperative radiotherapy (RT) is used in treatment of rectal cancer (RC) to enhance local control. Acute testicular failure with risk for permanent damage to T production is a less known adverse effect of RT. The aim was to elucidate long-term effects on T production, and the association of elevated luteinizing hormone (LH) and cancer recurrence. This was a longitudinal prospective cohort study including men with rectal- or prostate cancer stage I-III. Exposure was RT, quantified by mean cumulative testicular dose (TD). Testicular function was assessed by sampling of T, LH and follicle stimulating hormone (FSH) at baseline and at follow-ups after one and two years. Exposed men were additionally sampled preoperatively. Within two years after surgery, T levels recovered, but LH and FSH levels were significantly higher in exposed. Changes in LH and FSH were related to TD. Elevated LH one year after surgery inferred an incidence rate ratio for cancer recurrence in five years of 3·19 (95% C.I.: 0·97-11.2, mid-p=0·036)

Paper III. The aim was to analyse the impact of RT induced primary testicular failure on severe postoperative adverse events (AE, Clavien-Dindo grade 3+) in men treated for RC. 104 men were included from the previous cohort study. T and LH were sampled at baseline and after RT. The association between of primary testicular failure and severe postoperative AE was analysed using longitudinal regression. 25% had severe postoperative AE (AE+). Baseline data did not differ significantly between groups. The AE+ group had comparably higher LH/T-ratio after RT. 0.603 (0.2-2.5) vs 0.452 (0.127-5.926, p=0.035). The longitudinal regression analysis found that preoperative change in T (OR 0.844, 95% CI 0.720-0.990, p=0.034), LH/T-ratio (OR 2.020, 95% CI 1.010-4.039, p=0.047) and low T (<8 nmol/L, OR 2.605, 95 CI 0.951-7.139, p=0.063) were associated to severe postoperative AE. Preoperative RT induced decline in T seems to be a risk factor for severe postoperative AE in men with RC.

Paper IV. Sarcopenic signs have been related to worse cancer specific survival and the skeletal muscles in men are sensitive to T. The effect of RT induced testicular failure may therefore be of importance in men treated for RC. Based on the cohort study in Paper II, 102 men with RC were included. Using CT or MRI scans from routine examinations at baseline and one year after surgery, skeletal muscle (SM) area at 3rd lumbar vertebra was measured. Testicular function was evaluated by measurement of serum T and LH. The association between change in T (and calculated free T) and SM as well as systemic cancer recurrence and SM were analyzed. Change in free T level is associated with change in psoas major area (p=0.005) and abdominal muscle area (p<0.001). Systemic cancer recurrence was associated with changes in total SM area (-5.96 (-10.7 - -1.24) cm2, p=0.013)

In conclusion, Abdominal and pelvic muscles are as androgen sensitive as appendicular muscles, and impaired testicular endocrine function due to RT impacts muscle area. Preoperative decrease in T increase risk of severe postoperative AE. Elevated LH and decreased muscle area are associated with systemic cancer disease.

List of scientific papers

I. **Muscles of the trunk and pelvis are responsive to testosterone administration: data from testosterone dose–response study in young healthy men**
John Tapper, Stefan Arver, Karol M. Pencina, Anna Martling, Lennart Blomqvist, Christian Buchli, Zhuoying Li, Thiago Gagliano-Jucá, Thomas G. Travison, Grace Huang, Thomas W. Storer, Shalender Bhasin, Shehzad Basaria

II. **Long-term effects on testicular function and oncological outcome after preoperative radiotherapy in rectal cancer – a prospective cohort study**
John Tapper, Stefan Arver, Torbjörn Holm, Matteo Bottai, Mikael Machado, Ravi Jasuja, Anna Martling, Christian Buchli

III. **Acute primary testicular failure due to radiotherapy increases risk of severe postoperative adverse events in rectal cancer patients**
John Tapper, Stefan Arver, Torbjörn Holm, Matteo Bottai, Mikael Machado, Ravi Jasuja, Anna Martling, Christian Buchli

IV. **Body Composition changes and oncological outcome in men treated for rectal cancer**
John Tapper, Stefan Arver, Torbjörn Holm, Matteo Bottai, Josefin Segelman, Ravi Jasuja, Anna Martling, Lennart K Blomqvist, Christian Buchli

List of abbreviations

AE	Adverse events
AL	Anastomotic leakage
APE	Abdominoperineal excision
AR	Anterior resection
AR	Androgen receptor
ASA	The American Society of Anesthesiologists
BMI	Body mass index
CAG	Polyglutamine
CBG	Corticosteroid-binding globulin
CD	Crohn's disease
CI	Confidence interval
CR	Complete response
CRC	Colorectal cancer
CRM	Circumferential resection margin
CRT	Chemoradiotherapy
CT	Computed tomography
CTV	Clinical target volume
DHEA	Dehydroepiandrosterone
DHT	Dihydrotestosterone
DNA	Deoxyribonucleic acid
DXA	Dual-energy x-ray absorptiometry
ELAPE	Extralevator APE
EMAS	European Male Ageing Study
ER	Estrogen receptors
ERAS	Enhanced recovery programs
ESMO	the European Society of Medical Oncology
FAP	Familiar adenomatous polyposis
FDG-PET/CT	Positron emission tomography with fluorodeoxyglucose integrated with computed tomography
FSH	Follicle stimulation hormone
fT	Free testosterone
GEE	Generalized estimating equation
GI	Gastrointestinal tract
GnRH	Gonadotropin-releasing hormone
GTV	Gross target volume
Gy	Gray
HR	Hazard ratio
HU	Hounsfield units

IC/ICU	Intensive care/intensive care unit
IRR	Incidence rate ratio
LARS	Low anterior resections syndrome
LH	Luteinizing hormone
$L_{no.}$	No. lumbar vertebrae
LOH	Late-onset hypogonadism
LR	Local recurrence
LRA	Longitudinal regression analysis
MDT	Multidisciplinary team conferences
MRI	Magneti resonance imaging
OR	Odds ratio
PM	Psoas major
PTV	Planned target volume
QoL	Quality of life
RC	Rectal cancer
RR	Relative risk
RT	Preoperative radiotherapy
SARMs	selective androgen receptor modulators
SHBG	Sex hormone-binding globulin
SMI	Skeletal Muscle Index
SNCP-CRC	Swedish national care program for CRC
T	Testosterone
TAMIS	Transanal minimal invasive surgery
taTME	Transanal TME
TD	Testicular dose
TH	Thyroid hormones
TME	Total Mesorectal Excision
TNM	Tumor, lymph Nodes, Metastasis
TRT	Hormone replacement therapy
TV	Target volume
UC	Ulcerative colitis
xT	Tesla, x denoting field strength
"c-"	Clinical, preoperative, e.g. assessed by imaging
"p-"	Histopathological analysis of resected specimens

Contents

Background

Rectal cancer epidemiology

Rectal cancer (RC) was diagnosed in 2,145 people in Sweden 2016 and is thereby the 8th most common cancer, see Figure 1.[1, 2] Men are more likely to suffer RC than women, with an age-standardized incidence of approximately 26 versus 17 per 100,000 residents in Sweden, Figure 2.[1] Standardized for age, RC mortality has been stable the last decade, see Figure 3.

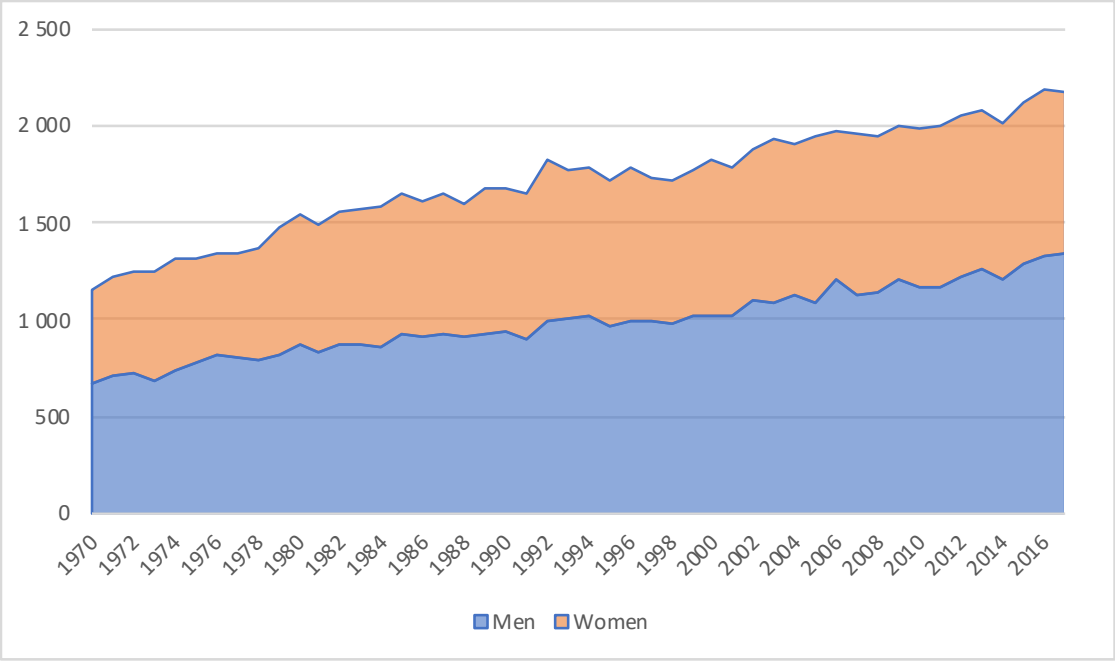

Figure 1. New cases of Rectal cancer in Sweden per year.

Source: The Swedish Cancer Registry, The National Board of Health and Welfare.

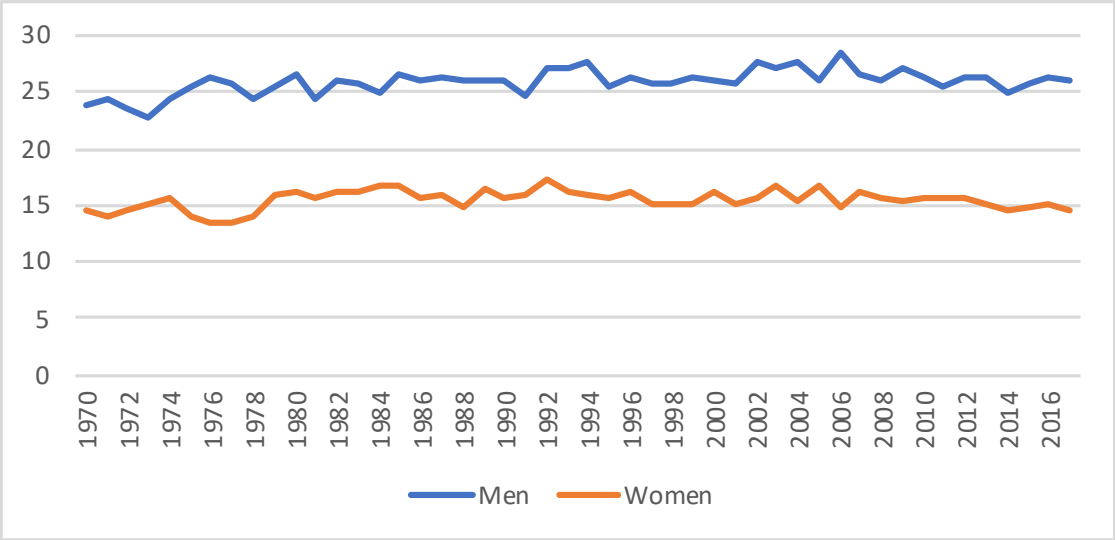

Figure 2. Age-standardized Rectal cancer incidence, per 100,000 persons in Sweden.

Source: The Swedish Cancer Registry, The National Board of Health and Welfare.

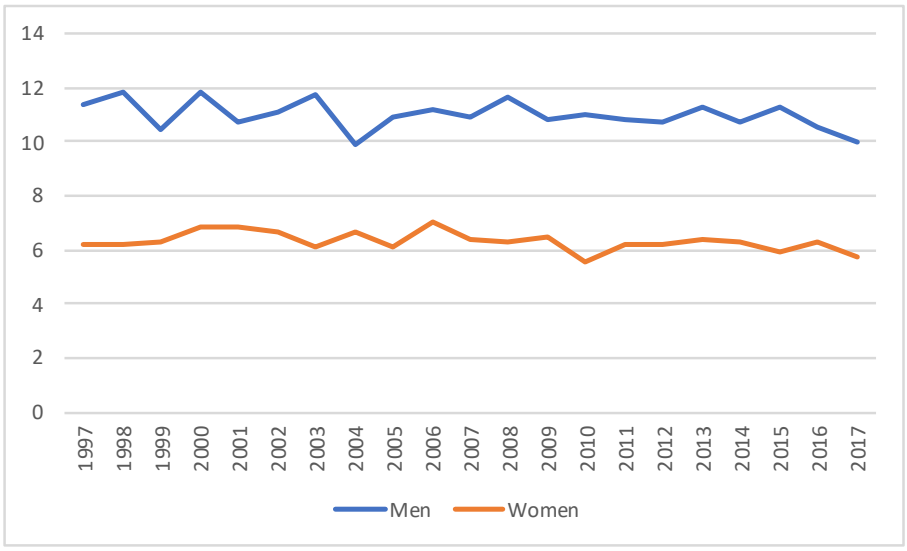

Figure 3. Age-standardized mortality rate due to Rectal cancer, per 100,000 persons in Sweden.

Source: The Swedish Cause of Death Registry, The National Board of Health and Welfare.

Worldwide, the incidence rate of RC differs between countries with generally higher rates in the more economically developed ones, illustrated in Figure 4.

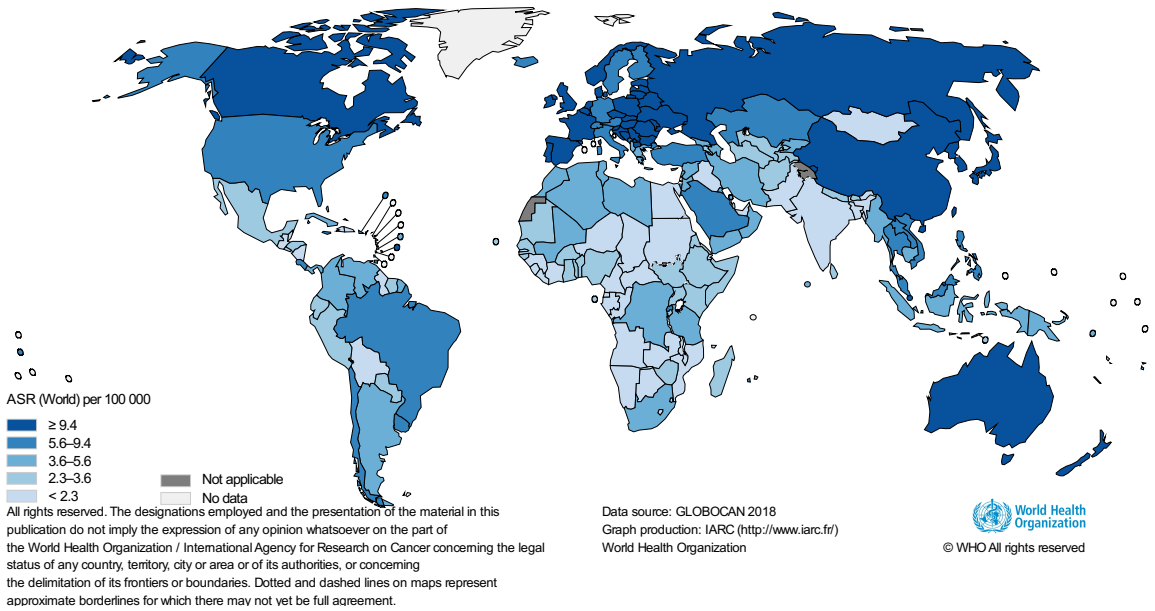

Figure 4. Estimated age-standardized incidence rates in 2018, rectal cancer, in both sexes of all ages. Source: GLOBECAN [3]

Risk factors for rectal cancer

As illustrated in Figure 4, the incidence rates of rectal cancer differ between regions, which is attributed to life-style factors including demographics, diet and physical activity. Differences in access to medical care and incomplete national registers may be a factor too. Indeed, it's been shown changes towards a more western life-style induce "western" incidence rates for cancer, e.g. migrants within few generations share the incidence rates of cancer of their new country.[4] Increasing age, smoking and high body mass index (BMI) is known risk factors for rectal cancer.[5, 6]

Dietary risk factors for rectal cancer are less clear than for colon cancer and fever specific studies have been conducted.[7] In a large meta-analysis, including 111 prospective studies, Vieira et. al

found a borderline association between processed meats and rectal cancer (Relative risk (RR): 1.08 for 50g/day increment, 95% CI =1.00–1.18, p= 0.77), a stronger one with alcohol (RR: 1.08 per 10g/day increment (95% CI = 1.07–1.10, p=0.54).[7] The Malmö Diet and Cancer Study found that, in men, high intake of beef has been related with increased risk of RC while intake of fish had an inverse relationship with RC risk in both sexes.[8] Comparison between non-drinkers versus drinkers of alcohol in a smaller meta-analysis yielded a RR 1.42 (95% CI 1.03–1.96).[9] The majority of studies report a combined risk of colorectal cancer (CRC), usually not stratified for adenocarcinoma of the colon and rectum separately. Red and processed meat and alcohol are generally accepted risk factors, however, vegetarians seem to have the same mortality from CRC as non-vegetarians.[7, 10, 11] High intake of beef was associated with less risk of colon cancer in women (men had a similar trend) while pork increased the risk in the Malmö Diet and Cancer Study.[8] Protective effects regarding CRC are attributed to whole grains, vegetables, dairy and fish.[7] The dietary factors in RC, colon cancer and, above all, the combined CRC, seems to be depending on sex, meat source and tumor location in the intestine, which could explain the sometimes conflicting results in different studies. The reason might be different embryological origins of the large intestine, dictating the local cell structure and function.[12]

Colorectal cancer risk is increased in persons suffering inflammatory bowel disease such as Crohn's disease (CD) and ulcerative colitis (UC).[13, 14] The risk of CRC increases even more if debuting at childhood (CD: Hazard ratio (HR) 5.8 (3.2 to 10.4), UC: HR 33.3 (23.1 to 49.1).[15] For rectal cancer specifically, UC but not CD, is a risk factor: IRR 1.84 (95% CI, 1.27-2.58) - 1.90 (95% CI, 1.05-3.43).[13, 14]

Oral antibiotic use is implicated in colon cancer risk, but seems protective regarding RC, findings recently verified in a large matched case-control study concluding that there was a dose-dependent risk increase in the proximal colon ($p<0.001$), especially regarding anti-anaerobic antibiotics.[16] A negative association was found between antibiotic use and RC ($p=0.003$), particularly if use exceeded 60 days. The association was detected with antibiotic use more than ten years prior to cancer diagnosis (OR=1.17 (1.06 to 1.31)).

