Imperial College London

ASSESSMENT OF SYSTEMIC EFFECTS OF SEX HORMONE ALTERATIONS IN ANDROGEN DEPRIVATION THERAPY FOR PROSTATE CANCER

A THESIS SUBMITTED TO IMPERIAL COLLGE LONDON IN FULFILLMENT OF THE REQUIREMENT FOR THE DEGREE

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DOCTOR OF PHILOSOPHY

BY

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Declaration of originality

I, Dr. Syed Imran Ali Shah, declare that this thesis is my own work and that it has not been previously presented for any award. Other sources of information have been duly accredited wherever used. The submission is based on the results of original research that I carried out myself and any assistance from individuals and/or institutions has been acknowledged in the relevant sections.

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Abstract

Sex hormonal variations cause bodily changes reflecting their systemic functions. This project was designed to define the influence of sex hormones on systemic function in men with prostate cancer (PC) receiving contemporary androgen deprivation therapy (ADT) with luteinising hormone-releasing hormone agonists (LHRHa). LHRHa induced suppression of sex hormones results in multiple serious toxicities of which the cognitive, skeletal and psychosocial aspects were addressed in this project.

Acute castration appears to cause cognitive problems in some men with PC. In order to identify the neuropathology of such cognitive decline, positron emission tomography (PET) was used along with a battery of validated neuropsychological tests, documenting for the first time an increased global and regional cortical neuroinflammatory response in patients with PC experiencing cognitive deterioration since being on LHRHa.

Bone mineral density, a commonly employed measure of bone mass, lacks accuracy in predicting bone strength and fracture risk. The present work showed bone volume fraction (a newer non-invasive metric of bone mass derived using computed tomography imaging) to be highly correlated with bone strength measured ex-vivo, thereby demonstrating its potential for use in clinical assessments. Levels of serum bone-specific alkaline phosphatase, a marker of skeletal metabolism, were also measured to assess bone changes in the early stages of LHRHa therapy but no change was observed.

Metabolic derivatives of sex hormones have been suggested to influence psycho-socio-sexual behaviour via odour signalling. The current work, investigating effects of castration on odour, showed no change in the olfactory perception of odour samples provided by men on LHRHa treatment.

These pilot data and observations will help shape future work that may not only improve quality of life outcomes in men with PC undergoing ADT but also benefit patients suffering from other clinical conditions involving physiological, pathological or therapeutic changes in sex hormone levels.

Acknowledgments

As I write these lines to recognise the help that I was fortunate enough to get in doing this job, I realise how tough it actually is to acknowledge all the people and institutions in a befitting manner. I will try my best to do justice.

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I wish to express sincere gratitude to all of my highly supportive co-investigators, collaborators, colleagues and consultants. This project couldn't be completed without their invaluable input. I am especially thankful to my good friend Hannah Wilson for her unconditional academic and moral support. I would also like to thank all the research subjects whose participation has provided me with important data that forms the basis of this work. I am forever grateful to Commonwealth Scholarship Commission and the UK government for funding my PhD studies and thus enabling me to undertake this life-defining training.

I cannot finish without thanking my parents, my family and my siblings whose unflinching love has always been the driving force for me. It is to them that I dedicate this piece of work.

Abbreviations

μA	Micro-ampere
μm	Micro-metre
[11C]PBR28	Carbon-11 peripheral benzodiazepine receptor ligand
®	Registered trademark
2D	Two dimensional
3D	Three dimensional
AD	Alzheimer's disease
ALP	Alkaline phosphatase
AR	Androgen receptor
BALP	Bone alkaline phosphatase
BMD	Bone mineral density
BMI	Body mass index
BRC	Biomedical research centre
BRCA1	Breast cancer antigen 1 gene
BRCA2	Breast cancer antigen 2 gene
BVF	Bone volume fraction
C	Carbon
CMIA	Chemiluminescent microparticle immunoassay
CT	Computed tomography

CVS	Cardiovascular system
CWIT	Colour-word interference test
CYP17	Cytochrome P450 isoform 17
DEXA	Dual energy x-ray absorptiometry
DHEA	Dehydroepiandrosterone
DHT	Dihydrotestosterone
DICOM	Digital imaging and communications in medicine
D-KEFS	Delis-Kaplan executive function system
dL	Deci-litre
DNA	Deoxyribonucleic acid
DRE	Digital rectal examination
EBRT	External beam radiation therapy
EIA	Enzyme immunoassay
EPIC	Expanded prostate cancer index
ERα	Oestrogen receptor alpha
ERβ	Oestrogen receptor beta
FBP	Filtered back projection
fMRI	Functional magnetic resonance imaging
FSH	Follicle-stimulating hormone
GNB3	Guanine nucleotide binding protein (G protein), beta polypeptide 3

GnRH	Gonadotropin releasing hormone
GnRHa	Gonadotropin releasing hormone analogue
GWAS	Genome-wide association studies
HDL	High density lipoprotein
HPG	Hypothalamic-pituitary-gonadal
HRQoL	Health-related quality of life
HRT	Hormone replacement therapy
HSP	Heat shock protein
ICHT	Imperial college healthcare NHS trust
ICHTB	Imperial college healthcare tissue bank
JRCO	Joint research compliance office
kDa	Kilo-dalton
kV	kilo-volts
L	Litre
LBM	Lean body mass
LH	Luteinising hormone
LHRH	Luteinising hormone releasing hormone
LHRHa	Luteinising hormone releasing hormone agonist
MBq	Mega-becquerel
MCI	Mild cognitive impairment

MHC	Major histocompatibility complex
mL	Milli-litre
mm	Milli-metre
MPa	Mega-pascal
MP-RAGE	Magnetisation-prepared rapid gradient-echo sequence
MRI	Magnetic resonance imaging
mRNA	Messenger ribonucleic acid
ms	Milli-second
mSv	Milli-sievert
N	Newton
ng	Nano-gram
NIHR	National institute for health research
nm	Nano-metre
nmol	Nano-mole
NRES	National research ethics service
NTX	N-terminal telopeptide of type I collagen
^{o}C	Degree Centigrade
OSEM	Ordered subset expectation maximisation
P1NP	Procollagen type 1 amino-terminal propeptide
PBR	Peripheral benzodiazepine receptor

PC	Prostate cancer
PCOS	Polycystic ovarian syndrome
PET	Positron emission tomography
pg	Pico-gram
pmol	Pico-mole
pNPP	Para-nitrophenylphosphate
PR	Progesterone receptor
PSA	Prostate-specific antigen
QoL	Quality of life
R	Repetition time
R&D	Research and development
R^2	Correlation coefficient
RANK	Receptor activator of nuclear factor kappa B
REC	Research ethics committee
ROI	Region of interest
RP	Radical prostatectomy
RT	Radiotherapy
SEM	Standard error of mean
SERM	Selective oestrogen receptor modifier
SHBG	Sex hormone binding globulin

SHR	Steroid hormone receptor
SNP	Single nucleotide polymorphism
SRY	Sex-determining region of Y chromosome gene
StAR	Steroidogenic acute regulatory protein
SUV	Standardised uptake value
SUVR	Standardised uptake value ratio
T	Tesla
TE	Echo time
™	Trademark
TNM	Tumour-Node-Metastasis
TRUS	Transrectal ultrasound
TSPO	Translocator protein
U	Units
UDP	Uridine diphosphate
UK	United Kingdom
USA	United States of America
VACURG	Veterans Administration Cooperative Urological Research Group
WASI	Wechsler abbreviated scale of intelligence
WMS-III	Wechsler memory scale, third edition
WTAR	Wechsler's test of adult reading

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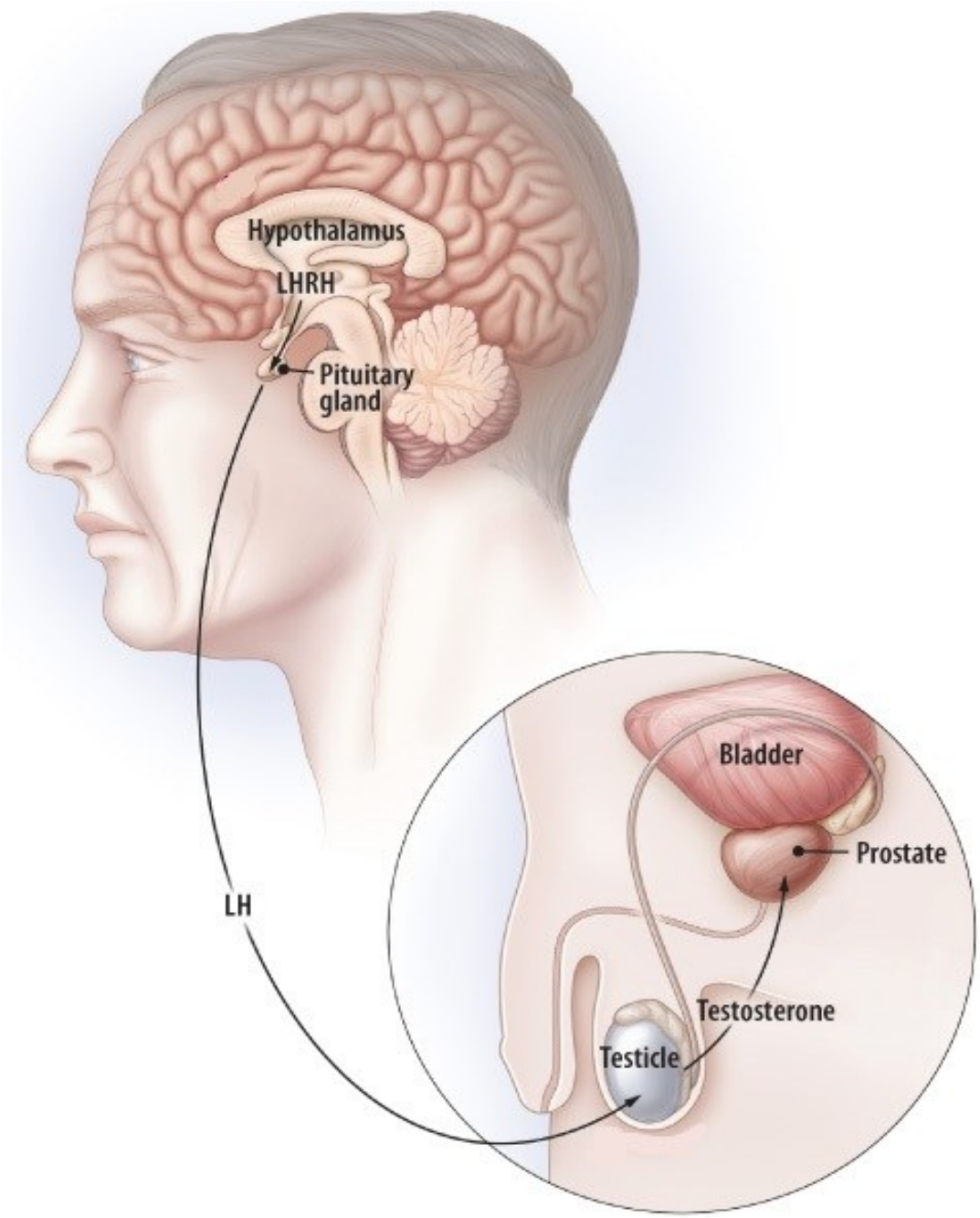