Heredity

Heredity is an important factor in CRC, and has been linked to 2-8% of all CRCs.[17] Having a first-degree relative with CRC induces a RR of suffering CRC of 2.24 (95% CI 2.06 to 2.43), and having two or more increases the RR to 3.97 (95% CI 2.60 to 6.06).[18] Hereditary syndromes also induce high risk contribution to approximately 5% of all CRCs. These cancer syndromes are usually separated into polyposis or non-polyposis syndromes. The non-polyposis Lynch syndrome is the most common syndrome, resulting in CRC risk of 43% at a median age of 47 in women, and 66% at a median age of 42 in men.[19] Familiar adenomatous polyposis (FAP) is the second most common syndrome overall, with penetration approaching 100% at 40 years of age if inherited (approximately 30% are de-novo mutations).[17, 20]

Anatomy of the rectum

The gastrointestinal tract (GI) runs from the mouth to the anus and is derived from endoderm. The primitive gut evolves into three different parts, foregut, midgut and hindgut. The foregut gives rise to the tract from esophagus to the upper duodenum and the part from the suspensory muscle of the duodenum up to the last third of the transverse colon stems from the midgut. The last third of the transverse colon to the upper part of the anal canal is stems from the hindgut and, when fully evolved, ends at the linea pectinate, marking the junction from columnar glandular epithelium of the rectum to the squamous epithelial cell of the anus. The areas with different embryological origins have differing arterial supply. Structures evolved from the foregut are supplied by the celiac trunk and midgut structures rely on branches from the superior mesenteric artery. The rectum, evolved from the hindgut, is supplied by the superior rectal artery, originating from the inferior mesenteric artery. The inferior rectal artery originating from the internal iliac arteries and, with individual variation, the middle rectal artery supplies the most distal part of the rectum. The differing embryological origins probably explain the differencing characteristics of the proximal colon and the distal part of the GI. The rectal mucosa produces a more acidic mucin compared to proximal colon, which in turn has a more extensive capillary network suggested to be important in water re-absorption.[12]

The rectum is located in the pelvis with the upper third anteriorly and laterally covered by peritoneum viscerale, the middle third only covered anteriorly and the last part is inferior to the peritoneum parietale. Between the rectum and the prostate in men and the vaginal wall in women the rectoprostatic fascia or rectovaginal fascia (Denonvillier), respectively, with unclear embryonic origin, is located. The rectum is supported by the pelvic diaphragm and musculus levator ani and the arterial, venous and lymphatic vessels and tissue sustaining it is contained in a fatty structure termed the mesorectum by Prof. Heald in 1982.[21]

Definition of Rectal cancer

Rectal cancer is defined by the Swedish national care program for CRC (SNCP-CRC) and in the guidelines from the European Society of Medical Oncology (ESMO), as adenocarcinoma with distal tumor border located within in the last 15cm of the large intestine, measured from the anal verge with rigid rectoscopy.[22, 23] This definition is not universally used and the measured distance in definition of rectal cancer varies. The 15cm definition, as other general values, is arbitrary and real rectal length is dependent on patient characteristics, such as sex, height and weight.[24] This might lead to misclassification of tumor location and thus impact treatment. Magnetic resonance imaging (MRI) has been mentioned as an alternative instrument to determine tumor location but anatomical landmarks need to be standardized.[24, 25]

Symptoms of rectal cancer

Rectal cancer symptoms can be local, such as change of stool habits/urgency, blood or mucus in the stool or a sensation of not being able to empty the bowel. Pain is relatively unusual but may herald a locally advanced tumor infiltrating other tissues. A tumor obstructing the lumen of the intestine may result in symptoms of congestion. General symptoms, such as fatigue, anemia, loss of weight may herald systemic disease where the rectal cancer has seeded metastases to other

organs. Synchronous distant metastases are present in approximately a quarter of persons diagnosed with rectal cancer.[26]

Workup

At presentation of symptoms a digital rectal palpation followed by rigid rectoscopy, including measurement of tumor distance from anal verge, shall be done.[23] The tumor is described according to distance from anal verge, "low" < 5 cm, >5-10cm "middle" and >10-15 cm "high".[22] Colonoscopy is recommended to detect synchronous lesions, as well as for taking biopsies for histopathological classification. Most rectal cancers are adenocarcinomas but there are instances of neuroendocrine tumors, sarcomas, lymphomas, melanomas as well as metastases from other primary cancers.[27] Computed tomography (CT) colonoscopy may be used as a second alternative. In order to detect metastatic disease, CT thorax and abdomen is performed and, in RC, MRI of the lower pelvic area is important to investigate the extent of the tumor. In the case of a locally advanced tumor or distant metastasis, where the curative potential may be threatened, a positron emission tomography with fluorodeoxyglucose integrated with computed tomography (FDG-PET/CT) is performed.[23] Use of FDG-PET/CT increased in Sweden during the last years.

Staging

Usually the TNM classification system is used to describe the characteristics of RC. "T" describes the invasion of the primary tumor, "N" the regional lymph node status and "M" eventual metastatic disease, see Table 1.[28] Staging is done both preoperatively, clinical staging denoted with the letter "*c*" in front of the TNM classification, and postoperatively from pathological evaluation of surgical specimens with the prefix "*p*" with an added "*y*" if neoadjuvant treatment was used. Currently the 7th edition of the TNM-classification is recommended in the SNCP-CRC but 8th edition, with minor differences, has been in place since late 2016.[28] Some important notions are that in 8th carcinoma in situ is termed intramucosal adenocarcinoma, less than 20 isolated tumor cells are N0 and micrometastases, clusters of more than 20 tumor cells or metastases measuring more than 0.2 mm but less than 2 mm in diameter, are denoted N1, see Table 1.[29]

Multidisciplinary team conference

Multidisciplinary team conferences (MDT) are mandatory in Sweden in regards to RC, according to the SNCP-CRC, and a case be addressed at several MDTs if needed during the course of treatment.[23] The MDT involves the specialties that are needed for its purpose: an evidence based best-of-care approach to RC treatment, and includes colorectal surgeons, radiologists, oncologists, pathologists and specialized nurses. If needed, a patient can be discussed at several stages of its treatment to assure best possible treatment.

The MDT recommends treatment regimens, including neoadjuvant treatment, according to criteria regarding perceived risk of local recurrence (LR) or systemic recurrence. A popular stratification is *the good*, *the bad* or *the ugly*, representing "early", "intermediate" or "advanced" local disease respectively.[30] This stratification has been used and modified for two decennia in Sweden. Recently, new ESMO-guidelines introduced five groups for a more tailored treatment.[22]

Table 1 TNM-classification of colorectal cancers, 8th edition.

T - Tumor		
TX	Primary tumor cannot be assessed	
T0	No evidence of primary tumor	
Tis	Carcinoma in situ, intramucosal carcinoma (involvement of lamina propria with no extension through muscularis mucosae)	
T1	Tumor invades submucosa	
T2	tumor invades muscularis propria	
T3	Tumor invades trough the muscularis propria into the pericolorectal tissues	
	T3a	Minimal invasion: <1mm beyond the borders of the muscularis propria
	T3b	Slight invasion: 1-5 mm beyond the of the muscularis propria
	T3c	Moderate invasion: >5-15 mm beyond the borders of the muscularis propria
	T3d	Extensive invasion: >15 mm beyond the borders of the muscularis propria
T4	Tumor penetrates the visceral peritoneum and/or directly invades other organs or structures	
	T4a	Tumor penetrates to the surface of the visceral peritoneum
	T4b	Tumor directly invades or is adherent to other organs or structures
N - Lymph nodes		
NX	Regional lymph nodes cannot be assessed	
N0	No regional lymph node metastasis	
N1	Metastasis in 1-3 regional lymph nodes	
	N1a	Metastasis in 1 regional lymph node
	N1b	Metastasis in 2-3 regional lymph nodes
	N1c	Tumor deposit(s) in the subserosa, mesentery or nonperitonealized pericolic or perirectal tissues without regional nodal metastasis
N2	Metastasis in ≥4 regional lymph nodes	
	N2a	Metastasis in 4-6 regional lymph nodes
	N2b	Metastasis in ≥7 regional lymph nodes
M - Metastasis		
MX	Distant metastasis cannot be assessed	
M0	No distant metastasis	
M1	Distant metastasis	
	M1a	Metastasis confined to one organ or site
	M1b	Metastasis in more than one organ/site or peritoneum
Adapted from Amin et al.[28]		

Radiotherapy

The effects of irradiation were first hinted as the research into the field of x-rays intensified in end of 19th century and dawn of the 20th. The irradiation caused cutaneous burns, and in analogue to other treatment modalities, radiation was used to treat skin diseases, including epithelioma and basal cell carcinoma, as well as benign outgrowths. The use of radiotherapy (RT) and, little later, radium was booming and subject to strong publication bias. However, evidence of adverse effects was piling up and the need to curb the, sometimes rampant, use of irradiation was acknowledged. In the 1920s Henri Coutard, building on the findings of Claudius Regaud, both working at the Curie Institute, presented data showing that fractioning RT had better effect with less adverse effects. In the early 1930ies, Coutard had refined a model with fractionated RT that is the basis of current RT.[31]

The two main objectives of preoperative RT in RC treatment are to decrease the tumor size and sterilize the excision margins. The decrease in size caused by RT is due to cancer cell death from; necrosis, uncontrolled death with highly inflammatory environment; apoptosis, controlled cell death with little or no inflammation; failed cell division at mitosis, mitotic catastrophe. Irradiation damages cells by various pathways, including directly destroying chemical bonds in molecules/proteins or by creating free oxygen radicals that in turn damage chemical bonds, both may disrupt cell membrane integrity, initiate apoptosis or induce DNA damage that will prevent proper mitosis. The radiation dose absorbed by the targeted tissue, joules per kg, is measured in Gray (Gy). Radiotherapy induces differing effects in different tissues and cancer types depending on their characteristics. Tissues with high cell proliferation, such as cancers in general, are more susceptible to damage as the effect of RT peaks at mitosis. Sterilization of the excision margin aims at removing undetected cancer cells outside of the main cancer mass in order to minimize the risk of LR. In order to achieve these objectives, the target volume of RT comprise the primary tumor and a safety margin, as well as regional lymphatic tissue. Fractioning RT allow for a differentiation between normal tissue and the tumor trough what is known as the four R´s: Repair of damage in normal and cancer cells, Reoxygenation of the cancer, Redistribution trough the cell cycle toward mitosis, and Regeneration of cells between fractions.[32] The mostly used RT regimens in Sweden today are both preoperative and consists of either 5 x 5 Gy "short course" (sRT) or 28 x 1.8 Gy with concomitant chemotherapy, "long course" or chemoradiotherapy (CRT).

Effect of Radiotherapy in Rectal Cancer treatment

The Stockholm Colorectal Cancer Study Group formed 1980 started the Stockholm I trial to elucidate the effect of RT in RC treatment. The study compared traditional surgery versus traditional surgery with preoperative sRT.[33] The trial found that sRT cut the local cancer recurrence risk in half, from 30 to 15%, but quadrupled the risk of postoperative complications and mortality from 2% to 8%.[33] The increased postoperative mortality was primarily driven by cardiovascular death in study participants older than 75, and the findings were incorporated in the next trial. Stockholm II trial included younger patients, used an evolved RT technique and the trial could repeat the relative risk reduction of 50% in RC recurrence but this time without increased mortality risk.[34] The study concluded that RT should be an alternative in RC treatment but age and cardiovascular co-morbidity must be considered at an individual level when recommending RT. The Swedish Rectal Cancer Trial found similar results regarding local recurrence and, additionally, a benefit in overall survival (HR: 0.79, 95% CI 0.66-0.92) and cancer-specific survival (HR: 0.69, 95% CI 0.55-0.83), findings that were deemed trustworthy in a follow-up study.[35,36] In the Dutch Total Mesorectal Excision (TME) -trial modern surgical technique was used as baseline and study participants randomized to no RT or preoperative sRT and surgery within one week.[37] The TME-trial found that even if LR in general was lower with TME technique compared to traditional surgery, sRT more than halved cumulative LR risk after ten years follow-up (from 5% to 11%).[37] Several studies, the Uppsala Trial among them, have found that preoperative RT seems preferable to post-operative RT in regards to LR, even if the latter minimizes the risk for unnecessary treatment.[38-40] Chemotherapy in CRT is a sensitizing agent, i.e. it makes the tumor cells more susceptible to RT. The use of full-dose chemotherapy after sRT is increasing, under the LARCTus-

study protocol (clinical trials no.: NCT03729687), based on the RAPIDO trial which had not been reported yet.[41]

Timing of Radiotherapy and Surgery

The timing of surgery, to operate directly after RT treatment or to delay surgery, have been up to debate. A delay between RT and surgery is that it allows for tumor downstaging/regression, sometimes to complete tumor regression, which is linked to improved survival.[42, 43] The possibility of complete tumor regression has been mostly attested to CRT, where the concomitant chemotherapy is attributed to a large part of the effect and in one study increasing the possibility for pathological complete response (pCR, from histopathological analysis of resected specimens) from 7% to 16%, p=0.04.[44-46] In sRT, tumor regression and even pCR has been observed if surgery is delayed more than 3 weeks, and in the Stockholm III trial sRT with delay had the highest frequency of pCR (10.4% vs 2.2% in CRT).[47-52] An optimal delay has not been found, studies comparing six to 12 weeks delay, and nine to 14 weeks delay, both found the longer delay beneficiary in regards to tumor regression.[53, 54] To the contrary, the GRECCAR-6 trial found no statistical difference between seven weeks and 11 weeks delay in regards to pCR.[55]

In analogy to organ preservation in anal cancer treatment, Prof Angelita Habr-Gama from Sao Paulo started to await with surgical resection in patients with clinical and radiological signs of complete tumor regression.[56] This selected group of patients is followed closely to detect cancer regrowth as soon as possible, a regimen termed "Watch-and-Wait". Estimates of local regrowth range between 21-25% and the possibility to surgical salvage in case of regrowth range from 90% to 93% in current literature. Due to differing study designs, a solid meta-analysis has been difficult to produce. Compiled data of 15 studies in 2017 shows an pooled overall survival of 92% after 23-68 months.[57] More recently, a systematic review and meta-analysis including nine studies reported surgical salvage in 10.5% of the 248 patients treated according to Watch-and-Wait, with a relative risk of overall mortality of 2.42 (95% CI 0.96-6.13) and cancer specific mortality 2.63 (95% CI 0.81-8.53) compared to traditional treatment.[58] A problematic facet of Wait-and-Watch is the patients whose tumors doesn't regress as they wait will have their surgery delayed without apparent benefit. Factors predicting a possible complete clinical response due to RT includes: lower tumor grade, lower cT and cN stage, higher radiation dose, and an interval of more than 6–8 weeks between RT and surgery.[59] Currently, data is compiled in the International Watch-and-Wait database for further evaluation.

Delaying surgery seems also beneficial regarding risk of postoperative complications. The Stockholm III trial recently concluded that delaying surgery 4-8 weeks after sRT compared to surgery within one week significantly decreased postoperative complications (OR 0.61, 95%CI 0.45-0.83).[60]

Radiotherapy recommendations and procedure

Radiotherapy in Sweden is administered the prone or supine position and in accordance to the MDT, general guidelines are shown in Table 2. A suprapubic reference point is tattooed and used throughout the planning and administration of the RT. Dose planning and target volume (TV) are

made using a dose-planning CT (dpCT) or MR, covering the volume between the 4th lumbar vertebrae and distal to the anus. The MRI information from workup complement the dpCT. The volumes of interest are: Gross Target Volume (GTV), Clinical Target Volume (CTV) and Planned Target Volume (PTV).

Table 2 Rectal cancer, neoadjuvant treatment.

Tumor distance from anal verge	T1-T2	T3a-b	T3c-d	T4a	T4b	N1	N2	mrf+	Lat. lgl	EMVI
-High, 10-15cm	0	0	5x5	5x5	5x5/CRT	0	5x5	CRT	CRT	5x5
-Middle, 5-10cm	0	5x5	5x5	5x5	5x5/CRT	5x5	5x5	CRT	CRT	5x5
-Low, 0-5cm	5x5	5x5	5x5	-	5x5/CRT	5x5	5x5	CRT	CRT	5x5

Notes: T = Tumor stage. N = Lymph node stage. (See Table 1) mrf+ = mesorectal fascia engaged (<1mm margin). Lat. lgl = Pathological lymph nodes outside mrf. EMVI = Extramural vascular invasion. 5x5 = Radiotherapy 25 Gy in five fractions. CRT = Chemoradiotherapy, 50.4 Gy in 28 fractions with concomitant chemotherapy. Adapted from the Swedish national care program for Colorectal Cancer.[23]

When including regional lymph nodes, CTV is denoted CTVN. The Clinical Target Volume includes the primary tumor and a 1-2 cm margin within the mesorectum, the primary and the closest secondary lymph nodes. The Planned Target Volume incorporates CTVN and adds a margin of 0.8cm to 1.3 cm, the latter on the ventral side and (denote PTVN. During planning, GTV is adjusted according to the stage of the tumor, with ugly tumors having expanded volume covering structures related to tumor overgrowths. Currently, organs at risk are not defined in sRT, but in CRT the bowel bag, bladder, pelvic bones and genitals are designated.

Surgery

Surgical intervention in rectal cancer historically had poor outcomes.[61] The complex area of the pelvis is difficult to operate in and, prior to the 1980ies, blunt dissection technique was standard, with high risk of massive bleeding and associated with high morbidity and mortality. Postoperatively, autonomic nerve damage was common with adverse impact on urinary-, sexual- and bowel functions. Blunt dissection often failed to completely remove tumor and which led to a high LR, and, compounded by the inability to remove regional lymph nodes increasing risk for systemic disease, resulted in a high rectal cancer mortality.

Abdominoperineal excision

At the beginning of the 20th century, Sir Willian Ernest Miles, from The Cancer Hospital in London and Gordon Hospital for Diseases of the Rectum, UK, found that in rectal cancer surgery the volume of possible outward spread of the tumour must be excised. This in effect means the pelvic colon, the pelvic mesocolon and iliac lymph nodes combined with a wide perineal excision. At the time, this was done bluntly and the postoperative mortality was 42% which was deemed acceptable as the most likely alternative was recurrent cancer and death.[62] With his new approach, Sir Miles

could decrease the frequency of LR from 95% to 30% and the procedure, abdominoperineal excision (APE) became the standard procedure until the introduction and spread of AR.

Total Mesorectal Excision

After introduction of the TME surgical technique, published in 1982 by prof. Bill Heald from the Basingstoke District Hospital, UK, local recurrence dropped from 30-40% to 5-10 %.[61, 63, 64] Total Mesorectal Excision infers visually controlled sharp dissection along embryologically defined planes, with the aim to remove the rectum and mesorectum, including blood vessels and lymphatic structures, *en bloc*, i.e. as a intact specimen.[21] By doing so, previously common adverse surgical complications, such as massive bleeding, nerve damages and local recurrence can be reduced substantially.[61, 64]

Currently, and still based on TME, the anterior resection (AR) is the standard procedure in rectal cancer surgery in regards to tumors situated in the upper or middle rectum, without engagement of skeletal muscle of the pelvic floor or external anal sphincter. Relative contraindications are diminished preoperative sphincter function and fecal incontinence and severe co-morbidity. Low tumors often engage skeletal muscle and are thus treated by APE. The inherent risk with an anastomosis is anastomotic leakage (AL), a postoperative complication with comparatively high morbidity and mortality. In order to mitigate the risk of AL a proximal, temporary, stoma is often created.[65] If an anastomosis is not deemed a viable alternative, usually dependent on poor physical status in the patient or emergency, Hartmann´s procedure or intersphincteric APE with an colostomy may be performed.[66-68]

APE evolved

With the introduction of the TME technique the pronounced improvements regarding LR did not really translate to the full extent in APE.[69] At that time, APE was performed according to TME standards in the abdominal segment but the perineal approach was done in part bluntly due to bad visibility. There were several problems with perineal part, one being that the procedure starts close to the externals sphincter and follows the perianal muscles towards the pelvic floor to meet up the abdominal part resulting in a waist at that location where there is a risk of compromised circumferential resection margin (CRM), compounded by the bad visibility. Another problem was that there was no clear standardization of the procedure making comparisons and refinement difficult.

To address the relative worse outcomes in APE compared to AR, the extralevator APE (ELAPE) technique was developed by Professor Torbjörn Holm, from the Karolinska University Hospital in Stockholm. In ELAPE the abdominal dissection is terminated before the pelvic floor at the top of the levator ani muscle and the perineal dissection is done outside the external sphincter, along the levator ani fascia up to its origin where it meets the abdominal dissection. This results in a tubular specimen without a waist. Observational studies confirm a better outcome regarding CRM and perforations.[70-72] Several meta-analyses have been conducted with differing results, one including 949 patients found that ELAPE had less perforations, less positive CRM rate and less LR compared to APE.[73] Another, larger, found no benefits regarding CRM or LR, but confirmed

significantly less perforations in ELAPE compared to conventional APE.[74A] A recent multicenter study did find significant benefits of ELAPE in overall, disease free and local recurrence free survival, particularly in advanced tumors.[75]

Intersphincteric APE is another variant, suitable when a low anastomosis is unfavorable. The perineal dissection is done between the internal and external sphincter and along the proximal surface of the levator ani and meets the abdominal part at the level of the puborectal muscle. In locally advanced cancer, if including levator muscles, ischioanal fat and/or perianal skin, or in the case of perforated cancer with abscess or fistulation in the ischioanal compartment, ischioanal APE is indicated. Ischioanal APE includes the levator ani muscle, the ischioanal fat (including a perianal fistula if present) and, if skin involvement, a wide skin excision.[69]

Laparoscopic surgery

Minimal invasive techniques, i.e. standard or robot-assisted laparoscopic surgery, are gaining ground and are currently applied in Sweden approximately 50% of rectal cancer patients. About 40 % of the laparoscopic surgeries are robot-assisted, an increasing trend.[76]
Minimal invasive surgery benefits, in relation to open surgery, include decreased time to recovery and length of hospital stay and quicker return of bowel function.[77, 78] The drawback of laparoscopic surgery is longer operating time and non-inferiority when compared to open surgery could not be established in T1-T3 rectal tumours in the ALaCaRT trial or the ACOSOG Z6051 trial including stage II-III rectal cancers, risk difference of -7.0% (95% CI, -12.4 - ∞) and -5.3% (95% CI, -10.8% - ∞) respectively.[79, 80]

A novel technique is transanal TME (taTME), laparoscopic abdominal surgery combined with transanal access to perform a bottom up TME trough a natural orifice. A systematic review and meta-analysis found favourable outcomes regarding positive CRM (OR = 0.39, 95% CI = 0.17–0.86), operating time (weighted mean difference = -23.45, 95% CI = -37.43 to -9.46) and overall postoperative complications (OR = 0.65, 95% CI = 0.45-0.95). [81] Given that the methodology of taTME is relatively new, more prospective studies are needed.

Local excision

In early stage cancers, such as Tis, or even in cases with cancers that have regressed enough from neoadjuvant treatment, local excision may be an alternative to organ preservations and thereby minimize treatment-related adverse functional outcomes.[82, 83] Local excision may be performed transanally with techniques allowing for direct visual control, with transanal endoscopic microsurgery (TEM), or with transanal minimal invasive surgery (TAMIS) using laparoscopic instruments. The benefits of local excision are less morbidity and earlier hospital discharge but the draw-back is increased risk of LR, with the more advanced TEM or TAMIS having a lower risk for LR than transanal excision.[84] Patient selection is of importance and in addition to MRI, endoscopic ultrasound should be used for correct assessment of T-stage.

Current results of rectal cancer treatment in Sweden

In Sweden the average risk of LR is below 3% after three years in regards to tumours classified as T1-T3 M0, with or without RT, and 6% for T4.[76] After five years the total LR risk is below 5%, but the coverage ratio is less than for the 3-year follow up, decreasing the possibility to draw conclusions from the material.[76] The relative three-year survival (any death), in all patients without metastases is 87%, and 94% in patients having a resection.[76] The stadium of rectal cancer have a large impact on survival, in patients with stadium I disease the relative five-year survival was 0.95 while for IV disease its 0.2.

Adverse effects and postoperative complications

The treatment for rectal cancer, both RT and surgery, also incurs adverse effects and the risk of potential complications as well as purely structural changes, e.g. removal of the rectum and its niche in the bowel function.

Functional loss

By removing the rectum and distal colon to a varying degree infers loss of their inherent functions: the ampulla recti store feces until stretch sensors in the rectal wall triggers the urge to defecate and the distal part of the colon, colon descendens and colon sigmoideum, reabsorbs water and helps in feces storage. In sphincter-preserving surgery, low anterior resections syndrome (LARS), characterized by urgency, frequent bowel movements and sporadic fecal incontinence, is not uncommon.[85] Risk- and severity factors for LARS are low remaining rectal volume, end-to-end anastomosis, AL, inflammation, neoadjuvant RT, female sex and age over 65 years.[86, 87] The symptoms are usually worse just after surgery and decreases during the first year until stabilizing. In surgery including the sphincter, the resulting permanent stoma may lead to negative body image impacting physical and social functioning as well as stoma-related morbidity.[85]

Radiotherapy toxicity

Radiotherapy treatment may infer short-term, or acute, as well as long-term adverse effects.[88] The symptoms of RT toxicity depend on effected organs, those included in the radiated volume, PTV, as well as those exposed to indirect or scattered radiation. Adverse effects presenting during RT, or within three months of RT, e.g. mucositis, are labelled short-term adverse effects and usually resolve within weeks to months. After the first three months the long-term effects, e.g. fibrosis, may be detected.

In rectal cancer, acute toxicity is more often present in CRT than sRT, chemotherapy being a known risk factor for mucositis for example.[89] The Stockholm III trial found that 7% of the study participants having sRT with delay developed acute toxicity symptoms requiring hospitalisation prior to surgery, compared to less than 1% in the group treated with sRT without delayed surgery, probably due to the fact they were admitted for surgery at the time (OR 24.7, 95%CI 3.3-183.7).[60] Urinary dysfunction, such as increased frequency and urgency, are more frequent in patients treated with RT.[90]

Long-term adverse effects of RT in rectal cancer are added onto the present surgical adverse impacts, compounding the symptoms. Specific long-term effects of RT include pelvic or femoral fractures, thromboembolic disorders, bowel obstructions, bowel dysfunction presented as faecal incontinence, evacuation problems or urgency, and sexual dysfunction.[87] Pelvic insufficiency fractures, presenting with chronic pelvic pain was detected in 12.2 % of 1100 patients during a follow up of 36 months in a Danish study.[91] Sexual dysfunction in men, including erectile and ejaculatory problems and decreased overall sexual function have been described, echoing findings in irradiated prostate cancer patients.[92-94] Persisting hypogonadism is potential risk after RT effecting the testes.[95] In analogy, women suffer dyspareunia, vaginal dryness and decreased overall sexual dysfunction due to RT.[96] Further, the ovaries may be impacted, adversely affecting fertility and hormonal levels resulting in reduction of sexual desire. [83,97] In the Dutch TME-trial, RT was linked to long-term decreased quality of life.[98]

Studies using data from 1980ies and -90ies suggested that RT, in rectal cancer, induced secondary cancers (RR 1.85, 95% CI 1.23-2.78).[99] This could not be verified in a large, more recent study, using the Swedish Colorectal Cancer Registry including over 13000 patients and covering 20 years.[100] There was decreased risk of prostate cancer. However, even as prostate cancer risk decreased, RT in rectal cancer treatment has been linked to gynaecological cancers, lung cancer and lymphoma.[101-103]

Postoperative complications

Current statistics for Sweden report 0.5% 30-day and 1.1% 90-day perioperative mortality in 2018 for rectal cancer patients that had been operated. The low mortality makes inference to causes difficult, but increasing age and comorbidity increases mortality risk.[76] Globally, in data analyzed 2010, perioperative mortality in rectal cancer treatment reached 2%.[104] Historically, AE have been reported by various means making comparisons problematic. In 2004 Clavien et. al published a system for reporting AE, it has since been widely adopted.[105] In global data, 11% suffered an AL and 12% contracted a pelvic sepsis.[104] Pelvic sepsis and AL greatly increases the risk of a permanent stoma. In a Swedish registry study, including 1442 patients treated with AR, 10% had an AL and 65% of those ended up with a permanent stoma.[106]

Abdominoperineal excision, and to an even larger extent ELAPE, infers perineal wound complications and infections, which are even more relevant if RT is used with an increase from 15% to 30% in regards to APE.[107-110] Preoperative RT increases the risk for postoperative adverse events (AE) in general.[33, 111]

The COLOR II study found that rectal cancer treatment negatively impacts sexual and urinary function, and while urinary symptoms usually improve within six months, sexual problems persists for at least two year in men.[112] Sex seems to be of great importance, e.g. male sex doubles the risk for AL, independent of tumor level, and increases the risk of wound disruption with 50 percent.[113-116]

Factors mitigating postoperative complications

Smoking cessation for at least 4 weeks prior to surgery have been found to slash the risk of postoperative complications almost in half, a risk reduction level that has also been found in enhanced recovery programs (ERAS).[117-120]

Androgens

Biosynthesis

Testosterone (T), the main androgenic hormone, is mainly produced by the Leydig cells in the testis in men and, to a far lesser extent, in the ovaries in women.[121, 122] Additionally, in both sexes, the inner and middle layers of the adrenal cortex produce dehydroepiandrosterone (DHEA) and androstenedione, and other androgens, which in turn are converted to T and estradiol in target tissues. Three to ten mg of T, equaling serum T levels of 10.4 to 34.7 nmol/L, is produced every day by adult men after regular puberty.[123] In men without Leydig cell function, by orchiectomy or androgen depravation therapy in prostate cancer, T levels range from below 0.69 to 1.73 nmol/L, highlighting the importance of the testis for T production.[124] Testosterone levels for castrate definition is suggested to be below 0.7 nmol/L.[125]

In the Leydig cells cholesterol, de novo synthesized inside the cells themselves using acetyl-coenzyme A or obtained from circulating low-density lipoproteins and stored in fat vacuoles, is converted to T. The initial, and rate limiting, step in the synthesis of T, and all other steroid hormones, is the conversion of cholesterol to pregnenolone on the inside of the mitochondrial membrane by the enzyme cytochrome P450scc. Subsequent steps in the synthesis of T are done in the Leydig cells' smooth endosomatic reticulum and in total five different enzymes are involved in the process. There are four different pathways in T production, the preferred in the testis is called 5Δ-synthesis pathway and where the 21 carbon pregnenolone trough progesterone, 17-α-hydroxypregnenolone, dehydroepiandrosterone (DHEA) and androstenediol is converted to the 19 carbon T. Testosterone, being lipophilic, passively diffuses trough the cell membrane after synthesis and the Leydig cells do not in general store T. The testes also produce dihydrotestosterone (DHT) from T, but most, 80 %, of DHT is produced in target cells. The testes also utilize aromatase to produce some estrone and estradiol.

Regulation

Testosterone production, and spermatogenesis, is regulated by the hypothalamic-pituitary-gonadal axis.[121] Kisspeptin, a peptide hormone mainly produced in the hypothalamus, controls, in a way not yet completely mapped, the pulsatile release of gonadotropin-releasing hormone (GnRH).[126] Every 60-90 minutes, GnRH is released in pulses from the hypothalamus and trough the hypothalamic-pituitary-portal system acts on gonadotroph cells in the anterior pituitary.[127] The gonadotroph cells produce the main gonadotropins, luteinizing hormone (LH) and follicle stimulating hormone (FSH). The release of LH and FSH, although pulsatile, are not on the same frequency: LH is released in pulses approximately eight to 14 times per 24-hours and FSH even fewer due to the longer half-life of circulating FSH.[121] The gonadotroph cells response to GnRH-stimulation require time to replenish GnRH receptors, if they are continually exposed to GnRH

or GnRH analogues as for example in chemical castration, the cells will cease excretion of LH and FSH. There is a circadian component in the release of LH and FSH as well, with a peak in early morning and decreasing levels of T during the day clearly visible in younger men but absent on older men.[128] Due to the diurnal variation, sampling of T should be done prior to 11 a.m.[129]

The gonadotropins are released into the circulation and act on the target organ, i.e. the testis in men. Luteinizing hormone, by binding to specific cell surface receptors on the Leydig cell, increases the conversion of cholesterol to pregnenolone by stimulating synthesis and activity of cytochrome P450scc as well as other enzymes and proteins that involved in the synthesis of T. The activation of the LH-receptor also results in expedited cholesterol availability, trough different pathways, to cytochrome P450scc, thereby increasing the substrate for T.

Follicle stimulating hormone primarily acts on Sertoli cells, the cells responsible for spermatogenesis. Sertoli cells and Leydig cells cross-talk and FSH, by acting on Sertoli cells does to some extent impact Leydig cell function.

In men in general, T levels peaks at puberty, levels out and then decline with increasing age.[130] This was verified in a large (n = 2,395) randomized longitudinal study, showing a mean serum T decline with 0.1 ± 0.95 nmol/L per year after the age of 40.[130] The decline is exacerbated by obesity and age-related chronic disease.[131]

Distribution

Ninety-five percent of the circulating T is produced by the Leydig cells in the testes. Testosterone is hydrophobic and thus does not passage readily on its own in the circulation, indeed, only about 2.9 percent is free (fT) or unbound in plasma according to the latest measuring/calculation techniques.[132] Approximately 45 % of T is tightly bound to sex hormone-binding globulin (SHBG), itself mainly produced in the liver. Testosterone bound to SHBG was earlier considered basically biologically inactive.[132] New evidence has shown that allostery between SHBGs two binding sites for T allows for a dynamic previously unknown. Thus, the free fraction of T is not strictly related to serum T concentration over a wide range. New findings suggest that SHBG may interact with cell membranes trough an androgen-binding protein and/or be internalized in cells and initiate a biological action.[133] Factors and conditions that increase SHBG, such as age, hyperthyroidism and liver disease decrease fT, but not to the extent earlier thought.[132] Certain conditions decrease SHBG, namely obesity and diabetes, probably due to low-grade inflammation and increased quantities of hepatic lipids.[133] About 33 to 54 % of T in circulation is compatibly loosely bound to albumin, the most abundant protein in the serum, and may dissociate at capillary level to varying degree depending on local conditions and thus, together with fT, termed bioavailable T.[133, 134] In addition, orosomucoid and corticosteroid-binding globulin (CBG) also transport T but their, to date incompletely comprehended, role is believed to be less significant.[133] Currently, the main theory is still that it's the free fraction of T that diffuses into target cells and binds to the androgen receptor (AR) in order to mediate its effect. In some cells, e.g. in the prostate, T is converted to the 30-50 times more potent DHT by the enzyme 5α-reductase. Certain target cells may also convert T to estradiol using aromatase. In contrast, possible target cells in peripheral

tissue have been found to have the capability to convert the, in circulation, more abundant DEHA to T or DHT, effectively increasing their possible intracellular concentrations.[135] Estradiol binds to its specific receptor but DHT binds the AR.[121] The androgen receptor is a homodimer in the family of nuclear receptors, T and the AR forms a complex that is a transcription factor.

Metabolism

Testosterone, and other androgens, are mostly and efficiently metabolized in the liver to 17-ketosteroids. The metabolites are primarily water soluble and excreted in the urine and to a lesser extent in the feces. Less than two percent are deposited as T in urine.[121] The half-life of fT in circulation is around 10 minutes.

Effects

The classical and widely acknowledged effect of the T-AR complex in the nucleus is increased transcription and production of proteins, with androgenic and/or anabolic properties.[136] Additionally, in the last few decades mounting evidence describes non-genomic signaling, with effect within seconds to minutes, interacting with cell membrane-bound or cytoplasmic proteins.[136] Possibly, this non-genomic effect mainly influences the classical AR, and other steroid receptors, genomic activity.[136]

Androgenic and anabolic response are tissue specific and varies during development and adulthood.[137] The androgen and estrogen receptors are widely distributed in tissue, including muscle, bone and the brain. Even different muscles have differing number of AR.[138] Androgenic effects are those related to development of the male sexual characteristics and anabolic are the growth-promoting effects on somatic tissue.[121] Mutations in the AR gene may result in varying grades of androgen insensitivity, ranging from infertility to having female genitals and no or little pubic hair.[139] Another example is polymorphism in the polyglutamine (CAG) repeat sequence in the AR, which affects the transcription intensity of the AR, fewer CAG repeats results in stronger androgen effect.[140] Having more repeats than 38 is associated with androgen resistance and spinal-bulbar muscular atrophy.[140] The androgenic effects are most significant during development in the embryo and during puberty, in adulthood its mainly maintaining the male phenotype.

Anabolic effects of T traditionally include dose-dependent increase of appendicular skeletal muscle mass, by growth of existent muscle fibers, and muscle strength in men of all ages, as well as loss of adipose tissue.[141-145] Additionally, bioavailable T has recently been shown in a pilot study to have an association with abdominal muscle area.[146] The hypertrophic effect of T on muscles has been abused since last century in order to gain muscle mass, increase performance and endurance, lose fat mass and to increase masculine appearance.[147] It is T, and probably not DHT, that seems to be the effective hormone in regards to muscle mass, as shown by Bhasin et al.[148] The mechanisms behind the hypertrophic effect of T on muscles are not fully elucidated but evidence suggest that T interacts in several steps in muscle remodeling.[142] Skeletal muscles are syncytium of multinucleated cells, fibers, surrounded by collagen matrix and linked to motor neurons. Adjacent to the muscle fibers are pluripotent satellite cells capable to differentiate into myoblasts, that in turn may fuse with the fibers in response to damage for example.[142] Testosterone influences the

synthesis and breakdown of fibers as well as pluripotent stem cell commitment and differentiation into satellite cells and myoblasts.[142] Further, it seems the initial number of motor neurons during growth and the maintenance of size is associated with T.[142] Testosterone within normal levels are mainly seen to halt protein degradation, and are by far not sole determinant of muscle size or power, physical exercise is a key component to muscle hypertrophy and possibly due to intracrine androgen action.[135, 149] As well as different muscles express different number of AR, they also react differently to exercise and steroid exposure.[138] Low T, and especially low fT, have been associated to frailty, even when adjusted for possible confounders such as morbidity.[150] Testosterone replacement therapy in older men with T in the lower half of normal ranges, a notably effect on muscle mass and power was found, with a difference depending administration intensity, weekly versus monthly, where the former had a stronger effect in type 1 or slow fibers.[151]

There seems to be a high individual variation in optimal T levels to maintain lean mass, fat mass, strength, and sexual function.[152] Eighty percent of the circulating estradiol in men comes from T, converted by aromatase, and T and estradiol levels in blood are positively correlated.[153, 154] In men, estrogen deficiency seems to increase body fat and decrease in sexual function.[152]

Androgen receptors are found in both osteoblasts and osteocytes and T has a large role in skeletal growth and homeostasis.[155] Testosterone promotes bone metabolism primarily through conversation to estradiol in the osteoblasts.[156] The conversion is critical for bone density, strength as well as for linear growth and closure of the epiphyses and individuals without aromatase or estrogen receptor deficiency will suffer osteoporosis, open epiphyses and high linear growth rate.[157-159] For periosteal growth, there seems to be an additive effect of T and estrogens.[155]

Hypogonadism

Diagnosis of hypogonadism, defined by The Oxford Dictionary as "reduction or absence of hormone secretion or other physiological activity of the gonads (testes or ovaries)" has increased during the last years in Sweden, see figure 5. Hypogonadism occurring before puberty, if left untreated, results in incomplete male phenotype, with eunuchoid phenotype including abnormal tallness, small penis and testes, sterility, impaired development of secondary sexual characteristics in addition to decreased libido and sexual potency. Post-pubertal debut of hypogonadism does not infer differences in already developed male characteristics but adversely impacts sexual function, body composition and psychological components, e.g. mood and motivation.

Diagnosing hypogonadism may be difficult, especially as some symptoms overlap with morbidity and with ageing, such as decrees in bone formation, loss of muscle mass and appetite.[121] Further, the individual differences in optimal androgen levels means that general, and adequate, cut-offs are difficult to set, making symptoms even more important. Hormone levels also depends on the type of hypogonadism, see below.