Adapted from: http://www.harvardprostateknowledge.org/hormone-therapy-for-prostate-cancer

1. Background

1.1 Reproduction

Reproduction is a fundamental property of living organisms ensuring continuity of their species, which from a biological viewpoint constitutes the foremost purpose of life. The two basic strategies employed by animals for reproduction include asexual and sexual reproduction.

Table 1 Comparison of sexual and asexual reproduction [1, 2]

Sexual Reproduction	Asexual reproduction
Combination of gametes in a process called fertilization	No gametes involved
Genetic information contributed by two parents	Genetic information contributed by a single parent
More resources are required e.g. time, energy, availability of partner	Lesser resources required
Genetic variation results from sexual reproduction only	No genetic variation via asexual reproduction
Genetic variation helps a species survive. During adverse environmental changes or increased competition for resources, organisms with varying genetic traits may exhibit a survival advantage	Fluctuating environment changes or increased competition may prove fatal for all organisms due to lack of genetic variation
Due to genetic recombination, offspring may lose the genes favourable to a particular stable environment	Offspring acquires same parental traits that are well adapted to a particular environment
Complex organisms tend to reproduce sexually e.g. humans	Simple organisms tend to reproduce asexually e.g. bacteria

In asexual reproduction, an individual produces genetically identical offspring by mitosis, thus allowing it to clone itself. Sexual reproduction involves the union of sex cells known as gametes. Gametes, having half the number of chromosomes of the original cell

(haploid), are produced by meiosis. Sperm (male gamete) and egg (female gamete) fuse together during fertilization to form a diploid cell, zygote, which is the first cell of the progeny. Table 1 gives a comparison between sexual and asexual reproduction. Sexual reproduction remains the most common method of reproduction in vertebrates including humans [1, 2].

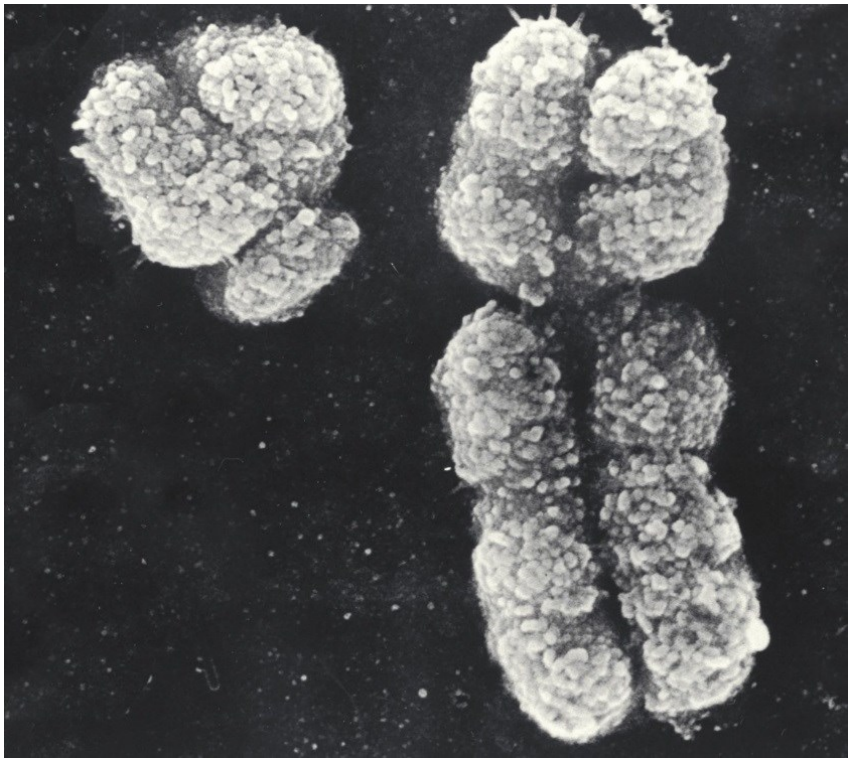


Figure 1 Scanning electron micrograph of human X and Y chromosomes
(Left: Y chromosome, Right: X chromosome)
Adapted from http://www.anatomybox.com/tag/sem/

Sexual reproduction evolved with the development of sex chromosomes. Humans have 46 chromosomes of which 44, called autosomes, are members of identical pairs. The remaining two i.e. X and Y chromosomes, however, do not form an identical pair and are referred to as the 'sex chromosomes' (Figure 1). In humans, females have two X chromosomes whereas males have one X and one Y chromosome. This difference in sex chromosomes gives rise to the anatomical and physiological dissimilarities in the two genders which are the pre-requisites for human sexual reproduction. Biochemical parameters also vary between males and females in addition to the structural and functional distinctions. Amongst

these biochemical factors are reproductive hormones which perform an important role in sexual development and reproduction.

1.2 Sex hormones

A hormone is a chemical messenger produced in one cell or gland that affects the function of its target cell or organ in another part of the body. The term hormone is derived from the Greek word 'horme' meaning 'impulse'. The major reproductive hormones, also termed sex hormones, control the growth and function of the reproductive organs and the development of secondary sex characteristics. Oestrogens and progestogens are typically referred to as female sex hormones while androgens are termed male sex hormones. However, these hormones are present in both sexes but at different concentrations, also varying with age and phases of menstrual cycle in females (Table 2). The word oestrogen takes its origins from the Greek words '*oistros'* meaning 'mad desire' and *'genein'* meaning 'to produce'. Similiarly, androgen is derived from the Greek '*andros*' meaning 'male'.

Table 2 Reference ranges for serum sex hormone levels in adults [3]

Sex hormone		Subject	Range
Androgens	Testosterone	Male	10-35 nmol/L 300-1000 ng/dL
		Female	0.7-3 nmol/L 20-85 ng/dL
	Dihydrotestosterone	Male	30-85 ng/dL
	Androstenedione	Male & Female	60-270 ng/dL
Oestrogens	Oestradiol	Male	50-200 pmol/L 14-55 pg/mL
		Female (Pre-menopausal)	70-600pmol/L 19-160 pg/mL
		Female (Post-menopausal)	< 130 pmol/L < 35 pg/mL
Progestogens	Progesterone	Male	0.27-0.9 ng/mL 0.86-2.9 nmol/L
		Female (Mid-Luteal phase of Menstrual cycle)	17-92 nmol/L 6-29ng/mL
		Female (Post-menopausal)	< 1 ng/mL < 3 nmol/L

1.2.1 Biosynthesis

The biosynthesis of steroid hormones involves multiple enzyme systems expressed in different tissues. Steroid hormones are synthesized from cholesterol which is a 27-C lipid molecule containing cyclopentanophenantherene steroid nucleus (Figure 2) [4].

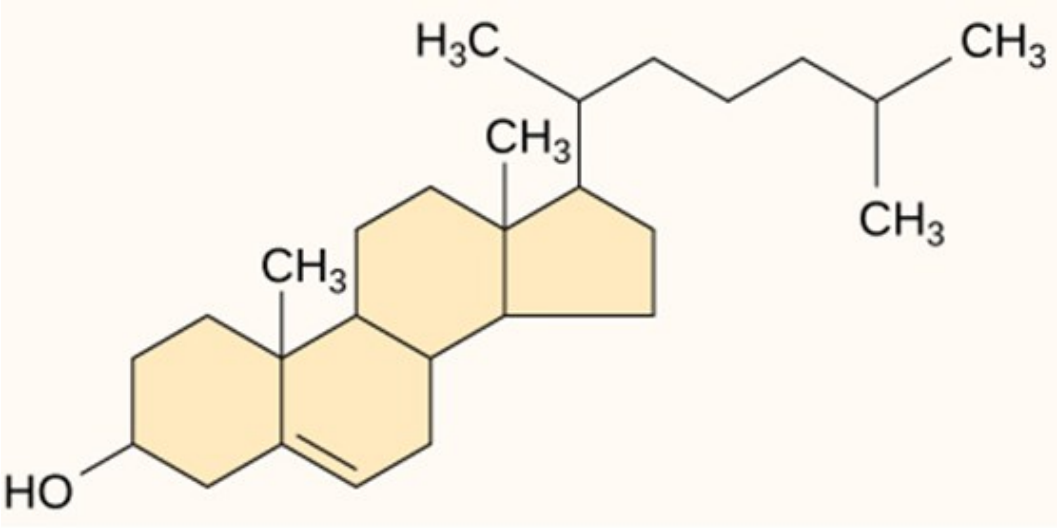


Figure 2 Molecular structure of cholesterol

Adapted from http://www.satorihealth.co.uk/articles.php?id=15

Cholesterol is composed of three regions; a hydrocarbon tail, a steroid ring structure nucleus with 4 hydrocarbon rings, and a hydroxyl group.