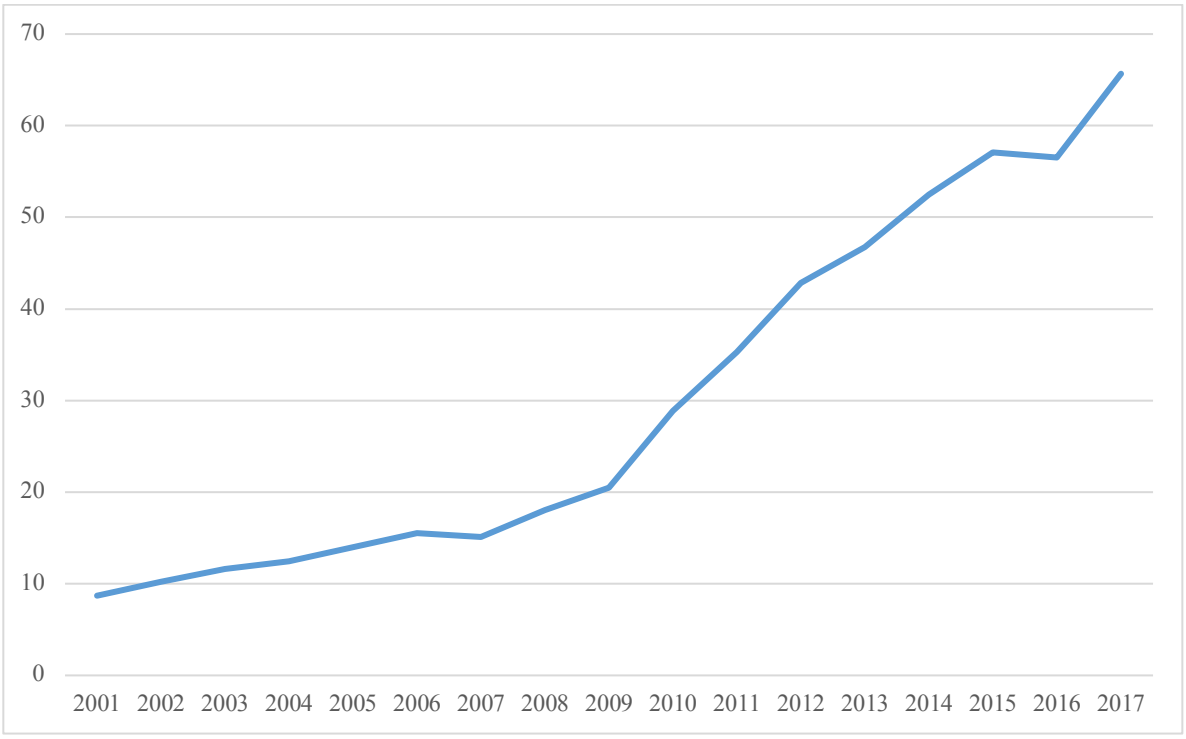

Figure 5 Testicular dysfunction, E29 (ICD-10), Cases per 100,000 Swedish inhabitants. Source: The National Board of Health and Welfare.

Primary hypogonadism

Hypogonadism, or testosterone deficiency, may be due to different pathologies. Primary hypogonadism is due to primary testicular failure, i.e. the testicular cells are unable to produce T at adequate levels and/or spermatogenesis is impaired, and results in a corresponding increase in LH/FSH as the hypothalamic-pituitary-gonadal axis responds. Primary hypogonadism is seen in Klinefelter's syndrome, testicular tumors, mumps-related orchitis, testicular irradiation, chemotherapy and testicular trauma.[123] Primary hypogonadism often presents with sexual symptoms, such as decreased libido, decreased frequency of morning erections and erectile dysfunction.[160,161] Table 3 includes other symptoms associated with hypogonadism. Serum T levels below 8 nmol/L are considered likely hypogonadal, while levels between 8 nmol/L and 12 nmol/L may be so, if combined with symptoms and/or if fT is below 220 pmol/L.[161-163]

Secondary hypogonadism

In secondary hypogonadism, or hypogonadotropic hypogonadism, the pathology is in the hypothalamus or pituitary and T levels and sperm count are subnormal, but LH and FSH are normal or reduced. Secondary hypogonadism may be congenital such as in isolated hypogonadotropic hypogonadism, Kallmann syndrome or genetic conditions such as Prader-Willi syndrome or Laurence-Moon syndrome.[123] Secondary hypogonadism may also be secondary to hyperprolactinemia, processes involving the pituitary (i.e. tumors; abscesses; infarcts; irradiation), Cushing's syndrome, substance abuse, serious or chronic morbidity or hemochromatosis.[123]

Late-onset hypogonadism

Hypogonadism after normal puberty is labeled late-onset hypogonadism (LOH). It seems to be a combination of primary and secondary hypogonadism.[164] In the European Male Ageing Study (EMAS) it is defined as serum T levels below 11 nmol/L, fT levels below 220 pmol/L combined

with three symptoms: (1) decreased number of morning erections, (2) erection insufficient for intercourse, (3) decreased sexual interest.[161]

Late-onset hypogonadism has been associated with cardiovascular diseases, metabolic syndrome, diabetes etc.[165-167] Thus, its it should be identified as soon as possible, to reduce risk of impaired quality of life and for subsequent morbidity.[131, 165-167]

Table 3. Symptoms of hypogonadism in men

A. More specific symptoms and signs	**B. Other less specific symptoms and signs**
Reduced sexual desire (libido) and activity	Decreased energy, motivation, initiative, and self-confidence
Decreased spontaneous erections	Feeling sad or blue, depressed mood, dysthymia
Breast discomfort, gynecomastia	Poor concentration and memory
Loss of body (axillary and pubic) hair, reduced shaving	Sleep disturbance, increased sleepiness
Height loss, low trauma fracture, low bone mineral density	Mild anaemia (normochromic, normocytic, in the female range)
Hot flushes, sweat	Reduced muscle bulk and strength
	Increased body fat, body mass index
	Diminished physical or work performance

Adapted from Bhasin et al. 2010.[168]

Compensated hypogonadism

Compensated hypogonadism, more recently termed and defined as increased LH, >9.4 IU/L, with generally normal T, is increasingly in focus as its been related to long-term cardiovascular disease and increased all-cause mortality.[169-172] In situations that infer chronic testicular stimulus by LH, the testicular reserve capacity may be overtaxed, which in turn may result in inability to cope to additional stress.[172, 173]

Treatment for hypogonadism

Hypogonadism may be treated with T, i.e. hormone replacement therapy (TRT), with some exceptions. Prostate or breast cancer, as they are responsive to T, must not be present and increased monitoring is advised if the individual 1st degree relatives with prostate cancer. Hematocrit above 53% should be investigated and treated before TRT. Congestive heart disease should be well medicated and in patients with cardiovascular disease, T dose should be carefully titrated. Reversible physiological and medical factors, e.g. obesity, glucocorticoid, spironolactone or ketoconazole treatment or substance abuse, that should be addressed first. Previously, TRT was considered able to induce prostate cancer, a notion that has been debunked.[174-176]

The effect of TRT has been studied extensively and positive effects have been noted. Lean body mass is increased in a wide range of study participants, in both primary and secondary hypogonadism as well as in LOH.[177-179] Studies have also shown increase in skeletal muscle strength in response to TRT, but not univocally so, and also functional outcomes have been harder to show.[175, 177, 180-182] Many older studies have been quite small, short duration and/or with differing outcome measurements.[175] In 274 older, intermediate-frail to frail men, with serum T ≤ 12nmol/L or fT ≤250 pmol/L Srinvivas-Shankar et al. found TRT to increase muscle mass in all men and physical function in frailer or older men.[183] Somatic and sexual function was increased. In conclusion, TRT increases muscle mass in hypogonadal men, with larger impact on those with lower T levels. The increase in muscle mass have not been clearly linked with increased strength or physical function, with conflicting results in the literature but some evidence points at effects, at least in older and frail patients. In regards to sexual function, TRT have been shown beneficial in regards to erectile dysfunction as well as in general quality of life (QoL) in patients with diabetes type 2.[123, 184]

In bone metabolism, TRT decrease bone loss and increases in bone density.[185-189] Snyder et al. found a relative increase in estimated spine trabecular bone strength, for TRT versus placebo, of 8.5% (95% CI, 6.0% - 10.9%) in older men.[187] However, the effect on fracture rate is unclear as present studies are too short.

In addition to T in TRT, selective androgen receptor modulators (SARMs) first described in the 1990ies may be an alternative with shown anabolic effects without androgenic effects, such as impact on prostate size, hair and skin.[190] Currently, there is no approval for their use.

Testosterone and Radiotherapy

A review of testicular exposure to irradiation in RT treatment found that 3-17 % of the prescribed dose, 50Gy, hit the testes directly translating to a mean testicular dose (TD) ranging from 1.24 Gy to 8.4 Gy.[191] The variation being dependent on field size, field type, tumor location, location of the testes at moment of RT and the type of shielding for the testes. This does not take scattered irradiation into account. Radiotherapy in RC treatment have been found to decrease in T levels to varying degrees.[191-196] Recently a dose-response association between testicular radiation and acute testicular failure was shown, with resulting decline in serum T.[95] The exact extent of RT induced T decrease have been hard to quantify, varying study design and small sample size making robust inference difficult.

One study included 25 men where only 16 submitted hormone samples at follow-up, four to six weeks after RT, and with a trend of increasing T levels with longer interval.[193] Another, smaller (n=10), study showed a 78 % decrease in serum T after long-course RT.[195] However, follow-up was conducted in different timespans, seven patients were analyzed three to eight weeks after RT and three were analyzed 11-12 months after RT.[195] A larger (n=290) study by Bruheim et al. used a cross-sectional retrospective design.[192] In this study, follow up time ranged from two to 12 years (median 3.9 years for irradiated patients and 5.5 years for non-irradiated patients). Several factors

differed between the studied groups, possibly impacting the results.[192] Using multimodal therapy with curative intent, Hennies et al. showed a 60 % decrease in serum T and a 91% increase in LH in 68 men one year after RT.[194] Chemoradiotherapy induced a mean T level decrease from 15.4 nmol/L prior to treatment to 8.0 nmol/L after more than 4 years.[197] In a sub-study including 40 study participants of a Swedish cohort of rectal cancer patients, the mean serum T did not differ significantly a year after RT, but LH did (11.5 nmol/L vs 10.9 nmol/L, $p = 0.16$ and 4.3 ± 1.9 vs 6.1 ± 3.1 $p<0.001$, respectively).[146]

In conclusion, there is compelling evidence that (C)RT results in potentially long-lasting Leydig cell damage in select individuals, with adverse impact on T production and, even if T regains levels, it requires abnormal stimulation by gonadotrophs.

Muscles

Function

Muscles generate the force needed for life. The human body uses three types of fundamentally different muscles: skeletal muscle, smooth muscle and cardiac muscle.[121] Skeletal muscle provides the force required for voluntary movement, ranging from minute corrections in eye direction to high jumps reaching 2.45m. Skeletal muscle. i.e. the diaphragm, also provides the work necessary for breathing and skeletal muscles, especially in the legs, help pump the venous blood back to the heart. Smooth muscle regulates, without conscious control, blood vessels and airways and controls the function and motility in the digestive, urinary and reproductive tracts. In the heart, cardiac muscle indomitably pumps blood into the circulation in order to provide the body with its needs in oxygen, nutrients etc.

Skeletal muscle, the muscle type observed in parts of this thesis, consists of special kind of elongated cells called muscle fiber or myofiber. Each myofiber, 10 to 100μm in diameter and up to 15cm long, have several nuclei located in the periphery and is enveloped by an endomysium. [198] Aligned myofibers form bundles, fascicles, sheathed by a perimysium. Each fascicle can include more than 100 myofibers and are what we see as "muscle fibers" in meat. Bundles of fascicles are contained in a fibrous sheath, epimysium, continuous with the fascia and with the associated tendons.[121] The myofiber have distinct nomenclature for its components: the membrane is called sarcolemma, the cytoplasm sarcoplasm and the mitochondria sarcosome. The "engine" in a myofiber, or indeed – in the muscle, is the myofibril. Each myofiber holds several hundreds of myofibrils, each sub-divided by Z-plates into approximately 2-3.65μm long sarcomeres. In the sarcomeres, thin actin filaments partly overlap and surrounds a central thick myosin II filament. The different fibers turn out black and white in a microscope, which originally gave skeletal muscle the name: "striated muscle". [198] Myosin II, using adenosine triphosphate (ATP) as fuel, moves along the actin fibers in a muscle contraction, in essence constituting the cylinders of the engine that provides power to all muscle movement. Genetic damage in proteins associated with the myofibers may result in muscular dystrophy, e.g. Duchenne muscular dystrophy.

Myofibers are controlled by motor neurons. One motor unit consists of one neuron and several to well over 1000 myofibers, and average skeletal muscles are innervated by around 100 motor neurons.[121] The cell body of somatic motor neurons reside in the anterior horn of the spinal cord, except for cranial nerves and are activated voluntary or by reflex. Motor neurons have split up and branched nerve endings so that a single neuron can cover up to one cm^2 of muscle cross-sectional area, and motor units intermingle with each other.[198] The innervation ratio is the ratio of muscle fibers per motor neuron, and determines the fineness of control.[121] The neuromuscular junction is where the motor end plate of the neuron's axon and the muscle cell synapse. A muscles action is initiated by neuronal release of acetylcholine, which triggers nicotinic receptors in the subsynaptic muscle membrane, subsequently leading to increase of calcium ion concentrations, that ultimately controls the interaction of myosin II and actin within the myofibers.[198] Interfering or blocking the synaptic activation results in muscular weakness or paralysis, e.g. curare-like substances used for muscle relaxation in surgery or botulism caused by a toxin produced by the anaerobic soil-dwelling bacteria Clostridium botulinum. Smooth and cardiac muscle have no motor end plates, only one nucleus per cell and several other specificities for their separate unique niches.

Motor units are divided into different types depending on their characteristics. Type I or slow-twitch motor units are highly effective at sustained, moderate intensity, work load, utilizing a large compliment of mitochondria and effective supply of oxygen and nutrients trough a highly developed network of capillaries. Type II, or fast twitch, motor units are generally suited for shorter bursts of high load, but are sub-divided into IIa and IIb types. Type IIa does have larger capacity for aerobic activity, i.e. higher mitochondria content and better supply, than type IIb that are adapted for rapid and powerful contractions and cope well with anaerobic conditions but for a limited time.

Fascicles are arranged differently depending on the muscle, i.e. muscle architecture. Longitudinal architecture, e.g. the sartorius muscle, refers to fascicles arranged parallel to each other and to the axis of force generation. Longitudinal architecture provides rapid shortening but results in less tension. In muscles with unipennate architecture have their fascicles at an angle to the axis of shortening, such as the soleus muscle, resulting in more but shorter myofibers producing added tension but at a slower speed.[121] Multipennate architecture refers to muscles with fascicles in differing angles to the axis of shortening, allowing for multiple functions as shown in the trapezius ability to both elevate and depress the shoulder.

Regulation

In 2004, Herbst and Bhasin concluded that T promoted muscle protein anabolism, the differentiation of pluripotent stem cells towards myogenic lineage and away from adipogenic and that motor neurons increase size in response to increasing T.[142] Testosterone support myogenesis by increasing myonuclear number, increasing protein synthesis while decreasing protein degradation, increasing satellite cell number as well as increasing AR in pluripotent stem cells and in motor neurons.[142] Type I myofibers have been found to be more T sensitive than type II.[142, 147] In men, T is the main anabolic hormone in skeletal muscle homeostasis.

Hormonal regulation of skeletal muscle development, fiber-type and contractility incorporates more than T: thyroid hormone and, mainly in women, estrogen are important.[199] Other factors such as myokines, including myostatin, are also important.[200]

Thyroid hormones (TH), termed T_3 and T_4, are released by the thyroid gland to the circulation where >99% binds to carrier proteins. T_3, accounts for approximately five percent of TH in circulation but, being 3-8 times as potent as T_4, is probably the most important TH. T_4, generally attributed as a plasma storage function, is in turn converted by many target organs, e.g. liver, to T_3. Converted T_3 constituted 80% of circulating T_3.[198] Thyroid hormones bind to both cytosolic and nuclear receptors similar to steroid receptors and are important in a multitude of bodily function, noticeably the intermediate metabolism.[121, 198] In regards to muscles, typical signs of hypothyroidism is low heart rate, muscle weakness and conversion of type II fibers to type I.[199] Hyperthyroidism induce fast and/or irregular heart rate and also muscle weakness among other symptoms. In rats, induced hyperthyroidism by injection of T_3, results in a reversible conversion of type I myofibers to type IIb, especially in females.[199] The levels of T_3 are positively associated with ATP turnover rate and animal studies have found faster contraction- and relaxion rates in hyperthyroid state.[199] Skeletal muscle has two types of estrogen receptors, ERα and ERβ, with functions similar to AR. In ERβ knockout male mice, contractile time is increased and contractile speed decreased. Female sex has been linked to better endurance, in humans as well, which has been attributed to sex hormone difference. However, estrogen is overshadowed in skeletal muscle function in men by T, but interestingly AR deficient male mice models show an increase in endurance in type I myofibers towards a level of female wild type mice.[199] Myostatin, and the counteracting follistatin, are recognized as major factors in muscle development and maintenance. Myostatin acts inhibitory on muscle growth, even degenerately on muscle tissue, and follistatin in turn blocks myostatin's receptor.[200] They seem to react to physical exercise, where myostatin decrease and follistatin increase, allowing for muscle growth.[201] Lack of physical exercise or disease may tip the scale in favor of myostatin, inducing atrophy.[200]

Sarcopenia

Sarcopenia is derived from the Greek terms σάρξ *sarx*, "flesh" and πενία *penia*, "poverty", it was termed by Rosenberg in the description of age-related loss of muscular function and first described by Evans & Campbell.[202-204] The exact definition of sarcopenia has been elusive, no doubt due to its multifactorial and still partly unclear genesis.[204] The historically vague definition might in part have led to the difficulties producing well defined and reproducible studies.[205, 206] In 2010, The European Working Group on Sarcopenia in Older People published a consensus report on sarcopenia, recommending "the presence of both low muscle mass and low muscle function (strength or performance)" for the diagnosis of sarcopenia.[207] In 2011 the International Working Group on Sarcopenia approved a similar definition: "Sarcopenia is defined as the age-associated loss of skeletal muscle mass and function".[208]

An exact definition of "low" muscle mass is still not generally set. There are proposed cut-offs set at muscle mass below two standard deviations of mean reference values in young healthy adults, 7.23 kg/m^2 – 7.26 kg/m^2 using dual-energy x-ray absorptiometry (DXA).[207, 209] The cross-sectional

total muscle area, measured at 3rd lumbar vertebrae (L_3) on CT or MRI, corresponds to total muscle mass, which facilitates assessment of sarcopenia in imaging modalities widely used.[210, 211] Currently, the golden standard for measuring muscle mass, in regards to sarcopenia, is CT or MRI, but in many settings, it may be too costly or impractical, and DXA is the secondary alternative.[207, 212] The Skeletal Muscle Index (SMI), the skeletal muscle cross-sectional area at L_3 divided by body height squared, has been proposed to assess sarcopenia and cut-off values ranging from 43.7 cm^2/m^2, to 55 cm^2/m^2 seem to be associated with a wide range of clinical outcomes.[213-215] The wide range gives a hint of the difficulty to set a general cut-off and different methodologies have been. Prado et al. for example, uses cross-sectional muscle area measurement at L_3 and optimal stratification in regards to mortality in their definition of sarcopenic obesity, 52.4 cm^2/m^2, in cancer patients.[216] Psoas sarcopenia, total psoas major area at $L_3 < 5$ cm^2/m^2 is another definition set by Peng et al. regarding hepatic resection for colorectal liver metastases.[217] The variation in methodology, and documentation, between studies is a problem, severely hampering comparisons and the need for standardization is apparent.[218] In a recent review including 8,895 CRC patients, only two out of 20 included studies showed high quality. Due to differing cut-offs and study population, sarcopenia prevalence ranged from 15 to 60% (myosteatosis ranged from 19-78%), but were still consistently related to worse survival outcomes.[219]

Longitudinal loss of muscle mass occurs naturally after the age of 40, with a 8% decrease per decade that increases after the age of 70 to 15% per decade.[220] Hence, muscle loss on individual level exceeding the norm, must be of interest. Muscle strength or function seems to be a more accurate predictor in regards to adverse outcomes than muscle mass, thereof the combined criteria for sarcopenia.[212] Different tests have been put forth in evaluation of physical fitness, such as the Short Physical Performance Battery, gait speed or stair climb power test.[212]

Decreased androgen level is recognized as of the main elements to drive sarcopenia, and TRT has been tried in order to treat the condition.[205, 207] As mentioned above, the results of TRT in older individuals in improving physical function have not been readily forthcoming, even if muscle mass and strength increases.[221] Sarcopenia, age and male sex are have been found to be risk factors for poor balance performance and thereby for fall trauma.[222] Abdominal muscles, "core muscles", are essential for posture and balance, and thus abdominal sarcopenia impact mobility and the risk of fall trauma.[223] Core strength, or stability, have been related to "successful performance of activities of daily living in old age".[223] The reports regarding T effect on abdominal muscles specifically are scarcer than reports regarding either appendicular muscles or lean body mass, as in reviews by Isadori et al. and Neto et al.[224, 225] Testosterone replacement therapy does seem to increase paraspinal muscles area significantly in severely hypogonadal men.[226] In rectal cancer patients treated with RT, acutely decreased bioavailable testosterone was related to decreased psoas major cross-sectional area.[146] The relative lack of data on abdominal muscles might be due to the lower accessibility using conventional muscle mass and strength measurements targeting them specifically, e.g. DXA measures total body lean mass, and even includes abdominal organs in that measurement. Using available CT/MRI scans generated in routine clinical practice may be a practical method to enhance patient treatment and outcomes.

Myopenia has been proposed as an alternative term to sarcopenia, sarcopenia being difficult to define and often used in relation to age related muscle loss, to describe muscle wasting more related to clinical factors.[227] In cancer, the term cancer cachexia is used to describe ongoing isolated skeletal muscle depletion, refractory to nutritional support, inducing progressive functional loss.[228] The diagnosis of cancer cachexia have three individual criteria, each enough for diagnosis: weight loss exceeding five percent over the past six months in a non-starving state and BMI <20 or sarcopenia in addition to weight loss exceeding two percent.[228]

Frailty

Frailty, a general age-associated physiologic decline in reserve and function of multiple systems that induces vulnerability, is a term increasingly used.[229] The frail state infers impaired capacity to handle everyday or acute stressors. Some of the proposed clinical markers for frailty: low grip strength, self-reported exhaustion, slow walking speed, low physical activity and/or unintentional weight loss, overlaps sarcopenic and/or hypogonadal symptoms.[230] Having three of the five frailty markers are deemed enough for being considered frail. Having two markers translates to a pre-frail stage with high risk of progress into manifest frailty. The prevalence of frailty varies between studies and in relation to geographic location, e.g. in a study of older community-dwelling adults in ten European countries the overall prevalence was 17%, but varied from 5.8% in Switzerland to 27% in Spain (8.6% in Sweden).[231] The risk of mortality is markedly increased in frail older adults, Fried et al. 2001 found adjusted HR to be 2.24 (95% CI 1.51 – 3.33) for death within 3 years compared to non-frail individuals in a large (n=5,317) US cohort.[230]

Radiology

Tomography is imaging in sections by use of a penetrating wave. The penetrating wave may be of differing origin. In the case of CT, tissue absorption of x-rays from a beam rotating around the supine body is measured and, by computer processing, reconstructed to cross-sectional images in 2D or 3D. Computed tomography was first used clinically in 1971, by Sir Godfrey Hounsfield in Hayes, UK, at EMI Central Research Laboratories. The method built on the Radon transform, a mathematical theory invented by Johann Radon, an Austrian mathematician, in 1917. The Radon Transform allows reconstruction of a function from an infinite set of its projections. Hounsfield shared the Nobel Prize in Physiology or Medicine 1979, with Allan MacLeod Cormack who independently developed theories for CT. Computed tomography is excellent in assessing organs and bone structures.

Modern Magnetic Resonance Imaging, MRI, build upon the insights and hard work of many contributors, e.g. the physicists Felix Bloch and Edward Mills Purcell got the Nobel Prize in Physics 1952 "for their development of new methods for nuclear magnetic precision measurements and discoveries in connection therewith". In 1977 Raymond Damadian, Larry Minkoff and Michael Goldsmith performed the first human MRI body scan. The first usable, clinical, MRI image was produced 1980 by John Mallards team at the University of Aberdeen, and depicted a tumor located in a patient's chest. In 2003, the Nobel Prize in Physiology or Medicine was awarded to Paul Lauterbur, of the University of Illinois at Urbana–Champaign, US, and Sir Peter Mansfield, of the University of Nottingham, UK, for their "discoveries concerning magnetic

resonance imaging". It uses magnetic fields and radiofrequencies, where the magnetic field (primary field) is measured in Tesla (xT). Currently, the usual field strength in clinical settings ranges from 1.5 to 3T, approximately 50,000 times the magnetic field of earth. The magnetic field influences protons in the body to align to the same axis as the field. A radiofrequency pulse is then used to stimulate the protons to shift out of axial alignment. After the pulse, the protons realign and in doing so, releases the energy induced by the pulse. The speed of realignment and energy released depends on the tissue type which is used to compute MRI images. It excels at imaging soft tissue, but fat content in muscle is more complicated to elucidate.[232]

CT and MRI in Body composition

As mentioned above, CT and MRI are golden standard to assess body muscle mass, and additionally subcutaneous and visceral adipose mass may also be quantified. The accuracy of skeletal muscle measurements for both CT and MRI has been verified in cadavers.[233] In CT, various tissues have differing attenuation in Hounsfield units (HU): skeletal muscle approximately 40 HU and adipose tissue has a value around -50 to -100 HU. Adipose tissue deposition in skeletal muscle, myosteatosis, will decrease average muscle area HU and is thus detectable on CT.[234] Radiological imaging can be used to assess individual muscles as well. Computed tomography and MRI have similar accuracy, up to approximately 5% difference between methodologies, with an error marginal of up to 2% in skeletal muscle, 4 % for adipose tissue.[211, 235] Comparing the modalities, CT and MRI, CT have some benefits: better spatial resolution, more accessible, faster and cheaper, but this comes at the cost of irradiation exposure. The exposure to irradiation has been decreased with better machines and protocols. Currently, an abdominal volumetric scan covering 1st to 4th lumbar vertebrae (L_{1-4}) results in less radiation, around two mSv, than the average annual background radiation at 2.5 mSv.[232]

Adipose tissue

Previously the level of L_{4-5} was deemed best regarding visceral adipose tissue measurements, and the level used for more than two decennia.[211] In the end of the 20th century, Abate et al showed that the best correlation of a single 10mm MRI slice to total abdominal adipose tissue was at L_{2-3} in men (n=49).[236] More recent studies have found that images between L_{2-3} to L_{3-4} are closer related to total abdominal adipose tissue and visceral adipose tissue as well as co-morbidity.[237-239] In 2007 a larger study (n=820) found that a cross-sectional MRI image at L_3 can accurately estimate the total visceral adipose tissue volume in both sexes.[240] The association of adipose tissue, both subcutaneous and visceral, of single slice area at L_3 with respective total abdominal adipose volumes were confirmed in CT by Irlbeck et al.[241] Using data from the Framingham Heart Study there, they could also find a strong association between adipose areas and cardio-metabolic risk factors.[241] Since visceral adipose tissue is associated with metabolic risk, measurements at L_3 could be of a high medical interest.[242] However, in regards to RC, it has been observed that area measurements of adipose tissue on a single abdominal slice is highly dependent on intraluminal gas, possibly effecting estimations.[146] Special MRI protocols, Dixon sequences, are used for quantification of fat fraction within muscles.[232]

Skeletal muscle

Skeletal muscle at L_3 have been found to relate to whole-body composition and is the level most commonly used.[211] Shen et al. found that muscle area on a single slice at a level 5 cm above L_{4-5}, i.e. approximately the L_3 given vertebra and disk height, correlated best with total skeletal volume.[243] Other studies have confirmed this, over a wide range of subjects, from healthy to patients suffering from diabetes or cancer, conditions known to potentially impact skeletal muscle mass.[210, 244, 245] Reproducibility of manual analysis of CT scans, both inter-reader and intra-reader, in regards to body composition have been found to be reliable.[211, 246] However, as mentioned above, standardization of skeletal muscle analysis is currently lacking.[219]

Impact of body composition in rectal cancer patients

The importance of body composition in clinical care is evident, with mounting reports of adverse effects of sarcopenia in regards to outcomes. In 805 CRC patients, Malietzis et al. found myopenia an independent prognostic factor for disease-free survival (HR: 1.53, 95% CI 1.06-2.39) and overall survival (HR: 1.70, 1.25-2.31).[247] In the same study, myosteatosis was related to longer hospital stay ($p=0.034$), and myopenia obesity related to higher 30-day morbidity ($p=0.019$) and mortality ($p<0.001$). In an earlier systematic review, Malietzis et al. pointed out the heterogeneity in the found studies, again highlighting the need for standardization.[248] However, some conclusions could be drawn in regards to CRC treatment: sarcopenia increases risk for developing chemotherapy toxicity; 30-day mortality; length of stay; complication rates as well as the need for postoperative rehabilitation. Sarcopenia may also result in worse disease-free survival and overall survival rates. Visceral obesity negatively impacted length of stay, operation time, risk of wound infection, complications rates, risk of anastomotic leaks and resumption of oral intake. The importance of body composition was highlighted by Martin et al. in a large (n=1,473) cohort of study participants with lung or gastrointestinal cancers using optimal stratification.[249] In that cohort, high weight loss, low muscle index and low muscle attenuation were found to be independent prognostic factors for survival. In study participants having all three factors, the median survival was 8.4 months (95% CI 6.5-10.3), and this regardless of BMI. In contrast, study participants lacking the three factors had a median survival of 28.4 months (95% CI 24.2-32.6).

By using existing radiological imaging examinations, performed as part of the workup of RC, body composition and, in follow-ups, longitudinal changes in body composition can be analyzed. This without the cost of a dedicated MRI scan or, in the case of CT, additional exposure to radiation. The gathered information may be used to individualize and/or risk-stratify the patients in effort to optimize clinical care.

Aims of the thesis

The general aim of this thesis was to investigate the impact of radiotherapy on testicular endocrine function and its possible adverse effects in men treated for rectal cancer. The motive being to optimize patient information, treatment and long-term quality of life.

I. To analyse the testosterone sensitivity of the abdominal and pelvic skeletal musculature in healthy men, using state-of-the-art methodology.

II. To assess long-term impact of radiotherapy on testicular function.

III. To analyse the risk of severe postoperative adverse events in relation to radiotherapy induced testicular failure.

IV. To verify testosterone sensitivity of skeletal muscle located at the 3rd lumbar vertebrae and the longitudinal impact of radiotherapy induced testicular failure on muscle area and oncological outcome.

Study Participants and Methods

The Boston Project

Original study

The 5α-Reductase Trial, a parallel-group, double-blind, randomized placebo-controlled trial, aimed at elucidating if 5α- reduction of T to DHT in fat-free mass is obligatory for mediating its anabolic effects. Thus, the main outcome was change in fat-free mass, measured by DXA. One of the secondary outcomes, prostate volume, required MRI. The trial spanned from 2005 to 2010, and included healthy eugonadal (serum T levels between 10,40 nmol/L and 41,64 nmol/L) men aged 18 to 50 years. Exclusion criteria included androgen deficiency, history of prostate cancer, lower urinary tract symptom score greater than 20, weight >135 kg, hematocrit > 51%, prostate-specific antigen (PSA) >4μg/L, creatinine > 176.8 μmol/L, aspartate aminotransferase or alanine aminotransferase > 1.5 times the upper normal limit. Men receiving glucocorticoids, growth hormone, androgens, or 5α-reductase inhibitors were also excluded. Upon completion of the study, the participants received $1000, else they received a prorated amount depending on participation time.

Ethical approval

The 5α-Reductase Trial was approved by the Boston University and Brigham and Women's Hospital institutional review boards (Protocol Numbers: H-24207 & 2013-P-000139/1). Informed consent was given by all study participants. The trial is registered at ClinicalTrials.gov, NCT00493987.

Interventions

The study participants endogenous T production was suppressed using a gonadotropin-releasing hormone agonist, and artificial levels of circulating T were created by administration of graded doses (50, 125, 300, or 600 mg) of T enanthate. The grading was planned to encompass the range from sub- to supraphysiological. The graded T doses were administered, additionally either the DHT suppressing dutasteride or placebo were given to crated eight groups. The trail lasted 20 weeks.

Results of 5α-Reductase Trial important for Study I

The resulting change in fat-free mass was related to T levels in a dose-dependent manner and there was no difference due to DHT suppression. As the suppression of DHT had no effect on fat-free mass, the two arms could be combined in Study I, yielding four groups with graded doses of T supplementation.

Study participants

Study I use data generated in the 5α-Reductase Trial, more specifically T and fT levels and study participants with complete MRI scans.

Outcome measurements

The primary outcome was change in cross-sectional axial area of total psoas muscle (PM) at L_3. The areas of both PM were manually traced, based on methodology by Taguchi et al., where the level on which both transverse processes were fully observed is used, and totaled, see Fig 6A.[250] Software used was OsiriX MD version 8.0.2 (Pixmeo, Bernex, Geneva, Switzerland).

Secondary outcomes were:

- paraspinal muscles (erector spinae and quadratus lumborum) at L_3. (Fig. 6A)
- total muscle area at L_3, including the muscle areas of psoas major; paraspinal; the anterior abdominal wall (transverse abdominis, internal and external obliques, and rectus abdominis muscles). (Fig. 6A)
- Obturator internus muscles, using the axial slice containing the largest combined trans-axial width of the muscle. (Fig. 6D)
- Ischiocavernosus muscles, using the axial slice containing the largest combined trans-axial width. (Fig. 6B)
- Pelvic floor muscles, i.e. pelvic diaphragm, including the levator ani muscle, using an axial slice at the level between the prostate and the penile bulb, excluding the rectum and the urethra. (Fig. 6C)

Muscle area measurements were examined both as standardized by division with height-squared (primary) and as absolute.

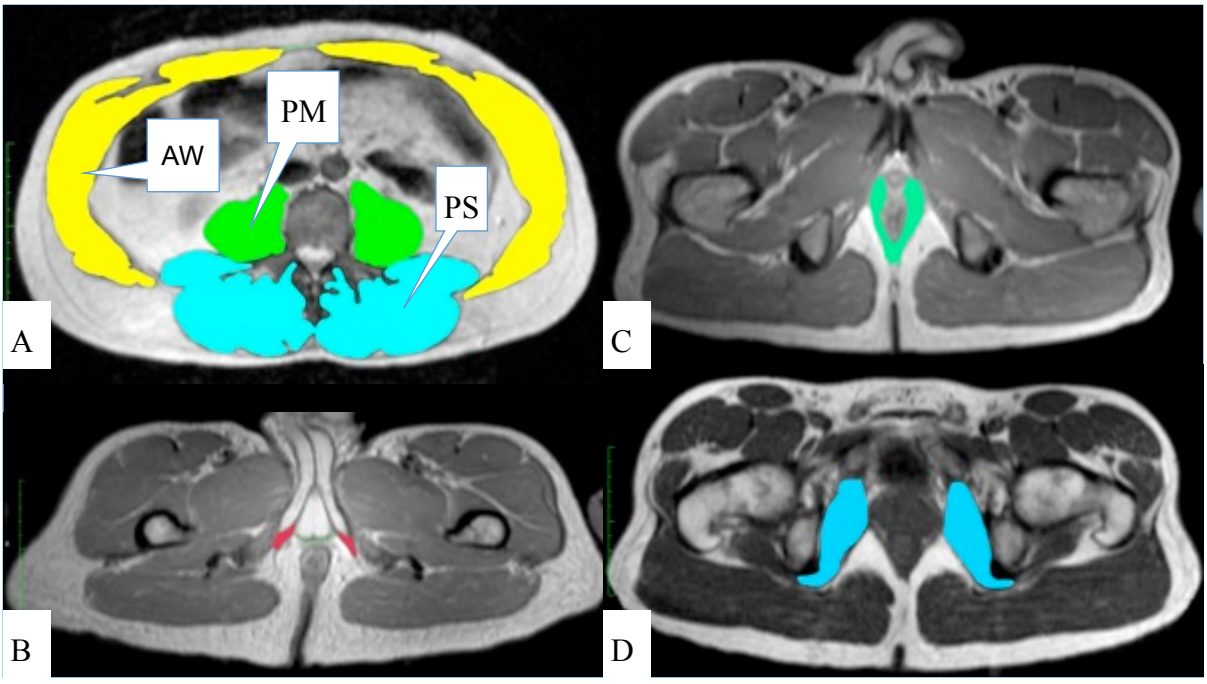

Figure 6. T1-weighted (A-C) and T2 weighted (D) Magnetic Resonance Images of areas measured. A) Muscle areas at 3rd lumbar vertebrae, psoas major (PM), paraspinal muscles (PS), abdominal wall (AW). 1B) The ichiocavernosus muscles. 1C) The pelvic diaphragm. 1D) The obturator internus muscles. Reprint from study 1.

Hormone measurements

Androgen sampling was done through morning blood samples collected at baseline and at the end of the study. Liquid chromatography–tandem mass spectrometry (sensitivity of <0.1 nmol/L) was used to measure serum T and DHT.[251] The interassay coefficient of variation for T assay was 7.7%

at 8.4 nmol/L, 4.4% at 18.5 nmol/L, and 3.3% at 35.3 nmol/L of T. Free T levels were calculated.[252] SHBG was measured using an immunofluorometric assay (sensitivity of 2.5 nmol/L).

Statistical analysis

Statistical analyses were made using SAS v.9.3 (SAS Institute, Cary, NC, USA) and R software v.2.15.1. Descriptive statistics of study participant characteristics and outcomes were presented as means and standard deviations. Statistical tests were done two-sided with alpha level 0.05. Model assumption and distribution of outcomes were examined graphically. Linear regression models were used to assess relationships of muscle areas with T dose and T levels. Linear regression models for standardized outcomes were adjusted for baseline measurements, age and SHBG. For absolute area measurements, BMI was included in regression models. Parameter estimates of outcomes were provided, with 95% CIs, per 100mg/week dose of T enanthate, and partial R-squared metrics calculated. Relation between serum T and fT levels and muscle outcomes were assessed with R-squared and corresponding p-values as well as presented graphically with scatter plots.

Sexual Function and Wellbeing in Males Diagnosed with Rectal Cancer – a cohort study

This prospective cohort study was set up after the findings in a the review by Buchli et al., concerning adverse impact on fertility and testicular function by RT.[191] The hypothesis generated by the review was that RT used in multimodal rectal cancer treatment induced acute and late adverse effects on testicular function. The adverse effects of RT in focus were infertility and hypogonadism, which would have significant impact on functional outcomes and QoL for the increasing number of long-term survivors of RC.

Cohort study design

Preoperative RT for RC, as determined by the MDT conference was the exposure in this longitudinal cohort study. The primary endpoint, serum T, and secondary endpoints included spermatogenesis, sexual function and QoL were assessed repeatedly. The study is registered at www.clinicaltrials.gov with Clinical Trial ID NCT01216202. The study was to run for two years, with yearly follow-ups. The initial sample size was set at 100, assuming a longitudinal decrease in mean serum T of 3 nmol/L (SD 6.5 nmol/L) with a two-sided CI of 0.95 and with the aim at exceeding a power of 0.80 using 40 pairs of observations and with predicted two thirds being exposed and one third lost to follow-up power.

Criteria

Inclusion criteria were RC stage I to III, planned for rectal resection, age over 18, fluency in Swedish, resident in the Stockholm County and given informed consent. Exclusion criteria were: planned for local excision, present or history of urogenital cancer, history of pelvic irradiation, androgen related medication or abuse.

Changes to protocol

During the second year of the study it was noted that less than ten percent of the study participants were unexposed, i.e. only underwent surgery. Prostate cancer, being an adenocarcinoma in the lower pelvis, were deemed similar enough to constitute part of the unexposed group. Thus, patients suffering prostate cancer and planed for robot-assisted prostatectomy were included as unexposed, given the same criteria.