Enzymatic alterations in the cholesterol molecule produce three classes of steroid hormones: 1) mineralocorticoids e.g. aldosterone 2) glucocorticoids e.g. cortisol and 3) gonadocorticoids e.g. androgens, oestrogens and progesterone. These gonadocorticoids are classed together because of their actions on reproductive physiology and commonly referred to as sex hormones or sex steroids [5]. Testosterone, dihydrotestosterone (DHT) and androstenedione are the major body androgens while oestrogens include oestradiol, oestrone and oestriol. Dehydroepiandrosterone (DHEA), derived from cholesterol, is the precursor molecule for the synthesis of androgens and oestrogens.

The conversion of cholesterol to pregnenolone, which occurs in the mitochondria, is the first and rate limiting step in the steroidogenic pathway catalysed by the enzyme cholesterol mono-oxygenase [6]. Microsomal cytochrome P450 isoform 17 (CYP17; having 17α-hydroxylase and 17,20-lyase activities) enzymes present in the endoplasmic reticulum act on pregnenolone to yield carbon (C)-19 steroid derivative dehydroepiandrosterone. Subsequently, a series of reductive and isomerisation steps lead to the production of androstenedione and testosterone (Figure 3).

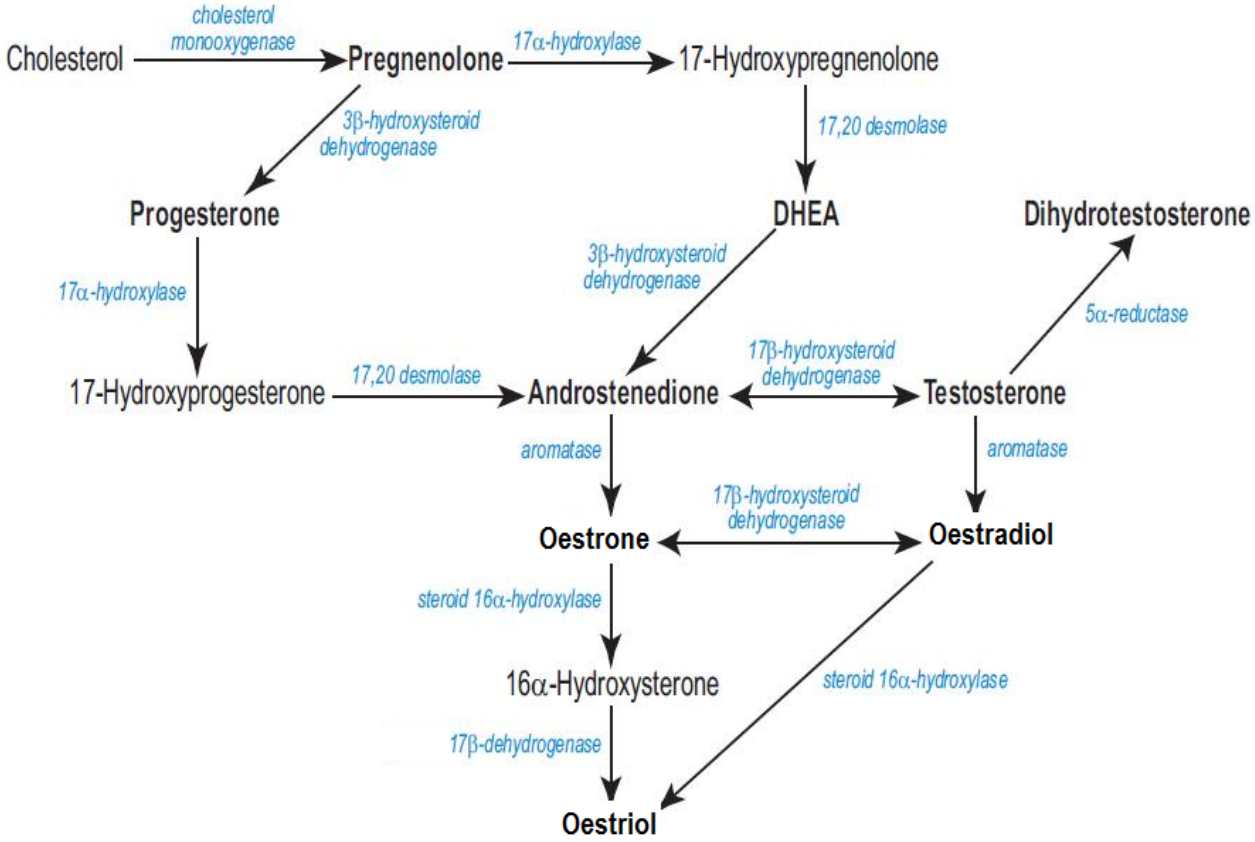


Figure 3 Biosynthetic pathway of sex hormones

Adapted from https://pharmaceuticalintelligence.files.wordpress.com/2015/02/steroid-hormone-synthesis.jpg

Testosterone is converted into DHT by the action of 5α-reductase. Androstenedione and testosterone serve as substrates for oestrogen biosynthesis and are converted into oestrone and oestradiol respectively by aromatisation (Figure 3). Pregnenolone also yields progesterone by the enzymatic action of 3β-hydroxysteroid dehydrogenase which is further converted into aldosterone, cortisol or androstenedione (Figure 3) [6-9].

1.2.2 Sites of synthesis

The activity of enzyme systems and the physiological nature of the steroidogenic cells determine the type of steroid hormones produced and secreted by them. In males, about 95% of testosterone production takes place in the Leydig cells of testes and the remaining 5% in adrenal glands. Around 85% of androstenedione in blood stream comes from testes and adrenals while the rest of it is produced in other tissues (adipose, skin, liver) from DHEA [8].

Ovaries are the principal sites for oestrogen biosynthesis in females, accounting for approximately 95% of systemic oestradiol. Extragonadal sites include adipose tissue, bone and brain which serve as the main sources of oestrogen following menopause while during pregnancy, placenta also contributes to the oestrogen pool [7, 10-12].

1.2.3 Quantitative and qualitative control

The quantitative control of steroid biosynthesis is provided by two proteins identified with cholesterol transport across the mitochondrial membrane which are the steroidogenic acute regulatory protein (StAR) and translocator protein (TSPO), formerly called peripheral benzodiazepine receptor (PBR). Both these proteins reside on the outer mitochondrial membrane and facilitate movement of cholesterol into the mitochondria [13].

Furthermore, the direction of pregnenolone towards sex hormone synthesis in the adrenal glands is dependent on the microsomal enzymes which provides the qualitative regulation. C-19 precursors of sex steroids are only produced when 17α-hydroxylase and 17,20 lyase activities of CYP17 are present (Figure 3). Absence of one or both of these activities leads to synthesis of glucocorticoids or mineralocorticoids [8].

1.2.4 Regulation and metabolism of sex hormones

The levels of sex hormones in the body are regulated primarily by hypothalamic-pituitary-gonadal (HPG) axis signalling which is involved in the production of most sex steroids. Local tissue level modulation of sex hormone metabolism is another important regulatory mechanism.

1.2.4.1 Hypothalamic-pituitary-gonadal axis

The principal regulation of sex steroid synthesis is carried out by a negative feedback effect on HPG axis exerted through downstream production of sex hormones (Figure 4) [6]. Luteinising hormone releasing hormone (LHRH), also known as gonadotropin releasing

hormone (GnRH), is a hypothalamic peptide transported via hypothalamic-hypophyseal portal venous system to the anterior pituitary gland. LHRH acts on the anterior pituitary which in turn secretes the glycopeptides luteinising hormone (LH) and follicle-stimulating hormone (FSH) into the circulation. In males, FSH regulates spermatogenesis in the testes by binding to its surface receptors on the Sertoli cells while LH acts on testicular Leydig cells to initiate androgen synthesis. In females, LH acts on ovarian theca cells to stimulate oestrogen synthesis while FSH affects granulosa cells to initiate follicular growth and maturation during menstrual cycle. Mid-cycle LH surge from the anterior pituitary triggers ovulation and LH also leads to the generation of corpus luteum from the residual follicle [14] [15].