Enrollment

The study started enrollment in April 2010 at the outpatient clinics of the colorectal units at Karolinska University Hospital (tertiary referral center) and Ersta Hospital (secondary referral center), Stockholm, Sweden. Enrollment ended in May 2014. Study participants with prostate cancer were enrolled at the Department of Urology at Karolinska University Hospital between May 2012 and January 2013.

In total 372 men with RC were assessed, 188 were found eligible, and 115 men were included. In men with prostate cancer, 298 were eligible and 63 were included. The main reason for non-inclusion was unwillingness to participate.

Data collection

Prior to any treatment, study participants visited the Center for Andrology and Sexual Medicine at Karolinska University Hospital (Stockholm, Sweden) to submit: clinical reporting form information, a fasting morning venous blood sample, questionnaires and a semen sample. The same material was collected approximately one and two years after surgery. In addition, cryopreservation of semen was offered if aged under 55. Study participants treated with RT had an additional pre-operative blood sample collected. Data regarding surgery and related events as well as oncological outcomes were retrieved from clinical records. At the one and two years after surgery, in addition to the data mentioned above, a test of testicular endocrine capacity was performed by injection of human chorionic gonadotropin (hCG), an LH analogue. Study participant had the option to meet an andrologist during the study to evaluate possible sexual dysfunction or symptoms of LOH.

Study participants could elect to opt out from semen/androgen sampling and/or questionnaires at any time without leaving the study. Study participants with prostate cancer were not pertaining in semen analysis.

Ethical approvals

The study was approved by the Regional Ethical Review Board in Stockholm (2009/1860-31/2), as were amendments 2010/1768-32; 2011/2097-32; 2012/2173-32 to allow for a wider inclusion and analysis of radiological examinations generated during treatment.

Exposure

The exposed group got RT in accordance with MDT recommendations, generating in total 101 exposed study participants. Short course RT was prescribed in 76, of which 12 had preoperative

full-dose chemotherapy (capecitabine and oxaliplatin) under the RAPIDO-trial. Long course consisted of mainly 50.4 Gy (n=22), two study participants received 50Gy and one 64.4 Gy. Long course RT, except 50 Gy, were administered with concomitant chemotherapy (capecitabine).

Cumulative mean testicular dose was derived from treatment planning CT and, by dividing TD by prescribed dose times 100, relative TD was calculated.[253] The LH-T ratio was used to assess the state of the hypothalamic–pituitary–gonadal axis. Biochemical hypogonadism was defined as serum T levels below 8 nmol/L.

Outcome measurements - Hormones

Total serum T, LH, FSH and SHBG was analyzed at Karolinska University Laboratory. Total serum T and SHBG were measured with chemiluminescence using the Beckman Coulters UniCel DxI 800 Instrument (Beckman Coulters, CA, USA), with a coefficient of variance (CV) <10% in regards to T levels ranging from 6.94 to 43.70 nmol/L and 5% for SHBG. Bioavailable testosterone levels (non-SHBG bound) were derived from measurements of total T, SHBG, and albumin. Free T levels were calculated according to Zakharov with a set albumin concentration of 43g/L. This new method allows for the allosteric characteristics of SHBG as mentioned above.
Luteinizing hormone was measured with fluorescence immunoassay using PerkinElmers AutoDELFIA immunoassay system (PerkinElmers, CT, USA), CV <2%.

Methods specific to Study II- IV

Descriptive statistics

In general, categorical data were reported as frequency (percentage) and continuous data as median (range). Groups were compared using Wilcoxon rank-sum or Fisher exact tests (cross-sectional). For longitudinal comparisons, Wilcoxon's signed-Rank test or McNamer's test were used. The data were analyzed with Stata version 14 (StataCorp LP, College Station, TX, USA).

Longitudinal regression analysis

In study II to IV longitudinal regression analysis (LRA) has been the statistical method of choice to assess change over time. Longitudinal regression analysis allows for consideration of possible confounding or effect modification. In study II and III, LRA based on generalized estimating equation (GEE) was used to model population-averaged outcomes accounting for correlation between repeated observations and enable evaluation of confounding or effect modification. In study IV, LRA based on random-effects models were applied to assess the association between T levels and muscle area, and muscle area and metastatic disease.

Study II

The effect of RT on long-term endocrine testicular function was assessed. The cohort included 91 men in the exposed group and 72 in the unexposed (59 with prostate cancer).

Outcome

Hormonal change was related to group, exposed or non-exposed, and to cumulative mean TD. Oncological outcome in RC study participants, time-to-recurrence, was collected from clinical

data. Detection of local recurrence, systemic disease and rectal cancer-related death were registered as events, non-cancer related death was censored. Oncological outcome was related to levels of LH at one-year follow-up to elucidate possible association between elevated LH levels one year postoperatively and development of cancer recurrence after uneventful one-year-follow-up. Elevated LH was defined as individual increase in LH of more than 50% between baseline and one-year follow-up.

Statistical analysis

Longitudinal regression analysis using GEE models. In group comparison, the reported coefficients represent the mean change differences between exposed or non-exposed. In TD analysis the coefficients describe the average change in androgen levels per one Gy increase of TD. Final models were adjusted for age, BMI and The American Society of Anesthesiologists (ASA) physical status classification. In the oncological analysis, the Kaplan-Meier method was used and groups were compared by log rank tests.

Study III

In Study III, a longitudinal observational sub-study using material from the cohort in Study II, severe postoperative adverse events were related to TD and preoperative change in T due to RT in 104 men with RC.

Outcome

Postoperative adverse events (AE) within 30 days after surgery, were recorded and graded according to Clavien-Dindo.[105] Grade of three or more was considered as severe, grade 3 defined as AEs that resulted in surgical, endoscopic or radiological intervention, grade 4 as AEs requiring IC/ICU management and grade 5 as death. The highest graded postoperative AE for each study participant was used.

Statistical analysis

Generalized estimating equation, with binomial distribution and robust variance estimator, were used. All models were adjusted for elapsed time between RT and surgery, to account for the initial decline in serum T and the subsequently responding increase in LH.[95] Final models were adjusted for age, BMI and ASA-score to reflect their impact on the hypothalamic-pituitary-gonadal axis.[254] Factors that did not change the point estimates more than 10% (smoking, tumor stage, distance from the anal verge and type of surgery) were omitted in the final models. [255]

Study IV

This study was also based on the cohort study described above. It included 102 study participants with RC and adequate CT/MRI examinations at baseline. Exposure was change in T, related to RT, and outcomes were change in select skeletal muscles. Further, change in skeletal muscle was related to systemic cancer recurrence.

Outcomes

The primary endpoint was the combined area of left- and right-sided psoas major (PM) at L_3. Secondary endpoints were total muscle area, sarcopenia (defined as total PM area $<5\ cm^2/m^2$), PM

height and width as well as average attenuation for each separate muscle area. All muscle measurements were manually traced on axial slices at the level of the 3rd lumbar vertebra of MRI or CT scans using OsiriX MD version 8.0.2 (Pixmeo, Bernex, Geneva, Switzerland). Oncological outcome, defined as cancer recurrence in the form of systemic disease, within five years of surgery, was collected from study participants journals.

Statistical analysis

Longitudinal regression models with random-effects were used. The models were adjusted for elapsed time between baseline and one-year follow-up imaging, and body weight. Body weight as it was found to have a stronger relation to muscle area than the usually considered height.
Models in oncological analyses were adjusted for fT, its association with muscle area verified in this study and previously shown. Possible confounders did not change point estimates more than 10%.

Results

Study I

Telephone screening covered 3,792 men of which 189 were eligible and 139 randomized. One-hundred-two study participants completed the study. In total 76 study participants had adequate MRI scans at baseline and at the end of study. Mean age was 37.6 (SD 8.7) and mean BMI 26.3 (SD 4.0).

Hormones

Concentrations of T and fT had a positive linear association with administrated T dose, ($p<0.001$, partial $R^2=0.215$ and $p<0.001$, partial $R^2=0.203$ respectively).

Longitudinal anabolic effect in skeletal muscle

The average standardized PM area decreased with 4.8% in the 50 mg/week T enanthate dose regimen, and increased with 9.7% in the 600 mg/week T enanthate dose regimen. There was a dose-dependent increase in muscle area, e.g. standardized PM estimated increase per 100mg T enanthate was 0.622 cm^2 (95% CI 0.394-0.850) (Table 4). The association between both absolute and standardized PM were repeated in the paraspinal muscles and in the abdominal wall and total abdominal muscles, see Figure 7 and Table 4.

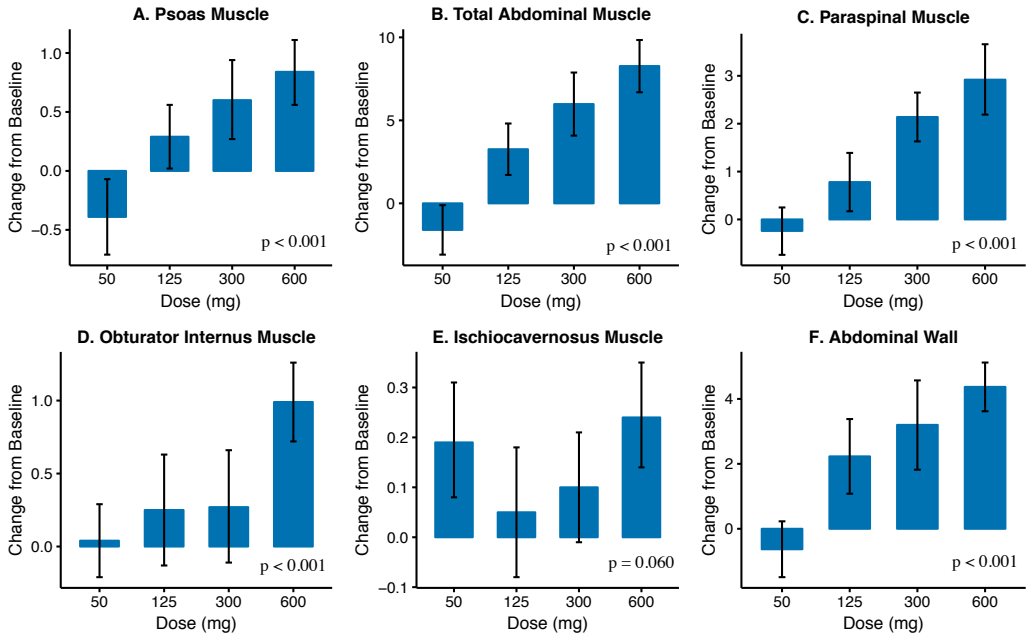

Figure 7 Testosterone dose effect on the cross-sectional area of abdominal and pelvic muscle groups. Bar charts indicate the change in standardized muscle area from baseline in each dose group. Results represent sample means and 95% confidence intervals. p-values for dose effect extracted from multiple linear regression model adjusted to baseline value, age, and SHBG. Reprint from Study I.

The findings of muscle area sensitivity visive T dose were similar for fT, see Figure 8.

In the pelvic muscles, the obturator internus and ischiocavernosus muscle, had comparable associations, even if the latter did not reach the same level of significance (Table 4). The increase in average area of the ichiocavernosus muscle in the highest T dose regimen was 22%. In the composite area of the pelvic floor muscles, there was an association between muscle area change and T dose ($p<0.001$, partial $R^2 = 0.402$).

Table 4 Estimated values of muscle area change per dose increase.

Muscles	Estimate (95% CIs)	*p*-value*	R-squared of the Model**	Partial R^2 of dose effect
Psoas Major, PM	0.622 (0.394, 0.850)	<0.001	0.307	0.297
Std PM	0.204 (0.133, 0.274)	<0.001	0.332	0.311
Paraspinal muscles, PS	1.789 (1.317, 2.261)	<0.001	0.466	0.450
Std PS	0.569 (0.426, 0.711)	<0.001	0.483	0.468
Abdominal wall, ABDW	2.530 (1.627, 3.434)	<0.001	0.408	0.340
Std ABDW	0.797 (0.527, 1.066)	<0.001	0.405	0.527
Skeletal muscle index	5.434 (3.989, 6.879)	<0.001	0.535	0.481
Std SMI	1.640 (1.210, 2.071)	<0.001	0.525	0.494
Ischiocavernosus, IC	0.082 (0.003, 0.045)	0.041	0.157	0.068
Std IC	0.025 (−0.001, 0.050)	0.060	0.117	0.059
Obturator internus, OB	0.455 (0.233, 0.678)	<0.001	0.279	0.175
Std OB	0.151 (0.082, 0.219)	<0.001	0.294	0.192

*Muscle area change is expressed in square centimetres. Standardized (Std)=cm^2/m^2. * = Values presented for parameter estimates and 95% CIs of muscle area change are per 100 mg increase of weekly dose testosterone enanthate. **Overall R-squared values for linear regression model with adjustments. Reprint from Study I.*

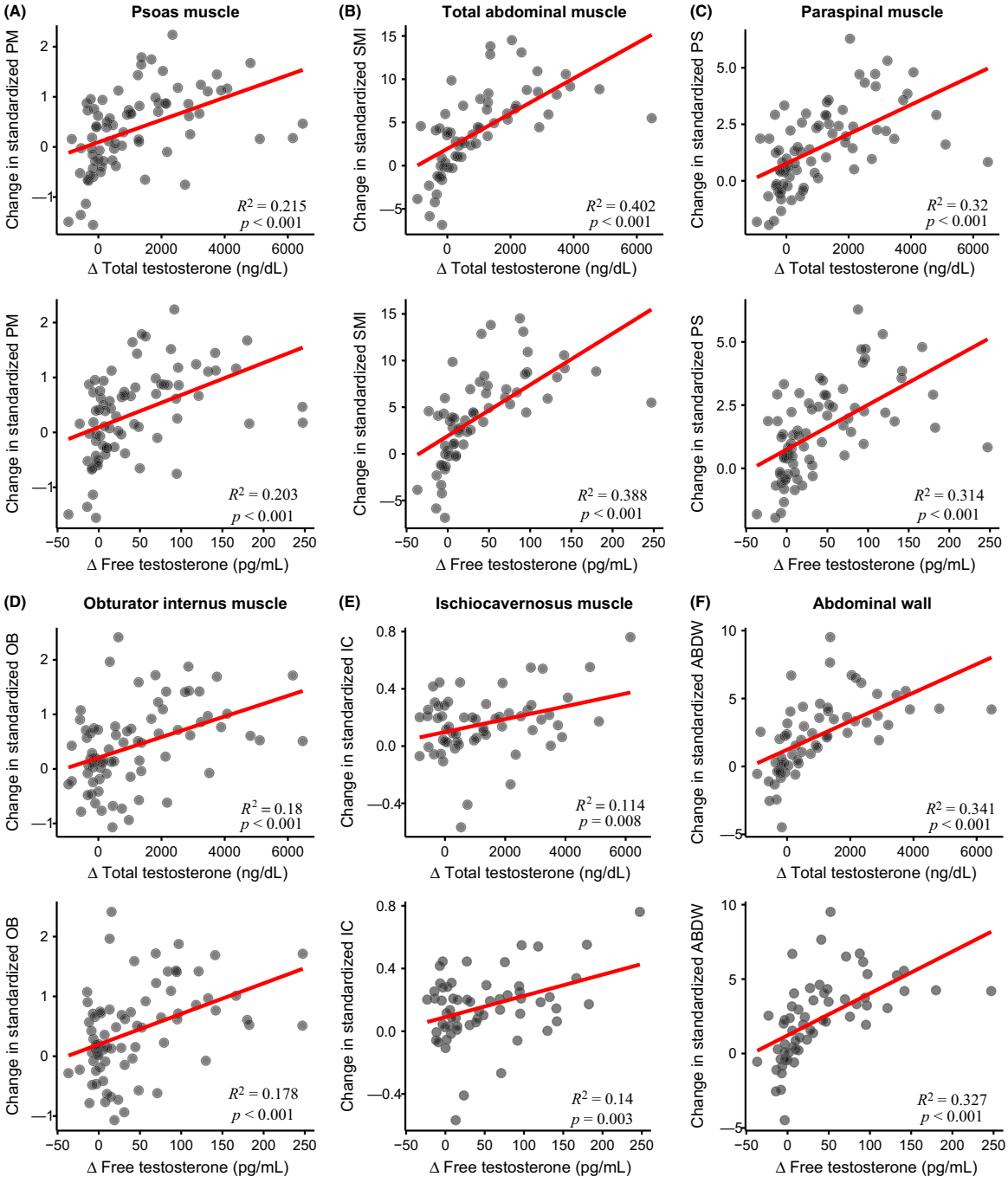

Figure 8. Scatter plots of change in standardized muscle area on total and free testosterone level change. R^2 and p-values represent association between standardized muscle area and total and free testosterone level change calculated from linear regression model. Reprint from Study I.

Study II

In total 91 irradiated and 72 unexposed men were analysed at baseline, see Figure 9.

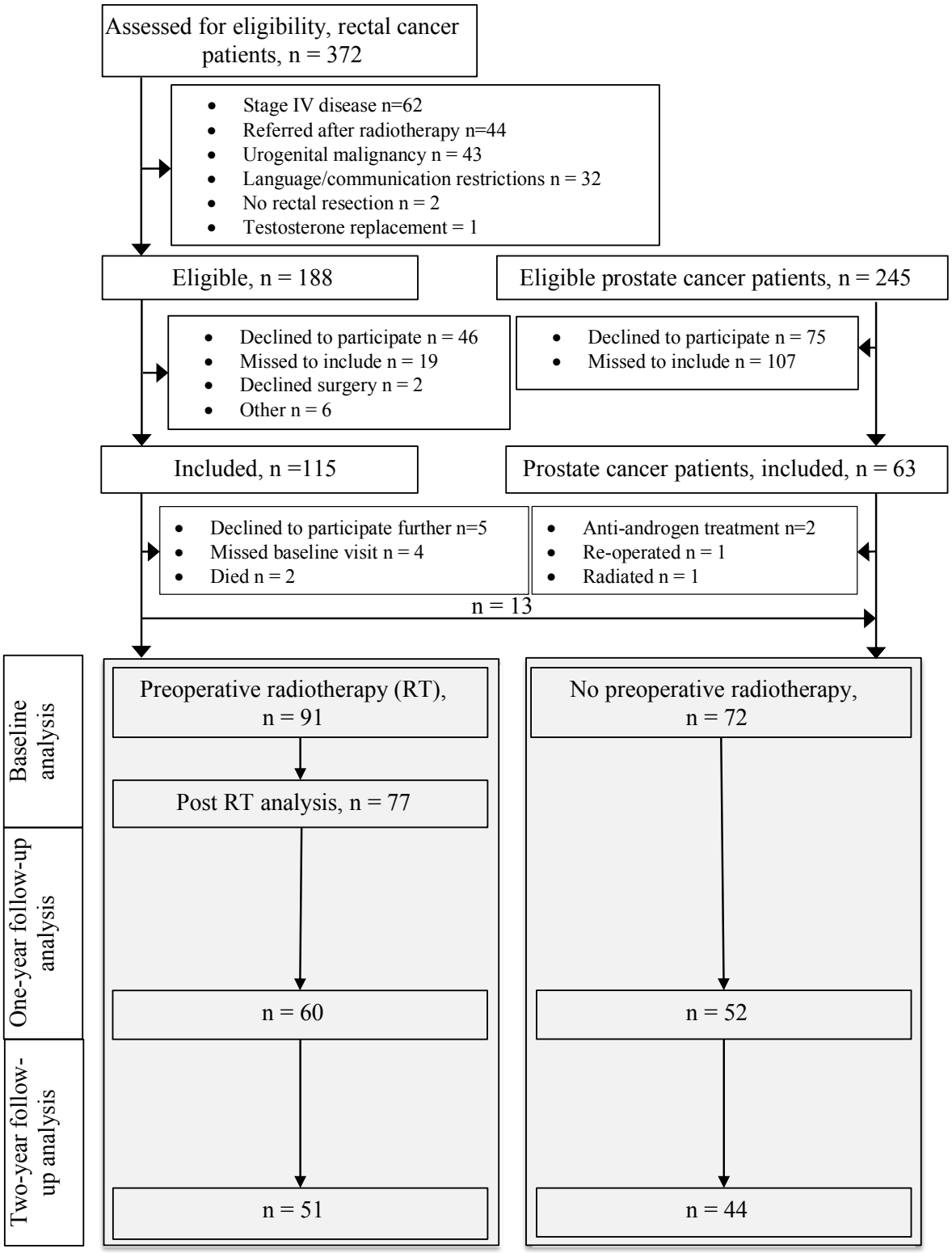

Figure 9. Study participant flow chart.

Median age was comparable in irradiated 63 (32-82) years vs. 63 (50-86) years in unexposed (p=0.336). The irradiated study participants had a lower BMI (25.2 kg/m^2 vs 26.5 kg/m^2, p=0.017). In the irradiated, there was a trend towards a higher ASA-score (p=0.062). Median T levels were

the same, 11 nmol/L (irradiated range 4-22 nmol/L vs unexposed 5.4-25 nmol/L, p=0.444), at baseline. Other hormonal levels did not differ either.

Out of the 163 men included, 95 had complete two-year follow-up. Hormone levels were comparable in men with complete follow-up versus men lost to follow-up of the non-irradiated group. In irradiated men, there was a larger proportion of biochemical hypogonadism in those lost to follow-up, 26.8 % vs. 5.77 %, p=0.004. They also had higher LH levels (4.25 vs. 3.6 IU/L, p=0.047) and, correspondingly, larger LH-T ratio (0.43 vs. 0.29, p=0.020) at baseline compared to men with complete follow-up.

Hormonal Change

One year after surgery, irradiated men had increased median levels of LH and FSH (5.1 vs 3.45, p<0.001 and 12 vs 5.45, p<0.001, respectively). The ratio of LH/T was higher in the irradiated compared to the unexposed as well, 0.52 vs 0.33 (p<0.001). After two years, there were no cross-sectional differences between groups. The levels of LH, FSH and the LH/T-ratio were significantly increased compared to baseline in irradiated men, see Table 5.

Table 5. Hormone profiles.

	RT-	RT+	p value, Wilcoxon rank-sum test
Baseline			
Serum T (nmol/L)	11 (5.4 - 25)	11 (4 - 22)	0.444
Free T (pmol/L)	332 (131 - 680)	319 (89.7 - 551)	0.390
LH (IU/L)	3.7 (1 - 16)	4.1 (1.6 - 13)	0.458
FSH (IU/L)	5.6 (1.6 - 33)	4.7 (1.2 - 19)	0.066
LH/T-ratio (IU/nmol)	0.33 (0.09 - 2.5)	0.35 (0.11 - 2.10)	0.165
Post RT follow up			
Serum T (nmol/L)		8.9 (0.8 - 18)**	
Free T (pmol/L)		0.243 (16.8 - 534)**	
LH (IU/L)		4.9 (1.6 - 16)**	
FSH (IU/L)		8.5 (2 - 30)**	
LH/T-ratio (IU/nmol)		0.56 (0.15 -5.93)**	
1-year follow up			
Serum T (nmol/L)	11.5 (5.5 - 22)	11 (5.5 - 18)	0.394
Free T (pmol/L)	334 (146 - 601)	302 (160 - 502)	0.077
LH (IU/L)	3.45 (1.6 - 9.7)	5.1 (1.7 - 13)**	<0.001
FSH (IU/L)	5.45 (1.4 - 27)	12 (0.07 - 40)**	<0.001
LH/T-ratio (IU/nmol)	0.33 (0.12 - 0.99)	0.52 (0.11 - 1.48)**	<0.001
dT, hCG-test (nmol/L)	11.05 (-6.1 - 30)	12.05 (-4 - 25.3)	0.941
2-year follow up			
Serum T (nmol/L)	10 (4.6 - 21)**	11 (4.2 - 16)	0.499
Free T (pmol/L)	272 (130 - 545)**	322 (132 - 493)	0.549
LH (IU/L)	3.75 (1.6 - 9.9)	4.1 (0.9 - 16)**	0.367
FSH (IU/L)	5.75 (2.6 - 27)	6.15 (0.09 - 41)**	0.727
LH/T-ratio (IU/nmol)	0.36 (0.14 - 1.09)*	0.39 (0.08 - 1.33)**	0.313
dT, hCG-test (nmol/L)	11 (1.7 - 23)*	14 (1 - 38)	0.105

Notes RT = Radiotherapy. Testosterone (T), Luteinizing hormone (LH), LH/T-ratio, Follicle stimulating hormone (FSH) and hCG (human chorionic gonadotropin, Pregnyl) response tests are reported as median, range. */** =Significant change from baseline p<0.05/0.005 respectively.

Longitudinal regression analysis

Longitudinal regression analysis confirmed a preoperative drop in T and fT levels, and increase in LH, FSH and LH/T-ratio. Figure 10 graphically displays predicted means with 95% CI.

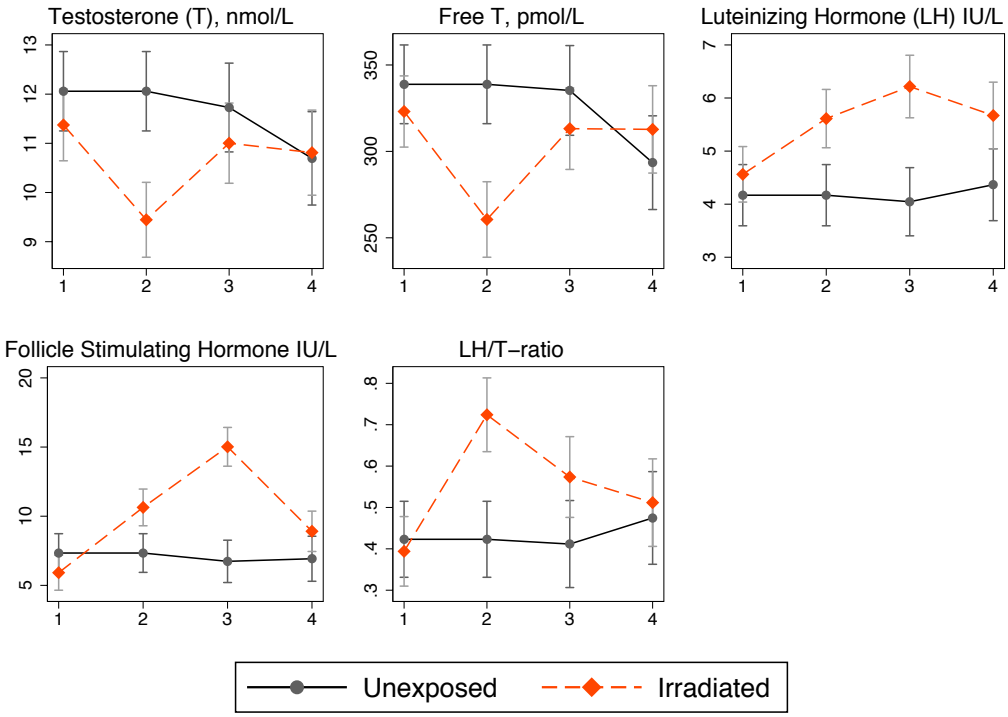

Figure 10. Predicted means of hormones. Range illustrates 95% confidence intervals.

The effect of TD on hormone levels are described in Table 6. Cumulative mean testicular dose was dose-dependently related to decrease in T preoperatively, decreasing mean T with 0.40 nmol/L per Gy (CI 95%: -0.66 - -0.14, p=0.002). Preoperative fT was similarly impacted. Luteinizing hormone, FSH and LH/T-ratio showed dose-dependent increases, both pre- and postoperatively.

Table 6. Longitudinal regression analysis of hormone changes over study period in relation to mean cumulative testicular dose.

	Mean change for each Gy of TD	95% Confidence interval	p
Testosterone (nmol/L)			
Preoperative	-0.40	-0.66 - -0.14	0.002
Postoperative	-0.01	-0.43 - 0.40	0.946
Free Testosterone (nmol/L)			
Preoperative	-15.2	-23.3- -7.06	<0.001
Postoperative	-0.51	-13.5 - 12.5	0.939
LH (IU/L)			
Preoperative	0.36	0.16 - 0.56	<0.001
Postoperative	0.62	0.31 - .0.93	<0.001
FSH (E/L)			
Preoperative	1.14	0.65 - 1.63	<0.001
Postoperative	2.94	2.17 - 3.71	<0.001
LH/T-ratio			
Preoperative	0.07	0.03 - 0.11	0.001
Postoperative	0.06	-0.00 - 0.12	0.057

Notes: TD = cumulative mean testicular dose. LH = Luteinizing Hormone. FSH = Follicle Stimulating Hormone. T = Testosterone. Models adjusted for age, BMI and The American Society of Anesthesiologists (ASA) physical status classification.

Oncological outcome

After one year, LH levels of 64 study participants treated for RC without clinical and radiological evidence of cancer recurrence were available. Twenty-one of them had an LH increase by more than 50% (LH-elevated), the remaining 43 men had a less prominent change in LH (LH-stable). The groups, LH elevated and LH-stable, were comparable regarding age, ASA-score, cTNM, proportion of RT, type of surgery and perioperative markers for extensive surgery.

In the LH-elevated group there was eight failures (distal metastasis n=7 and LR n=1), with a rate of 9.90 per 100 person-year. In the LH-stable group there was six failures (distal metastasis) and a rate of 3.10 per 100 person-year. The incidence rate ratio of RC recurrence in LH-elevated was 3.19 (95% C.I.: 0.97-11.2, mid-p=0.036) compared to LH-stable. Kaplan-Meier plot is displayed in Figure 11 (log-rank test: p=0.032).

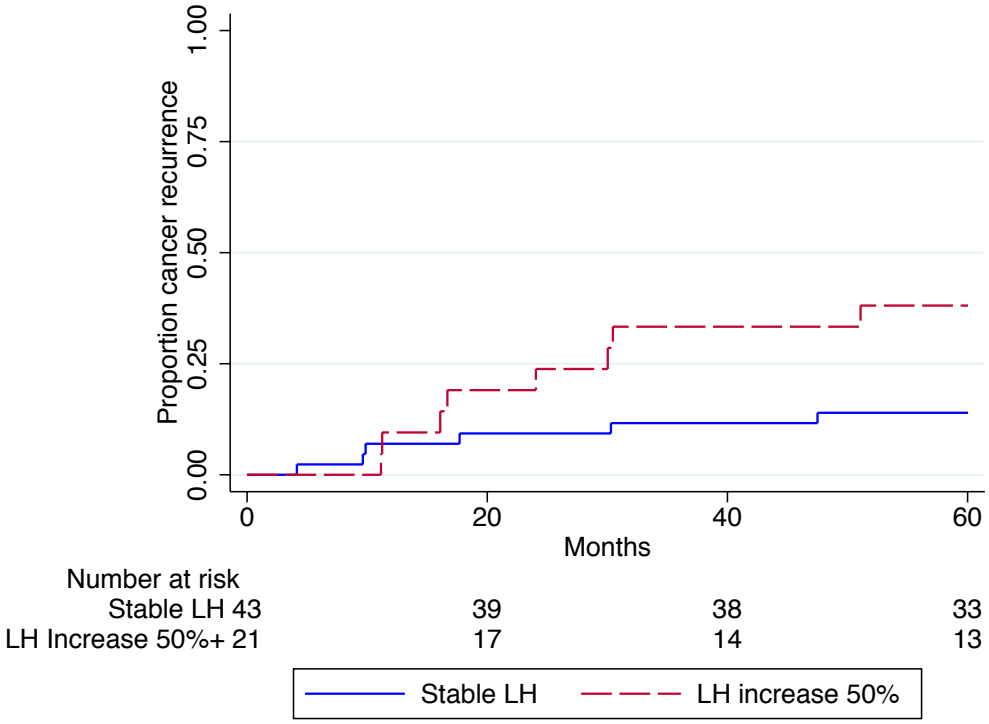

Figure 11. Cumulative incidence of rectal cancer recurrence, months after surgery.

Study III

Out of 104 study participants, 26 had severe postoperative AE (AE+), equaling a cumulative incidence of 25%. Baseline characteristics, including clinical tumor stage and treatment, did not differ, but AE+ had longer median hospital stay 16 (range: 4-46) days vs. AE- with 9 (4-31) days ($p < 0.001$) (Table 7).

Table 7. Baseline characteristics and treatment specific factors. Reprint from Study III.

	Clavien 0-2	Clavien 3+	*p*
Number of participants	78	26	
Age (Years)	63.5 (36-82)	63 (32-86)	0.816†
BMI (kg/m2)	24.8 (16.1-35.3)	26.2 (20.8-40.6)	0.145†
ASA-score			
I	17 (22)	4 (15.5)	
II	41 (52.5)	18 (69)	0.372*
III	20 (25.5)	4 (15.5)	
No. of comorbitidies			
0	30 (38.5)	10 (38.5)	
1	18 (23)	9 (34.5)	0.446*
≥2	30 (38.5)	7 (27)	
Current smoker			
No	68 (87)	22 (85)	0.746*
Yes	10 (13)	4 (15)	
Radiological tumor stage			
T2	9 (11)	6 (23)	
T3 a/b	35 (45)	11 (42)	0.539*
T3 c/d	10 (13)	3 (12)	
T4	24 (31)	6 (23)	
Radiological lymph node status			
N0	29 (37)	13 (50)	0.573*
N+	49 (63)	13 (50)	
Tumor distance from anal verge (cm)	8 (1-15)	7 (1-14)	0.595†
Neoadjuvant treatment			
None	10 (12.8)	3 (11.5)	
25 Gy, direct surgery	30 (38.5)	11 (42.3)	
25 Gy, delayed surgery	8 (10.2)	7 (26.9)	0.258*
25 Gy, chemotherapy and delayed surgery	10 (12.8)	1 (3.9)	
50 Gy	2 (2.6)	0 (0)	
50.4 Gy and concomittant chemotherapy	18 (23.1)	4 (15.4)	
Cumulative mean testicular dose, TD (Gy)	0.660 (0-14.369)	0.461 (0-14.193)	0.571†
Realtive TD (TD/Total dose*100)	2.301 (0-57.477)	1.513 (0-56.773)	0.571†
Time between radiotherapy and surgery (days)	34 (1 - 192)	41 (3 - 188)	0.755†
Type of resection			
Anterior resection	54 (69)	15 (58)	0.340*
Abdominoperineal excision	24 (31)	11 (42)	
Surgical operation time (minutes)	304 (163-605)	332 (141-857)	0.504*
Blood loss during surgery (mL)	600 (25-2800)	500 (150-16600)	0.783*
Length of hospital stay (days)	9 (4-31)	16 (4-46)	0.000†

Notes: Continous variable reported as median (range), Categorical data reported as frequency (percentage), *=Fisher´s exact test, †=Wilcoxon rank-sum test, ASA = American Society of Anesthesiologists, Gy = Gray.

Hormones and laboratory measurements

Preoperative levels of T fT and LH were similar in men with and men without severe postoperative AE. The median LH/T-ratio was higher in AE+ with 0.603 (0.2-2.5) vs 0.452 (0.13-5.93) for AE- ($p = 0.035$). In the AE+ group, median T decreased with 24% from baseline to preoperative samples, in AE- the same comparison yielded 13%. Preoperative laboratory markers, such as C-reactive protein and white cell blood count, did not differ between groups, see Table 8.

Table 8. Hormones and laboratory markers. Reprint from Study III.

	Clavien 0-2	Clavien 3+	
Baseline hormones			*p*
Testoterone (nmol/L)	11 (4.2-22)	11.5 (4-17)	0.949†
Free testosterone (pmol/L)	318.0 (89.7 - 665.7)	311.7 (100.5-496.3)	0.828†
Luteinizing Hormone (IU/L)	4 (1.6-13)	4.7 (2-16)	0.127†
LH/T-ratio	0.342 (0.11-2.097)	0.460 (0.182-2.5)	0.118†
Proportion with Testosterone < 8 nmol/L	11 (14)	5 (19)	0.539*
Preoperative hormones			
Testoterone (nmol/L)	9.6 (0.8-22)	8.7 (2.4 - 17)	0.286†
Free testosterone (nmol/L)	0.266 (0.017-0.666)	0.254 (0.046-0.534)	0.276†
Luteinizing Hormone (IU/L)	4.6 (1.6-16)	5.3 (2.1-16)	0.107†
LH/T-ratio	0.452 (0.127-5.926)	0.603 (0.2-2.5)	0.035†
Proportion with Testosterone < 8 nmol/L	22 (28)	10 (38)	0.337*
Labratory markers			
Preoperative			
C-reactive protein	3 (1-38)	4 (1-50)	0.243†
White cell blood count	5.45 (3.1-11.8)	5.6 (3.7 - 11)	0.190†
Albumin	38 (28-45)	38 (30 - 43)	0.905†
Postoperative, day 1 after surgery			
C-reactive protein, mg/L	85 (22-196)	100 (45-219)	0.341†
White cell blood count, Units x 10^9/L	7.8 (3.7 - 11.4)	8.5 (4.5 - 23.8)	0.432†
Albumin, g/L	26.5 (22-35)	26.5 (22-30)	0.918†

Notes: Continous variable reported as median (range), Categorical data reported as frequency (precentage) † = Wilcoxon rank-sum test, * = Fischer´s exact test.

Longitudinal regression analysis

The longitudinal regression analysis found that change in T had a suggested inverse relationship with severe postoperative AE (OR: 0.878, 95% CI 0.759-1.015, p = 0.078). Adjusting for age, BMI and ASA-score strengthened this to a significant inverse association between preoperative change in T and severe postoperative AE, OR 0.844 (95% CI 0.720-0.990, p = 0.034), illustrated in Figure 12. This translates to a OR of 1.18 to suffer a severe postoperative AE for one unit decrease of T between baseline and preoperative sampling. Increase in LH/T-ratio induced a OR 2.020 (95% CI 1.010-4.039, p = 0.047) for severe postoperative AE.

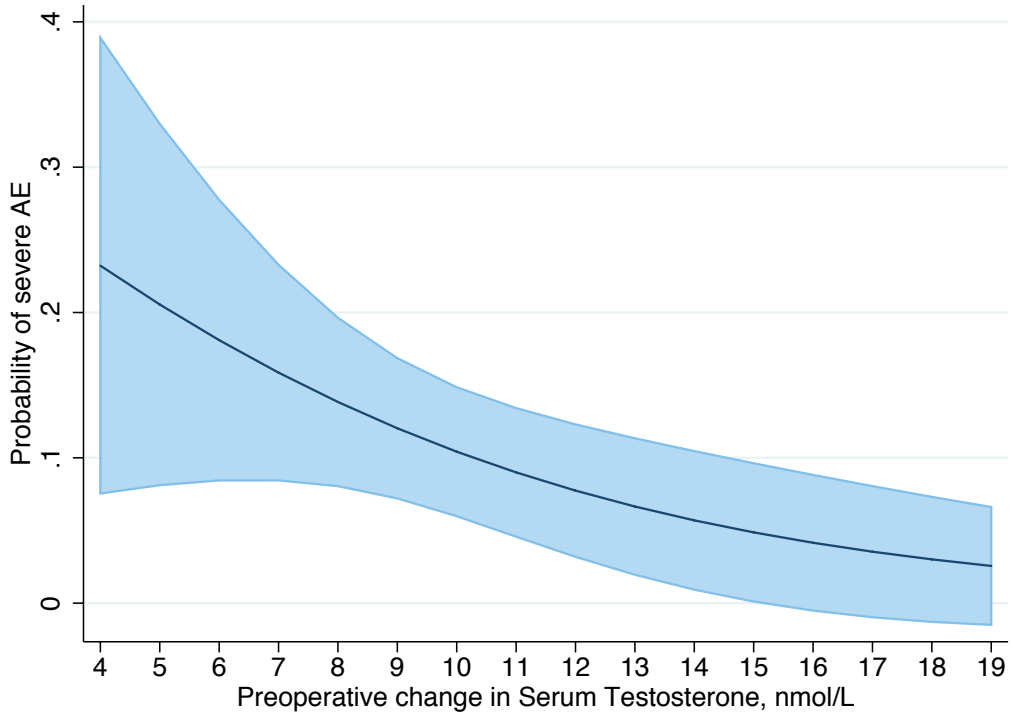

Figure 12. Predicted risk of severe postoperative adverse events graded 3+ according to Clavien-Dindo in relation to the preoperative change in serum testosterone. The curve describes the expected change in risk for severe postoperative adverse event with 95% confidence interval related to the preoperative change in serum testosterone. Reprint from Study III.