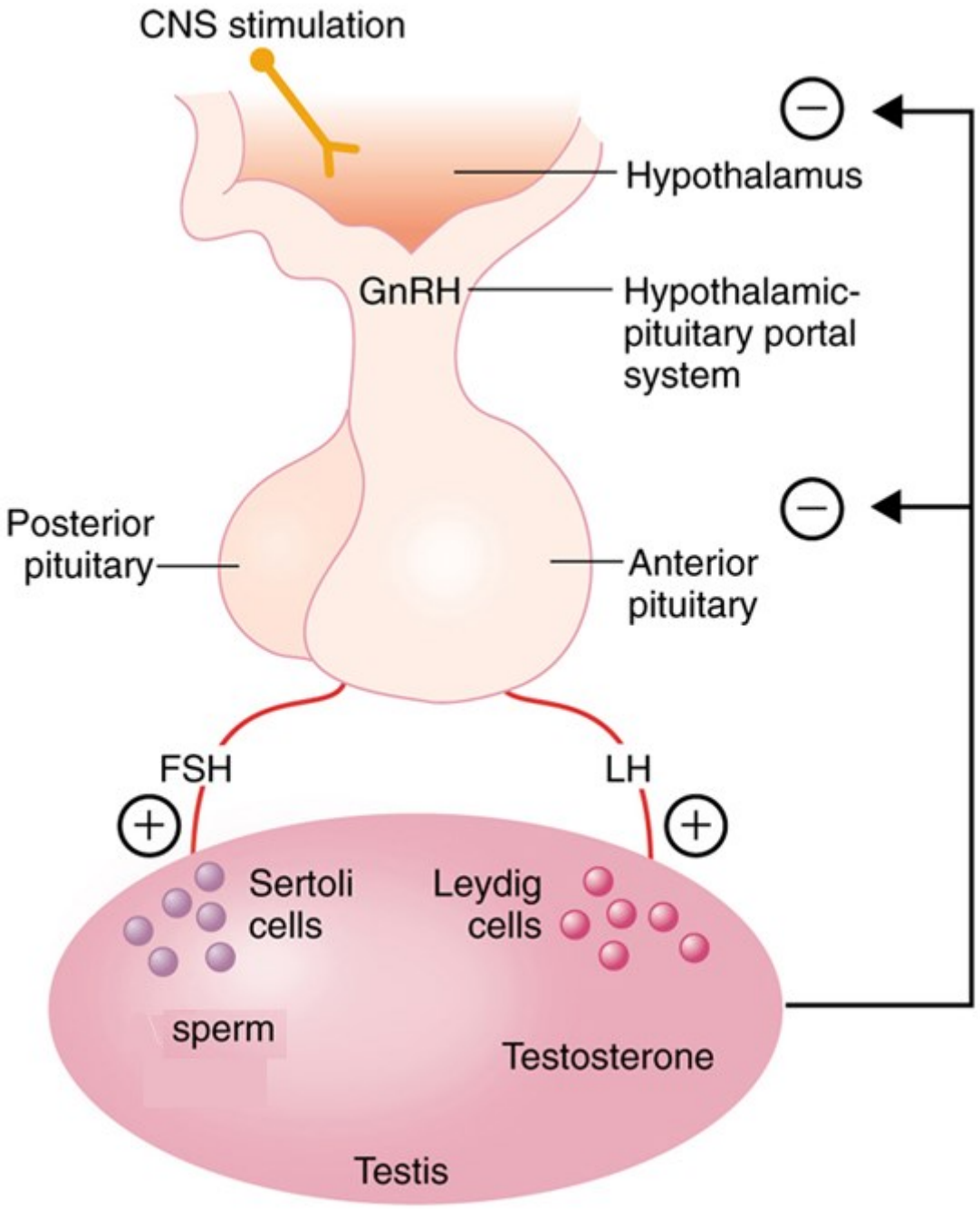


Figure 4 Feedback regulation of gonadal hormone production via HPG axis

Adapted from http://www.clevelandclinicmeded.com/medicalpubs/diseasemanagement/endocrinology/male-hypogonadism/

Negative feedback inhibition of GnRH (LHRH) and LH release results from binding of testosterone to androgen receptors present in the hypothalamic neurons and anterior pituitary

1.2.4.2 Tissue level regulation

Sex hormones undergo tissue level metabolism which is important for inactivation and excretion of excess hormones. The inactivation involves metabolism of testosterone into oestradiol and dihydrotestosterone. Oestradiol is metabolised to yield oestrone and dihydrotestosterone is converted into androsterone and 3α-diol, which possess little sex steroid activities. Irreversible conjugation of oestrone, androsterone and 3α-diol with uridine diphospho- (UDP) glucuronic acid inactivates these metabolites by impeding their binding to steroid receptors. Glucuronidation also converts these metabolites into a water-soluble form for excretion in the urine and/or faeces [16].

1.2.4.3 Transport

Sex hormones in the circulation are transported bound to plasma proteins including sex hormone binding globulin (SHBG) and albumin. The specific transporter SHBG is synthesised in the liver and has a higher affinity for testosterone than oestradiol. SHBG binds two-thirds of the circulating testosterone and one-third of oestradiol while only 2% of testosterone and oestradiol is free. The remaining testosterone and oestradiol are bound to albumin but they are considered bioavailable due to low binding affinity [9].

1.2.5 Sex hormone receptors

Sex hormones exert their biologic actions mainly by interacting with their receptors. Sex hormone receptors belong to a subgroup of nuclear receptors (Figure 5) called steroid hormone receptors (SHR) which include the androgen receptor (AR), the oestrogen receptors alpha and beta (ERα and ERβ), and the progesterone receptor (PR) [16, 17].

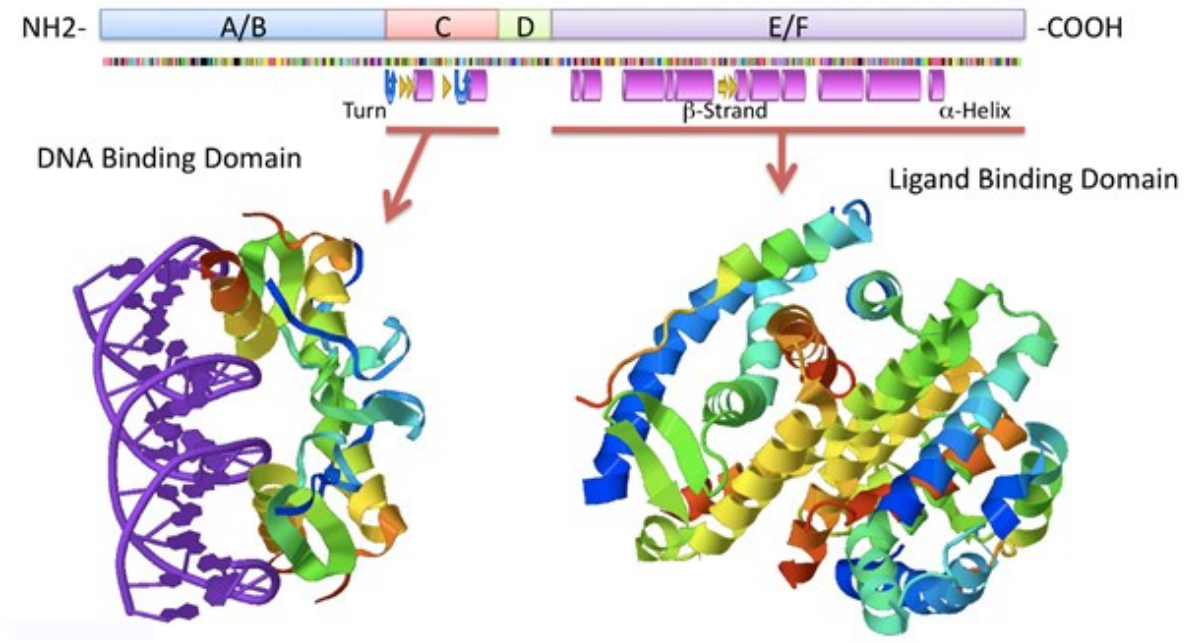


Figure 5 Structural organisation of a typical nuclear receptor

Adapted from [17]

Nuclear receptor comprises a variable NH_2-terminal region (A/B), a conserved deoxyribonucleic acid (DNA)-binding domain (C), a variable linker region (D), and a conserved E/F region that contains the ligand-binding domain

1.2.5.1 Mechanism of action of sex hormones

The classical genomic mechanism of sex hormone action involves binding of the sex hormone to its receptor. SHRs in the absence of ligand are sequestered in large complexes with heat shock proteins (HSP). The binding of hormone brings about a distinct conformational change in receptor structure, eliciting dissociation of HSPs. The hormone-receptor dimer then binds to the target genome at highly specific hormone response elements (HREs), initiating gene transcription and production of specific messenger ribonucleic acids (mRNA). Translation of these mRNAs in the cytoplasm leads to synthesis of proteins which bring about physiological changes in the target tissue that are characteristic of the sex hormone (Figure 6) [17, 18]. Sex steroids have also been shown to alter cellular activity by exerting acute non-genomic effects through their interaction with plasma membrane-bound non-nuclear receptors such as G protein-coupled receptors and ion channels [18, 19].

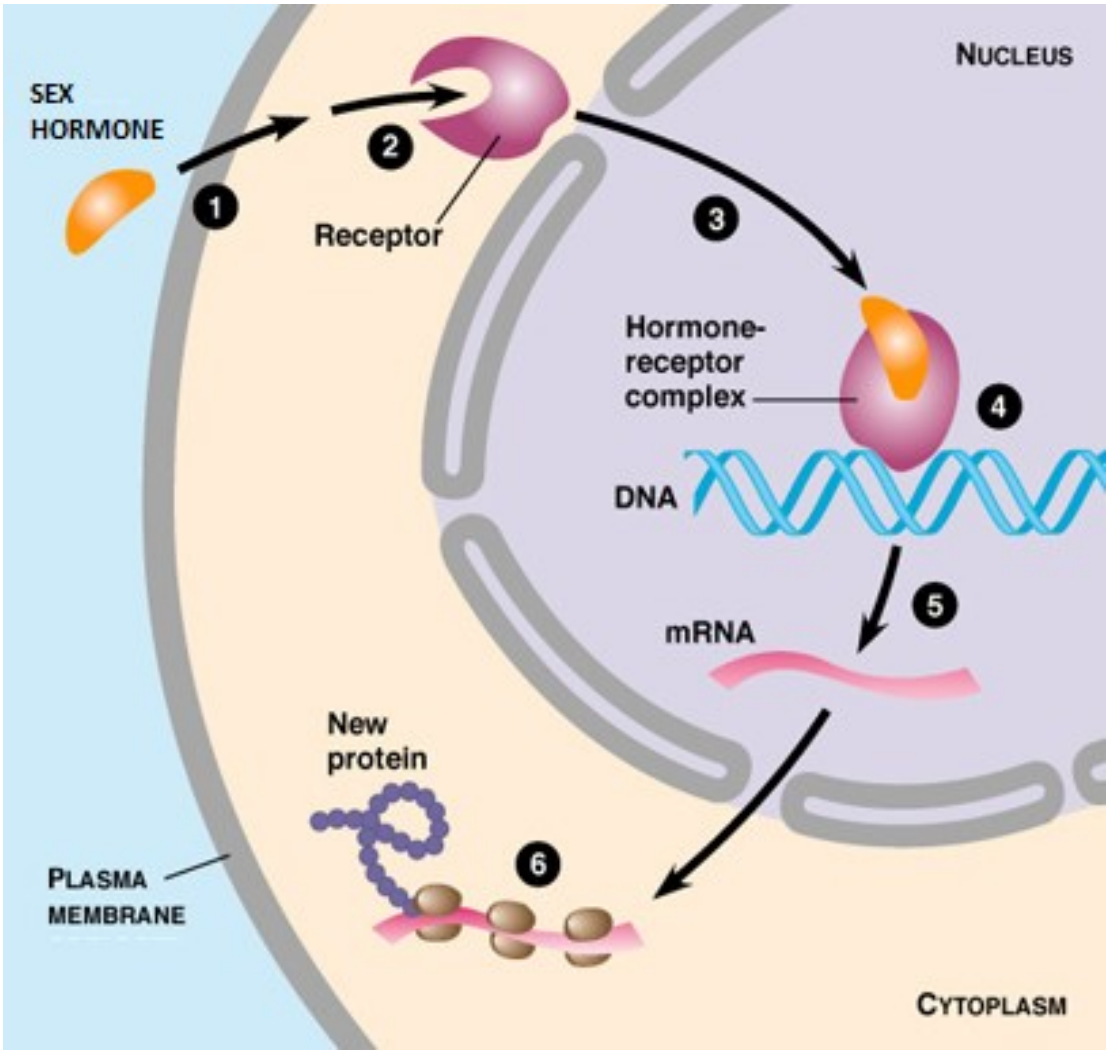


Figure 6 Mechanism of action of sex hormones

Adapted from http://biologysemester58.wikispaces.com/Homeostasis

1) Sex hormone diffuses into target cell 2) binds to its receptor 3) Nuclear translocation of hormone-receptor dimer 4) the dimer attaches to DNA at response element 5) transcriptional activation of specific gene 6) protein synthesis

1.3 Sex hormones in foetal development

The embryo remains in a sexually indifferent stage till about the sixth week of embryonic development whereby it has the potential to develop into either male or female phenotype. Sex chromosomes direct gonadal differentiation in the seventh week after conception. The embryo at that stage has Müllerian and Wolffian ducts and rudimentary external genitalia. Embryonic development into male phenotype is triggered by expression of the Y-chromosomal SRY gene (sex-determining region of Y chromosome) which controls the formation of testes (Figure 7) [20]. Anti-Müllerian hormone secreted by Sertoli cells of the testes causes degeneration of Müllerian ducts, while the production of high amounts of testosterone from foetal Leydig cells in males directs differentiation of Wolffian ducts into epididymis, vas deferens, seminal vesicles and prostate (Figure 7). In the females, absence of SRY gene and subsequent lack of testosterone and anti-Müllerian hormone production leads to regression of Wolffian ducts while Müllerian ducts differentiate into fallopian tubes, uterus and vagina with simultaneous development of female external genitalia [21].

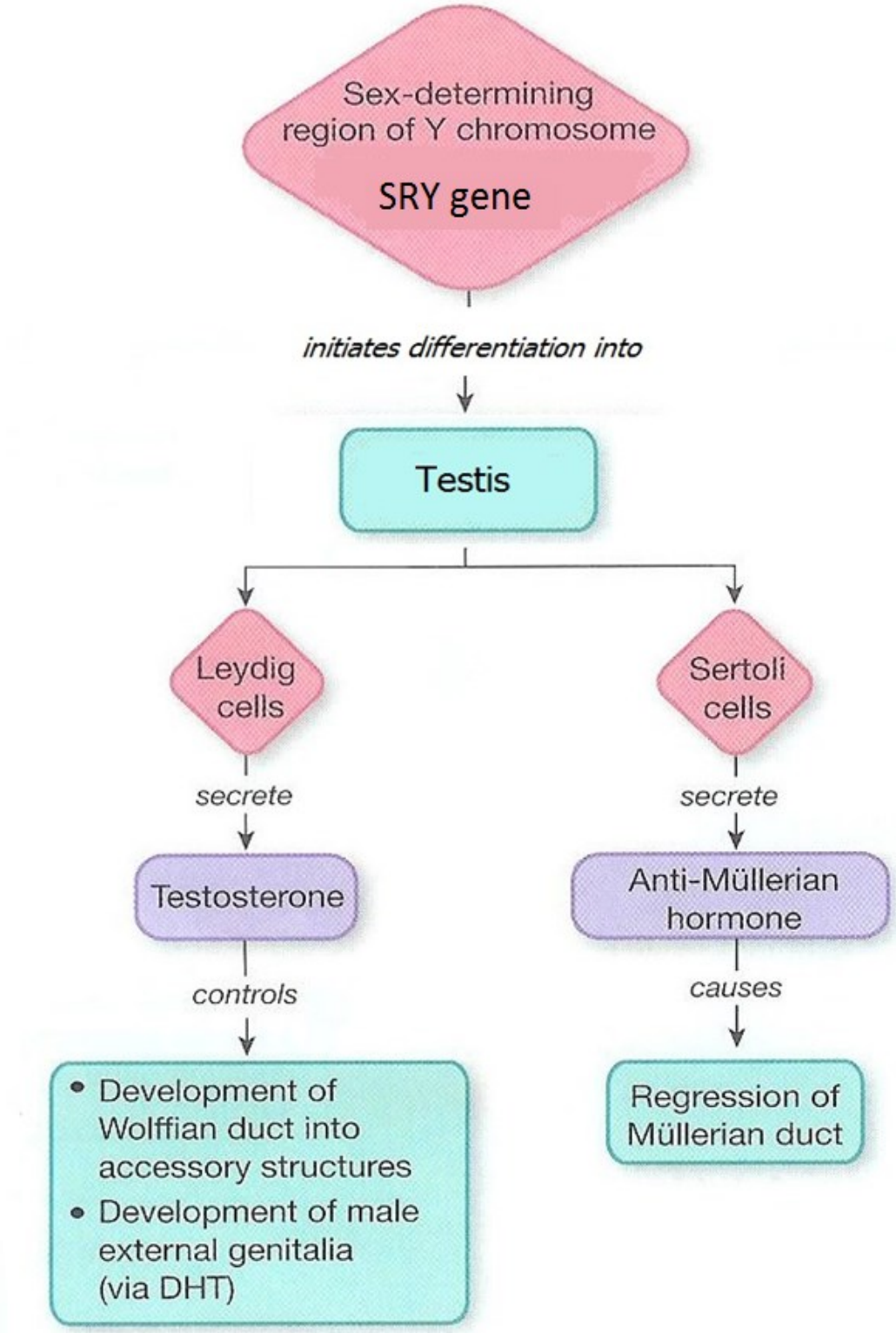


Figure 7 Role of SRY gene in the development of male phenotype

Adapted from http://en.wikipedia.org/wiki/Sexual_differentiation_in_humans

1.4 Sex hormones in pubertal development

Puberty is the period of attaining sexual maturity that encompasses changes including growth of the reproductive organs and development of secondary sexual characteristics [22]. These physical changes at puberty occur as a result of elevated secretion of gonadal sex hormones by activation of the HPG axis (Figure 8). The HPG axis at pre-pubertal stage is highly sensitive to negative feedback inhibition from very small concentrations of sex hormones. Closer to puberty, the sensitivity of HPG axis to negative feedback from sex hormones is decreased. This leads to a gradual rise in the levels of gonadotrophic hormones and subsequent growth of the gonads, which in turn causes accelerated production of sex hormones. There is a 45-fold increase in testosterone in adult boys as compared to childhood levels while oestradiol levels are up to 9 times higher in adult girls as compared to pre-pubertal levels [23]. In adolescent males, testicular growth is driven primarily by testosterone while other androgens cause growth of pubic hair and long bones and the development of characteristic masculine voice and body odour. In adolescent females, oestradiol causes growth of breasts, promotes fusion of long bones following growth spurts and female-type fat distribution, and initiates ovulation and menstruation (Figure 8) [23, 24].