Study IV

Complete CT scans and hormone analyses of 102 men were available with RC stage I to III at baseline. Median age was 63.5 (32-86) years, median BMI 25.5 (16.1-40.6) kg/m^2 and 77.5% had ASA score I-II. At one-year follow-up, 66 study participants had submitted hormone samples. Comparing to baseline, median LH level and LH/T-ratio had increased.

Longitudinal muscle change

Psoas major could be traced for 92 study participants (CT n=78 and MRI n=14), and SMI could be analyzed in 86. Some examinations were done at the pelvic and upper abdominal/lung areas separately, omitting the L_3 level.

Psoas major area did not change statistically, 18.6 (7.5-32.0) cm^2 at baseline compared to 19.4 (9.4-34.0) cm^2 at follow-up (p=0.743) but attenuation increased from 41.4 (16.5-60.0) HU to 47.6 (27.2-71.3) HU (p<0.001). The proportion of sarcopenic PM decreased from 19.6% to 12.0% (p<0.001). Median total skeletal muscle area and SMI decreased (-11 cm^2, p=0.042 and -2.7 cm^2, p=0.029 respectively), while attenuation of total muscle area increased (p<0.001).

Longitudinal regression analysis found significant associations between changes in PM area and changes in fT, LH and LH/T-ratio in men with complete hormone samples at one-year follow up (Table 9). There was also a relationship between PM attenuation and fT. Changes in total muscle area was associated to changes in all hormones. Excluding right psoas width, fT was associated with all muscle measurements. Change in all muscle areas were associated with the LH/T-ratio.

Table 9. Longitudinal analysis of muscle changes in relation to hormones.

	Longitudinal change per unit in image analysis							
	Serum Testosterone	*P*	Free testosterone	*P*	Luteinizing Hormone	*P*	LH/T-ratio	*P*
Psoas major								
area	11.7 (-3.51 - 26.8)	0.132	0.70 (0.21 - 1.18)	0.005	-20.6 (-38.5 - -2.71)	0.024	-223 (-376 - -70.5)	0.004
attenuation	0.27 (-0.16 - 0.69)	0.218	0.02 (0.00 - 0.03)	0.011	-0.36 (-0.83 - 0.11)	0.137	-3.58 (-7.42 - 0.27)	0.068
Total muscle								
area	0.79 (0.11 - 1.47)	*0.022*	0.04 (0.02 - 0.06)	*<0.001*	-0.85 (-1.58 - -0.12)	*0.022*	-8.43 (-14.7 - -2.11)	*0.009*
attenuation	0.47 (0.00 - 0.94)	*0.049*	0.03 (0.01 - 0.04)	*0.001*	-0.32 (-0.85 - 0.21)	*0.239*	-3.33 (-7.77 - 1.12)	*0.142*
Left psoas major								
area	6.83 (-0.97 - 14.6)	0.086	0.36 (0.11 - 0.61)	0.004	-8.22 (-17.5 - 1.03)	0.082	-99.6 (-178 - -20.4)	0.014
height	0.24 (0.01 - 0.48)	0.042	0.01 (0.00 - 0.02)	0.021	-0.23 (-0.52 - 0.06)	0.114	-2.45 (-4.83 - -0.07)	0.043
width	0.19 (-0.06 - 0.43)	0.132	0.01 (0.01 - 0.02)	0.001	-0.15 (-0.45 - 0.15)	0.335	-1.78 (-4.22 - 0.66)	0.153
attenuation	0.23 (-0.23 - 0.69)	0.327	0.02 (0.00 - 0.03)	0.038	-0.33 (-0.83 - 0.18)	0.210	-3.33 (-7.50 - 0.83)	0.116
Right psoas major								
area	5.25 (-2.96 - 13.5)	0.210	0.35 (0.08 - 0.61)	0.009	-12.3 (-22.0 - -2.64)	0.013	-123 (-206 - -41.4)	0.003
height	0.20 (-0.04 - 0.43)	0.100	0.01 (0.00 - 0.02)	0.016	-0.24 (-0.52 - 0.03)	0.084	-2.72 (-5.02 - -0.42)	0.021
width	0.03 (-0.22 - 0.28)	0.822	0.01 (-0.00 - 0.01)	0.095	-0.27 (-0.57 - 0.04)	0.084	-2.01 (-4.50 - 0.47)	0.113
attenuation	0.41 (0.01 - 0.80)	0.044	0.02 (0.00 - 0.03)	0.001	-0.42 (-0.89 - 0.05)	0.077	-4.37 (-8.15 - -0.60)	0.023

Notes: Total muscle area is measured in cm^2, psoas major area is measured in mm^2, height and width are measured in cm, attenuation in Hounsfield units. Adjusted for weight (kg) and time (days) elapsed between hormone samples.

Oncological outcome

Out of the 102 participants, 28 had distant metastases and one had a LR registered as first cancer related event postoperatively. Two study participants had systemic disease discovered already during the perioperative period and were excluded from this analysis. Of the 28 study participants suffering distant metastasis, 22 had complete CT/MRI examinations at baseline and at, and six of those 22 had distant metastasis discovered prior to the one-year follow-up. Psoas major attenuation and right psoas height were related to metastases in all 22 with complete CT/MRI examinations, see Table 10. A negative change in total muscle area and SMI were associated with distant cancer recurrence. Sub-analysis of later metastases, in those 16 study participants diagnosed after to the follow-up, found a more distinct association between total muscle area measured at one-year and later metastases (-7.88 (-13.6 - -2.21) cm^2, p=0.006).

Table 10 Longitudinal analysis of muscle change and recurring systemic rectal cancer.

	Longitudinal change in image analysis due to metastases	*P*
Psoas major area	-53.3 (-164.5 - 58.0)	0.348
attenuation	5.34 (0.91 - 9.78)	0.018
Total muscle area	-5.96 (-10.7 - -1.24)	*0.013*
attenuation	4.14 (-0.32 - 8.60)	*0.069*
Skeletal Muscle Index	-1.96 (-3.48 - -0.43)	*0.012*
Left psoas major		
area	-16.2 (-73.1 - 40.7)	0.577
height	-1.38 (-3.18 - 0.42)	0.132
width	1.59 (-0.50 - 3.68)	0.136
attenuation	5.55 (0.91 - 10.2)	0.019
Right psoas major		
area	-36.6 (-97.7 - 24.5)	0.240
height	-1.89 (-3.65 - -0.12)	0.036
width	0.55 (-1.57 - 2.67'9	0.611
attenuation	5.16 (0.78 - 9.54)	0.021

Notes: Total muscle areas are measured in cm^2, psoas major areas are measured in mm^2, height and width in mm, attenuation in Hounsfield units, height/width in cm. Adjusted for weight (kg) and free Testosterone

Discussion

Androgen sensitivity of appendicular muscles has been shown before, which was not the case for human abdominal and pelvic muscles. **Study I** demonstrated a dose-dependent relationship between T and specific skeletal core muscles. This allows for wider considerations of the impact of hypogonadism, e.g. loss of balance and falls with subsequent morbidity, as well as possible treatment for pelvic floor weakness.

The long-term effects of RT, using modern RT techniques, were explored in **Study II**. On average, T and fT levels recuperated within two years after surgery. The median levels of LH and FSH as well as the LH/T-ratio were increased after two years in irradiated men, compared to baseline, indicating an increased degree of compensated hypogonadism. Longitudinal changes in hormones were associated with TD, previously not shown in RC. Elevated LH levels after one year tripled the risk of systemic cancer recurrence, a possible reaction to systemic disease before metastases are discernible with current follow-up regimen.

In the short term, **Study III** presented the novel finding that preoperative testicular failure due to RT is related to severe postoperative AE. Anabolic effects of T may play an important role in limiting the systemic inflammatory response on surgical trauma.

Study IV validated earlier findings that PM and abdominal muscles are associated with androgen levels in study participants treated for RC with RT. Loss of total muscle area at the 3rd lumbar vertebra and changes in PM composition were associated with later systemic cancer recurrence, another possible indication of systemic disease before discernible metastases.

Interpretation

The finding that abdominal and pelvic muscles are at least as responsive to androgens as the appendicular muscles were somewhat anticipated due to the known anabolic effects of T. A small study had also shown similar results in hypogonadal men in regards to paraspinal muscles.[226] Atrophy of the core muscles, e.g. in wasting conditions such as liver cirrhosis or prolonged bed rest, may lead to instability and predisposition to falls.[256-260] Common chronic low back pain, associated with atrophy of the abdominal muscles, predisposes to mobility limitation and loss of balance.[261] This new evident data allows for educated further studies of these muscles in differing conditions, including sarcopenia and frailty, where treatment might be beneficial for short- and long-term outcomes. Especially, core stabilizing abdominal muscles and the pelvic muscles could possible targets for treatment in older men suffering from frailty or pelvic floor disorders.[222, 223] In regards to surgical settings, for total knee arthroplasty there seems to be faster recovery in study participants treated with anabolic steroids, but so far studies have been small and no general recommendation exists.[262] As shown in Study I, MRI allows for solid and reproducible research in this field.

The general knowledge regarding RT in RC treatment was that outdated RT induced Leydig cell damage to varying, individual, degree and within differing timespans. In the present cohort study, a larger and/or more structured group was studied than previously done. The results of average recuperation of T and fT levels tells us that, with current RT methodology, the average T levels

recovers within two years. The novel finding that TD does have an association with long-term hormonal change verifies the casual relation between RT and adversely affected hormone levels. The signs of increased compensated hypogonadism do also suggest that, even if average recuperation of T levels is present after two years, Leydig cells may not be not fully restored. Permanent Leydig cell dysfunction may result in increased long-term risk of morbidity and mortality linked to strained or overtaxed androgen production. Increased levels of LH have been associated with several morbidities as well mortality, causation still remains uncertain however, but not to RC cancer recurrence prior to this study. The process linking elevated LH and RC recurrence cannot be elucidated in this material but LH seems to be a possible marker for later systemic disease and could compliment decision making regarding treatment and follow-of regimens.

A preoperative decrease in T levels after RT resulted in increased risk of suffering severe postoperative AE. This finding perhaps mirrors part of the results in the Stockholm III study, showing that the group with sRT and delay suffered fewer complications than those that were operated without delay. The delay may have allowed for Leydig cell recuperation, if possible. It could also be part of an explanation to why men suffer more postoperative adverse events than women. In the literature, endocrine testicular function in regards to RC treatment outcomes is limited. In a study encompassing abdominal surgery, low T was a risk factor for postoperative complications and T levels decreased by surgery have been shown to recover slower after postoperative AE.[263] It is possible that RT-induced T level decrease, T being the main anabolic hormone in men, adversely impacts postoperative healing by shifting the body towards a more catabolic hormonal state and also by modulating systemic inflammatory response on surgical trauma.

Body composition changes in RC patients were linked to changes in androgen levels, in line with the findings in Study I regarding generally younger, healthy men. This finding adds further to the knowledge regarding androgen effect on muscles in men. Due to damages to nerves and microvasculature in multimodal RC treatment, the symptoms used in diagnosis of hypogonadism may be unreliable. In this, longitudinal muscle loss measured on routine CT/MRI examinations might be a supplementary marker of hypogonadism. Muscle loss may also herald occult cancer disease, it has been shown in pancreatic cancer up to five years prior to diagnosis. [264, 265] In CRC patients, metastatic seeding can occur in some patients before the carcinoma is detectable using routine examinations.[266] This means that sarcopenic signs present at baseline or follow-up may point at occult metastases, and that under those circumstances, a more advanced treatment regimen than recommended today may be beneficial.

Limitations and validity

The MRI examinations in Study I were not optimized for the analysis performed, hence not all examinations covering all areas of interest, but using manual tracing allowed for exact tracking of muscle boundaries and compensation for potential image artifacts making the most of the existing images. Given that the trial was not designed for evaluation of trunk and pelvic muscles, the result of this study should be viewed as exploratory. By using a single experiences investigator, inter-observer variability was nullified. Interobserver variance was tested well within standard. The study

was relatively small but still the largest to date in this setting. The randomized double-blind study design is a strength as well as the comprehensive range of T dosage used.

The Cohort

The androgen levels at baseline were not associated with group assignment, which was in effect decided independently at the MDT conference, type of cancer or type of preoperative oncological treatment. This reduces the risk of selection bias. The study was not large enough to distinguish eventual differences between RT regimens. Misclassification of exposure was not an issue, and exposure was quantified by calculation of cumulative mean TD from planning CT scans.

Treatment with APE was 37% in the exposed group, a larger proportion than in the general population of men presenting with RC. As PTV thereby will include the pelvic floor, leading to higher TD, the effect of RT on endocrine testicular function may not be representative for the general population. Type of surgery was not associated with the amount of testicular failure. The exposed men with incomplete follow-up had higher ASA-score and impaired testicular function at baseline: higher proportion of T below 8 nmol/L, higher LH and LH/T-ratio. In the unexposed group there was no difference in baseline characteristics between those that completed the study versus those that did not, which may result in an overestimation of the testicular recovery of the exposed group. The unexposed group surprisingly had decreasing levels of T, which complicates the comparison.

At baseline serum T levels were lower than expected, median 11 nmol/L, whereas the EMAS study (n=2736) showed a mean of 16.5 nmol/L.[130] In comparison, young healthy males participating in the Framingham Heart Study had a median (quartile range) serum T of 24.2 (10.3) nmol/L .[251] The diagnosis of RC may in itself be a reason for low serum T levels, as psychological stress is known to have a negative impact on serum T levels.[267-269] The inflammatory reaction to the tumor may also adversely impact T levels.[270, 271] Prostate cancer patients of comparable age have been shown to have low T baseline levels (n = 25, 10.6 ± 0.94 nmol/L).[272] However, the median T levels after two years in the treated men returned to baseline levels. As T levels have diurnal fluctuation and are dependent on feeding and sexual habits, standardized sampling is important (fasting morning sample, no sexual activity). Serum T levels may decrease approximately 30% if eating before sampling and serum T seems to increase with sexual activity.[273-276] Collection of blood samples and laboratory analyses for androgen assessment were done according to the guidelines of the Endocrine Society. The used longitudinal regression analysis accounts for the covariance of repeated samples in the study population and final models were adjusted for important confounders and effect modifiers.

The oncological and AE analysis were based on a subgroup thus not following the design of the original trial. The results should be validated in dedicated studies. However, the respective sub-groups did not differ in essential baseline- or perioperative characteristics or TD. As for image analysis, the intra-observer variability was low (<2 %).

Future perspectives

The findings of postoperative implications of testicular failure due to RT promotes a study designed to follow hormone levels during the pre- and postoperative period to validate the current finding as well as develop understanding of hormonal impact in wound healing. The next step is to investigate if TRT might be of value in the perioperative period in men with adversely impacted T levels.

Evaluation of muscle mass as a possible marker for hypogonadism in men lacking possibility to express the typical symptoms needs to be evaluated in a dedicated study, and if successful could be integrated in the ordinary follow-up in RC patient treatment.

Sarcopenic signs, or muscle mass loss, as well as increased LH as markers for occult metastases or cancer recurrence may complement current practice as it seems they predate metastatic detection by current methods.

Conclusions

Overall conclusion

Testicular endocrine function is threatened by RT, with both short- and long-term implications, and the testes should be considered an organ at risk in sRT as they currently are in CRT.

Specific conclusions

- Abdominal and pelvic muscles are as responsive to androgens as appendicular muscles in young healthy men, with clear dose-response relationship. Changes are clearly evaluated on MRI.

- Radiotherapy treatment in RC results in impact on the hypothalamic-pituitary-gonadal axis, related to TD. Average T level are regained within two years after surgery but select patients does not recover and signs of compensated hypogonadism is increased. Increased LH after one-year is associated with cancer recurrence.

- Decline in preoperative levels of T due to RT is a risk factor for adverse postoperative adverse events graded 3+ according to Clavien-Dindo. This could hypothetically explain in part why men suffer more postoperative complications than women.

- Abdominal and PM muscle area are androgen dependent in older men with RC, and decrease in total abdominal muscle area is related to later metastatic disease. Data can be evaluated using routine radiologic examinations and might be of clinical as well as prognostic value.

Sammanfattning på svenska

Rektalcancer, den åttonde vanligaste cancerformen i Sverige, drabbar ca 2100 personer per år. Fler män än kvinnor insjuknar i rektalcancer. Rektalcancer definieras med att tumören sitter inom 15 cm från ändtarmsöppningen. Behandlingen är i första hand kirurgisk, med avlägsnande av mer eller mindre av rektum inklusive tumör, omkringliggande blod- och lymfkärl, lymfknutor och en viss marginal av vävnad. Man strävar efter att följa embryonala strukturer för att minimera risk för att cancerceller finns kvar samt att minska blödning och nervskador. Beroende på tumörens läge och omfattning kan hela rektum inklusive tarmöppningen behöva tas med, vilket nödvändiggör permanent stomi, i annat fall kan tarmen sys ihop i en s.k. anastomos. I modern rektalcancerbehandling ingår ofta preoperativ strålbehandling, som i sig visats sänka risken för lokalrecidiv med ca 50%. Dock medför strålbehandling diverse bieffekter, däribland risk för testikeldysfunktion och påverkade hormonnivåer.

Denna avhandling redogör för testosterons effekter i kroppen och sätter det i relation till strålterapi.

Studie I genomfördes i Boston, med material från en randomiserad kontrollerad studie och analyserade effekterna av olika doser av administrerad testosteron på muskler i buk och bäcken hos yngre friska män. Inga tidigare studier har genomförts med sådant material och med MR som metod. Skelettmusklers relation till det anabola hormonet testosteron är väl studerat i framförallt armar och ben samt i ”fettfri vävnad” vilket dock inkluderar bukens organ. Studien kunde påvisa ett dos-beroende samband mellan muskelarea, vilket har visats relatera till total kroppsmuskelmassa, och testosteron. Detta möjliggör fortsatta studier och tanken att kunna behandla svaghet i dessa muskler, som kan innebära dålig balans och ökad risk för fall liksom bäckenbottenproblematik, med testosteron.

Studie II-IV arbetade med ett material från en kohortstudie inkluderande rektal- och prostatacancerpatienter i Stockholm.
Studie II tog vid en tidigare studie som påvisat akut testikeldysfunktion efter strålningsterapi. I den strålade gruppen så återgick den mediala testosteronnivån till samma nivå som innan behandling inom två år. Dock så var luteiniserande hormon, det hormon som styr testosteronnivån, påverkat på ett sådan sätt som tyder på att testiklarna i snitt inte var helt återställda. Vidare så fanns det en relation mellan förhöja nivåer av luteiniserande hormon ett år efter kirurgisk behandling och ökad risk för cancerrecidiv.
I *studie III* så undersöktes risken för svåra postoperativa komplikationer i relation till minskade nivåer av testosteron på grund av strålning. En testosteronminskning innebar en nivåberoende ökad risk för svåra postoperativa komplikationer, detta oberoende på andra behandlingsmässiga faktorer, såsom typ av kirurgi.
Studie IV validerade en tidigare pilotstudie från kohorten och fann samband mellan fritt testosteron och muskelarea mätt på CT eller MR. Detta ger att samband mellan androgener och bukmuskler gäller även i denna grupp av i snitt äldre män med cancersjukdom. Vidare var minskande muskelarea under första året efter operation relaterad till systemiskt recidiverande cancersjukdom.

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