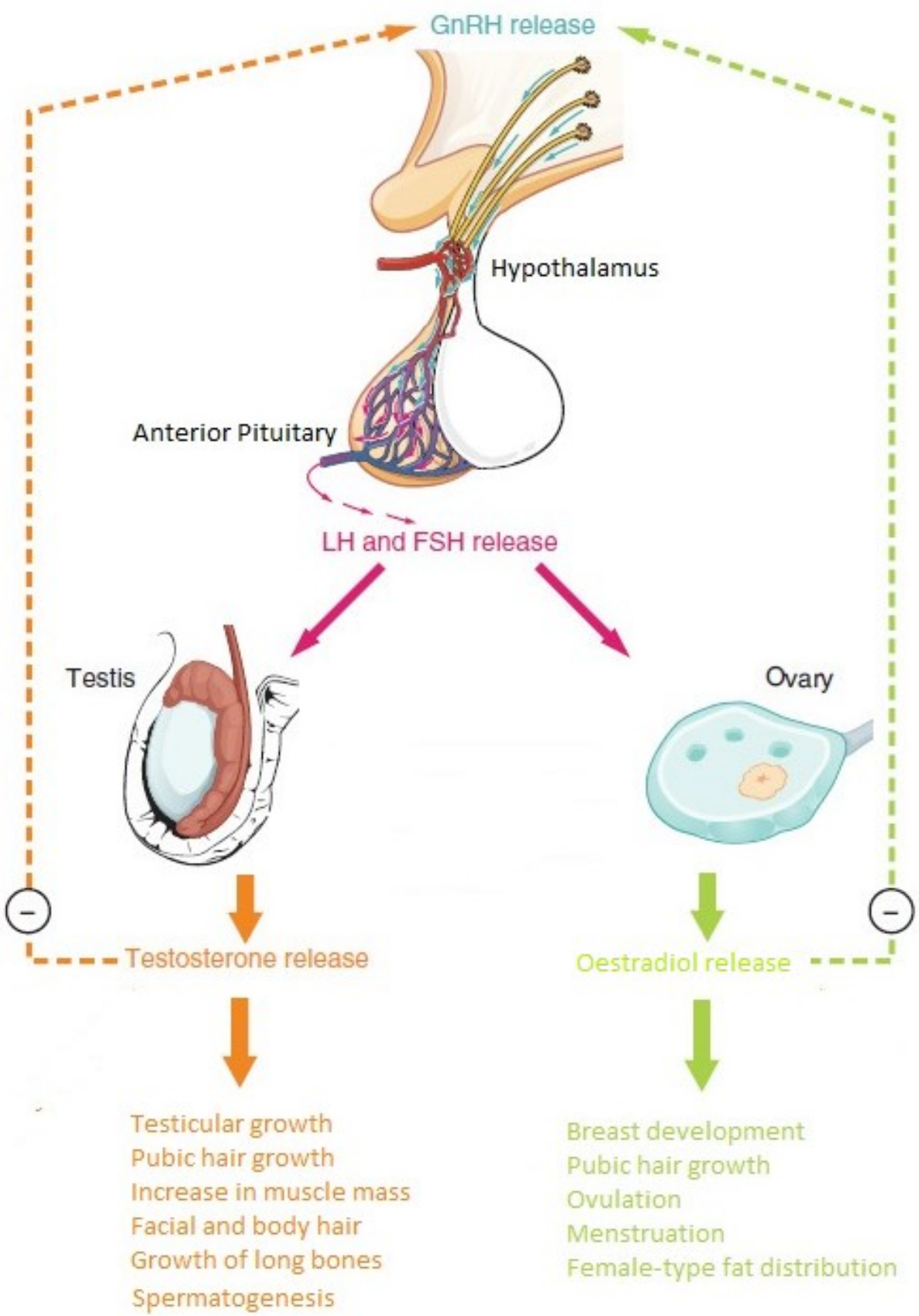


Figure 8 Pubertal changes as a result of increased sex hormone production

Adapted from http://cnx.org/contents/14fb4ad7-39a1-4eee-ab6e-3ef2482e3e22@6.27:190/Anatomy_&_Physiology

More than half a century ago, Tanner described five stages of puberty based on visible secondary sexual characteristics which is still considered the gold standard for assessing pubertal development [24]. Table 4 describes key features of Tanner staging.

Table 3 Tanner staging of pubertal development [24]

Tanner Stage	Genitalia (Males)	Breast (Females)	Pubic Hair (Both males and females)
I	Childhood size	Pre-adolsecent	None
II	Enlargement of scrotum / testes	Breast bud	Sparse, straight, long
III	Penis grows in length, further enlargement of testes	Areolar diameter enlarges	Darker, curling, increased amount
IV	Penis grows in length and diameter, scrotum darkens, testes enlarge	Secondary mound, separation of contours	Coarse, curly, adult type
V	Adult shape and size	Mature female	Adult, extend to thighs

1.5 Role of sex hormones in adult physiology

Androgens and oestrogens are present in both sexes but at different concentrations and there are temporal changes in concentrations as well e.g. during menstrual cycle, menopause. Further, sex hormone receptors are distributed in reproductive (including testes, prostate, uterus, ovaries) as well as many non-reproductive organs e.g. brain, bone, heart etc. The biological role of sex hormones in adults is therefore, not limited to reproduction and they affect all tissues where their receptors are distributed.

1.5.1 Cardiovascular system

The expression of ERs and AR in hearts and blood vessels suggests a role of sex hormones in cardiovascular system (CVS) physiology [25, 26]. Studies have demonstrated an atheroprotective role of oestrogen, facilitated by its beneficial effects on lipid profile (reduced low-density lipoprotein and elevated high-density lipoprotein) as well by its mediation of vasodilation through increased production of nitric oxide and decreased production of endothelin-1 from arterial endothelium and decreased intracellular calcium in arterial smooth muscle [26, 27].

Premenopausal women have a relatively lower incidence of CVS disease than age-matched men but the rates become similar with the loss of oestrogen in postmenopausal women [28]. Previously, observational studies showed a decline in CVS events in postmenopausal women receiving hormone replacement therapy (HRT) with oral oestrogen but later trials demonstrated an increase in the risk of thromboembolism and CVS events with oral oestrogen given as HRT [25, 26, 29].

The role of androgens in CVS physiology is complex and far less studied than oestrogen. Androgens have been associated with atherosclerotic CVS disease in men through their adverse effects on lipids metabolism and hypertension. Conversely, the gradual decline in androgen levels in elderly males as a result of the ageing process has also been linked to hypertension, diabetes and atherosclerosis [28]. Studies in athletes using anabolic steroids

have suggested detrimental effects of testosterone on heart function, showing it to be associated with cardiac hypertrophy, impaired cardiac function and an increased risk of myocardial infarction [25]. However, studies of long-term testosterone replacement in hypogonadal elderly men have demonstrated reduced risk of CVS morbidity and mortality [30].

1.5.2 Body composition

Sex steroids are involved in regulating muscle metabolism and function as well as body composition. Women have a higher body fat percentage and subcutaneous adipose tissue as compared to men [31]. In men, androgens are known to play a critical role in maintaining muscle mass, strength and body composition which is mediated by activation of ARs found in myocytes and adipocytes. The direct anabolic effects of androgens leading to skeletal muscle hypertrophy have been shown in numerous studies. [32]. In the first 3 years after menopause, there is a decrease in lean body mass (LBM) of about 4% which as a consequence leads to decline in muscle strength in postmenopausal women. Androgen therapy in such women has been shown to increase muscle mass and decrease fat mass [33]. Hypogonadism in elderly men is associated with sarcopaenia and increased adiposity which result in physical frailty, reduced mobility and increased risk of falls [34]. Testosterone levels in elderly males have been shown to have a positive correlation with muscle mass and a negative correlation with fat mass [32].

Results from an experimental study of healthy adult male volunteers undergoing androgen suppression followed by androgen replacement with or without aromatase inhibitors showed that loss of muscle mass and strength occurs because of androgen deficiency while body fat gain results mainly from the lack of oestrogen (owing to suppression of aromatisation) [35]. Cross-sectional analysis from another recent study showed testosterone (free and total) to be inversely correlated to anthropometric parameters including body weight, body mass index (BMI: weight in kilograms / height in metres squared), fat mass and waist-to-hip ratio.

Free oestradiol was positively correlated with fat mass and oestradiol:testosterone ratio was positively correlated with all measured variables of body composition [36].

1.5.3 Cognition

Sexual differentiation of the brain occurs during intrauterine and neonatal period. Masculinisation of brain anatomy and physiology during these early stages of development is brought about by secretion of testosterone and its actions either directly through AR activation or via aromatisation to oestradiol and subsequent ER activation [19, 37]. Sex based structural dimorphism is seen in many parts of the brain including hypothalamus, limbic system, higher cognitive centres and structures of the midbrain, brainstem and basal forebrain. Both ERs and ARs are located in these brain regions but their regional expression is not uniform [37, 38]. Gender differences in cognitive functioning have been demonstrated with men exhibiting a superior visuospatial ability and women displaying superior ability in verbal fluency and perceptual speed tasks [39].

Oestradiol, in addition to its ovarian synthesis, is also synthesized in the brains of adult males and females where it serves to regulate several functions related to cognition, pain perception and behaviour. A neuroprotective function of oestradiol has been suggested in neurodegenerative disorders (e.g. Alzheimer's disease; AD) and traumatic brain injury [38, 40, 41]. Specific cognitive domains such as verbal memory and fluency are influenced by oestrogen as reflected in the performance variations on these tasks seen across the menstrual cycle as well as the decline observed at menopause. Some studies have shown that symptomatic postmenopausal women receiving HRT with oestrogen show improvements in verbal memory and reasoning. Association of HRT with a decreased risk of dementia has also been shown [42, 43].

The age-related decline in testosterone levels is associated with a decline in cognitive functions in men and the cognitive domains most commonly affected include memory and visuospatial function [44]. A neuroprotective role of testosterone has been suggested in

laboratory and animal studies. Compared to healthy controls, lower levels of testosterone are seen in patients suffering from AD, a neuropathological condition characterised by impairment of cognitive ability [45]. However, testosterone supplementation in hypogonadal men does not seem to offer cognitive benefits, possibly because of the inability of standard neuropsychological assessments in picking up the subtle improvements [45, 46].

1.5.4 Bone health

Androgens and oestrogens are involved in normal bone physiology and skeletal homeostasis. Rapid bone growth and development of skeletal differences in adult males and females during adolescence are regulated by sex hormones [47]. Both ARs and ERs are expressed in all bone cells including osteoblasts, osteoclasts, osteocytes and bone marrow stromal cells, reflecting a role of both androgens and oestrogens in preserving bone health [48].

Oestrogen preserves bone integrity through its anti-resorptive function mediated predominantly by activation of ERα. It inhibits osteoclast formation and increases osteoclast apoptosis via modulation of signaling pathways including FAS/FAS ligand, and receptor activator of nuclear factor kappa B (RANK)/RANK ligand [49]. The bone-sparing properties of oestrogen have been studied widely in the setting of female menopause. Accelerated bone loss and subsequent development of osteoporosis occur as a result of oestrogen deficiency seen in postmenopausal women. HRT with oestrogen has been shown to prevent this bone loss and improve bone mineral density (BMD) [47, 50].

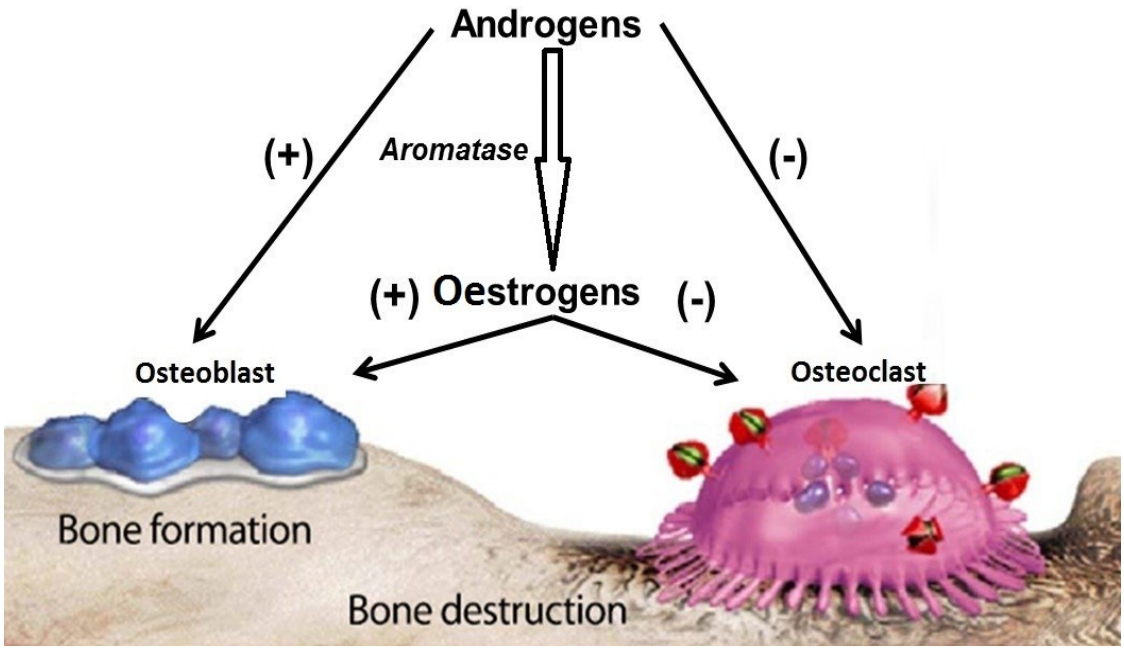


Figure 9 Physiological effects of sex hormones on bone cells

Adapted from Skeletal Care Academy; Cancer Treatment induced bone loss DMO-IHQ-AMG-187-2012- August-NP

Androgens promote bone formation and restrain bone resorption directly or indirectly through conversion to oestrogens

Androgens stimulate the differentiation and proliferation of osteoblasts causing increase in mineralisation and also lead to a surge in the synthesis of extracellular matrix proteins (type 1 collagen, osteocalcin, osteonectin). Testosterone has been suggested to have a dual mode of action for its proskeletal effects involving both AR and ER through its conversion into oestradiol via aromatisation (Figure 9) [33, 51]. A recent study of testosterone replacement in postoperative hypogonadal males with pituitary tumours showed significantly improved lumbar spine BMD compared to baseline BMD (4.5% improvement, p-value 0.028) over a mean observation period of 56 months [52]. Osteoporosis and bone fractures are common among the elderly due to age-related androgen deficiency. Androgen replacement has been shown to improve BMD in these men [34].

1.5.5 Sexual function

Human sexual function is complex and involves integration of several neural, vascular and muscular processes. Sex hormones coordinate these processes and contribute to sexual

function in both males and females. These effects are mediated by ARs and ERs present in areas of central nervous system regulating sexual behaviour as well as in the reproductive organs [53]. Testosterone and DHT maintain spermatogenesis during adult life by activation of ARs in Leydig and Sertoli cells [4].Testosterone has an important role in maintaining sexual interest and arousal in men. Testosterone replacement in hypogonadal men has been shown to improve duration of erection, nocturnal erections, penile rigidity and sexual satisfaction [54, 55].

Oestrogens are known to maintain normal female sexual and reproductive physiology. Both oestradiol and testosterone peak at ovulation and studies have shown sexual interest in females to rise in the follicular phase and reach maximum at ovulation [56, 57]. Oestrogen deficiency following menopause negatively alters libido, sexual arousal and satisfaction but the degree of these changes varies considerably. Menopausal reduction in oestrogen is also associated with genital shrinkage and HRT with oestrogen has been shown to improve these aspects of female sexual dysfunction [58]. In men, oestradiol is also produced in the testis from testosterone via testicular aromatase activity. Moreover, ERs are present in male genital apparatus suggesting their role in normal male sexual function [53]. Studies in aromatase deficient men have suggested exogenous oestrogen replacement to improve sexual interest [59].

1.5.6 Metabolic effects

Sex differences in metabolic profiles of men and women suggest specific roles of sex hormones in energy metabolism. In young adult males, lipid deposition is predominantly visceral as compared to females where lipids are deposited subcutaneously as well in the lower body [31]. Androgen deficiency in men is linked with the development of insulin resistance, impaired glucose tolerance, central adiposity and adverse lipid profile (elevated total cholesterol and triglycerides and low high density liporotein (HDL)-cholesterol. On the other hand, low testosterone levels are commonly observed in men with type 2 diabetes

mellitus and metabolic syndrome [60, 61]. Testosterone replacement therapy in hypogonadal men has previously been shown to improve glucose metabolism and reduce fat content [62]. However, a recent randomized, placebo-controlled trial of testosterone therapy in obese diabetic men has shown no beneficial effect on insulin sensitivity or visceral adiposity [63].

1.5.7 Odour signalling

Pheromones are airborne chemo-signals secreted by an individual and having specific hormonal, neural and behavioural influences on conspecifics (individual belonging to the same species). Pheromones are known to affect animal behaviour and recent evidence suggests that humans can detect pheromones through olfaction and show specific responses [64].

Humans possess uniquely discernible body odours which are affected by pheromones, gender, age and several other factors. Exposure to body odour of other individuals has been shown to elicit psychological and physiological responses. Body odour provides cues about a potential sexual partner's health, reproductive status and genetic quality. It is considered a signal of physical attractiveness and sexual appeal and is associated with masculinity and dominance [65]. Men have been shown to perceive female scents as pleasant and sexually attractive during the high fertility phase of the menstrual cycle as compared to the low fertility phase [66]. Women show a greater preference for body odours of males with low fluctuating asymmetry during the high fertility phase of their menstrual cycles [67] and these enhanced sexual preferences are associated with testosterone levels in males [68]. Monozygotic twins have matching body odours suggesting important genetic influences [69, 70]. Major histocompatibility complex (MHC) genes have been shown to affect body odour [71]. Age differences in odours of men have been documented [72] but it is not known whether that is due to changes in their gonadal hormone profile, which in fact, is known to diminish with advancing age [34].

Axillary odour, readily identifiable due to its pungent nature, is generally referred to as body odour [73]. Axillary secretions are known to contain various odoriferous volatile

chemicals which may act as pheromones [74]. Androstadienone, a steroidal metabolite yielded from the sex hormone biosynthetic pathway, is one such compound that has been implicated as a human pheromone. It is synthesised from androstadienol, a derivative of pregnenolone [64]. Pregnenolone also serves as the precursor molecule of human sex hormones [6]. Androstadienone is found in bodily secretions including sweat, semen and saliva and its concentration is particularly high in axillary sweat of men [64]. Studies have reported favourable effects of androstadienone, particularly in females, on sexual behaviour, attraction, mood and mate selection. Exposure to androstadienone has also been shown to enhance concentration and pain perception, increase cortisol levels and activate brain regions involved in social cognition [75-80].

Animal studies suggest a potentially direct role of sex hormones as pheromones. Behavioural and physiological changes have been observed in female mice through cutaneous or nasal absorption of oestradiol excreted in male urine [81].

1.6 Sex hormones and their clinical implications

Owing to the wide array of biological functions carried out by sex hormones, the aetiopathology, symptomatology and therapeutic management of many clinical conditions are linked to sex hormone homeostasis. These include menopause, andropause, contraception, gender dysphoria, polycystic ovarian syndrome (PCOS) and hormone-sensitive cancers. Surgical and/or pharmacologically induced alterations in sex hormone levels in these conditions offer an opportunity to study the complex biological roles of sex hormones. Variations in sex hormone levels, whether physiological, pathological or pharmacological, lead to bodily changes which are reflective of their systemic functions.

1.6.1 Menopause

The physiological cessation of menstruation in women (at a mean age of 51 years) occurring as a result of exhaustion of ovarian follicles and consequent decline in the levels of

oestrogen and progesterone is termed the menopause [82]. Secondary effects due to menopausal sex hormone changes include vasomotor symptoms (hot flashes), depression, sleep disturbance, vaginal atrophy, osteoporosis and cognitive dysfunction [83-88]. HRT with oestrogen alone or in combination with progesterone is used to relieve menopause-related symptoms and dosage, timing of administration, duration all affect the outcomes [50, 82, 89].

1.6.2 Andropause

Andropause, also termed late-onset hypogonadism, is the male counterpart of female menopause. Androgen levels in men begin to diminish in the mid-thirties at an annual rate of 1-1.5%. Nearly a third of men between 70-79 years have testosterone levels lower than normal, and more than two-thirds have reduced levels of bioavailable testosterone [90]. This reduction in androgens as a result of ageing manifests as andropause which is characterised by symptoms including reduced libido, erectile dysfunction, loss of musculature, increased adiposity, osteopaenia, lack of energy, psychological disturbance and cognitive impairment [91, 92]. Testosterone replacement therapy is used for treating andropausal symptoms but definitive evidence about its benefits and risks is lacking currently [93].

1.6.3 Contraception

Hormonal contraception is widely used by women of reproductive age. In the United States of America (USA), half of the women use some form of hormonal contraception including short-term (oral contraceptive pills, transdermal patches) or long term methods (depot injections, subcutaneous implants and intrauterine contraceptive devices) [94]. Oral contraceptive pills are the most common form of hormonal contraception used by a quarter of women of reproductive age in the United Kingdom (UK) while 1 in 10 women use long-acting hormonal contraceptive techniques [95].

Hormonal contraceptives, containing combined oestrogen/progestogen or progesterone only preparations, work primarily by reducing endogenous ovarian production of oestrogen and progestogen via negative feedback inhibition. The resultant drop in circulating levels of oestrogen and progesterone leads to suppression of ovulation [96, 97]. Some experimental studies have shown the feasibility of using testosterone preparations (given in oral, gel and injectable forms) as a means of hormonal contraception in men [98].

1.6.4 Polycystic ovarian syndrome

Polycystic ovarian syndrome (PCOS) is an extremely common endocrinopathy affecting 5% to 15% of the women of reproductive age the world over [99]. The aetiology of this heterogeneous disorder is complex with genetic influences, life-style factors and gestational environment all implicated in its development [100]. PCOS is classified by the presence of two of the three diagnostic traits namely 1) biochemical or clinical features of hyperandrogenaemia 2) Chronic ovulatory dysfunction and 3) polycystic ovaries, after ruling out other causes that may have similar features [100, 101].

Hyperandrogenaemia in PCOS usually results in hirsutism, acne and male pattern alopecia. Oligo-anovulatory cycles often manifest as oligomenorrhea, amenorrhea, dysfunctional uterine bleeding and problems with fertility [102, 103]. PCOS is also associated with psychological disturbances, increased risk of endometrial carcinoma, obstructive sleep

apnoea and features of metabolic syndrome including obesity, insulin resistance, impaired glucose tolerance and dyslipidaemia [104]. Management of PCOS is mainly directed at alleviating the symptoms and includes pharmacological interventions and lifestyle modifications to correct hormonal imbalances and metabolic disturbances [100, 101].

1.6.5 Gender dysphoria

Gender dysphoria, also referred to as transgenderism or gender identity disorder, is a condition in which the individual identifies with the gender opposite to his/her own biological sex. Such individuals exhibit cross-gender behaviours, express constant uneasiness with their assigned gender roles and desire to alter their physical appearance to conform to their self-perceived gender [105]. Unlike disorders of sexual development, gender dysphoria is not associated with any structural or functional abnormality [106]. Management of affected persons is aimed at making their bodies congruent to their internal sense of gender and enhancing their psychological well-being [107]. Treatment requires a multidisciplinary approach requiring psychological, hormonal, cosmetic and surgical interventions [108, 109].

Hormone therapy is an important component of the treatment and involves alteration of the sex hormonal milieu of the subject to bring about the desired physical and psychological change [110]. Hormone therapy for male-to-female transgenderism requires administration of oestrogens and androgen-suppressing agents such as luteinising hormone releasing hormone agonists (LHRHa) or anti-androgens. LHRHa suppress testicular testosterone production by inhibiting the HPG axis whereas anti-androgens block the effects of circulating androgens by binding to ARs. Female-to-male transformation requires testosterone administration [111]. Depending on the type of sex hormone administered and its formulation, dosage and route of administration, hormone therapy in transgender subjects can result in adverse metabolic and/or thromboembolic side effects [112].

1.6.6 Hormone-sensitive cancers

Sex hormones regulate the normal development and function of many organs, particularly the reproductive organs, and are also implicated in the development and progression of cancers in these organs [113, 114]. Prostate and testicular cancers in men and breast, endometrial and ovarian cancers in women are the typical hormone-sensitive cancers. These cancers are very common and are a major cause of cancer-related mortality worldwide [115]. It has been suggested that cellular proliferation driven by sex hormones increases the likelihood of genetic mutations which can get accumulated over time and lead to the development of a malignant phenotype [116]. Endocrine therapies targeting endogenous sex hormones are employed for treatment of hormone-dependent cancers [6] e.g. oestrogen levels are lowered to treat breast cancer by agents causing ovarian ablation such as LHRHa, aromatase inhibitors or selective oestrogen receptor modifier (SERM). Anti-oestrogens, which competitively bind ERs, are also used in the management of hormone- sensitive breast cancer [117].

1.7 Prostate cancer

1.7.1 Prostate gland

The term ‘prostate’ has its roots in the Greek word ‘prohistani’, meaning ‘to stand in front of’. Herophilus used this term in 335 B.C. to describe the organ located ‘in front of the urinary bladder’ [118]. The prostate is situated just inferior to the bladder in the pelvic cavity (Figure 10). It is a fibromuscular and glandular organ, normally weighing about 20 grams and roughly conical in shape. It has a base, an apex, an anterior, a posterior and two lateral surfaces. Prostatic secretions contribute to volume of semen and serve as nourishment for sperms. Prostate gland has four zones; anterior, transitional, central and peripheral (Figure 11).

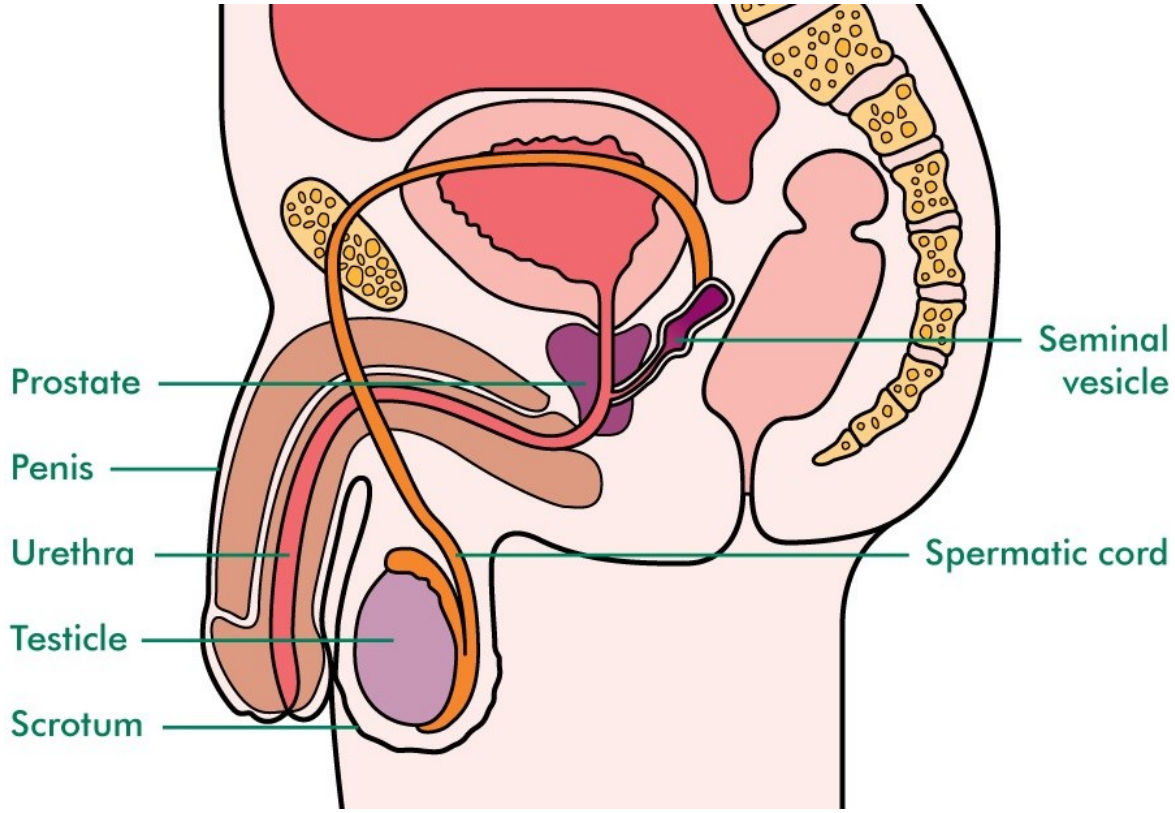


Figure 10 Anatomical relations of prostate gland

http://www.macmillan.org.uk/Cancerinformation/Cancertypes/Prostate/Aboutprostatecancer/Theprostate.aspx

The unrestricted irregular proliferation of abnormal cells of prostate gland origin is called prostate cancer (PC). Prostate gland is a common site of cancer development in males and nearly two-thirds of all PCs are found within the peripheral zone [119]. Almost all PCs are adenocarcinomas and other rare types of cancers in the prostate gland include neuroendocrine tumours, small cell cancers, transitional cells cancers and sarcomas [120]. The characteristic pathological features of PC include genetic aberrations, resistance to apoptosis, chronic inflammation, angiogenesis and metastasis [121].

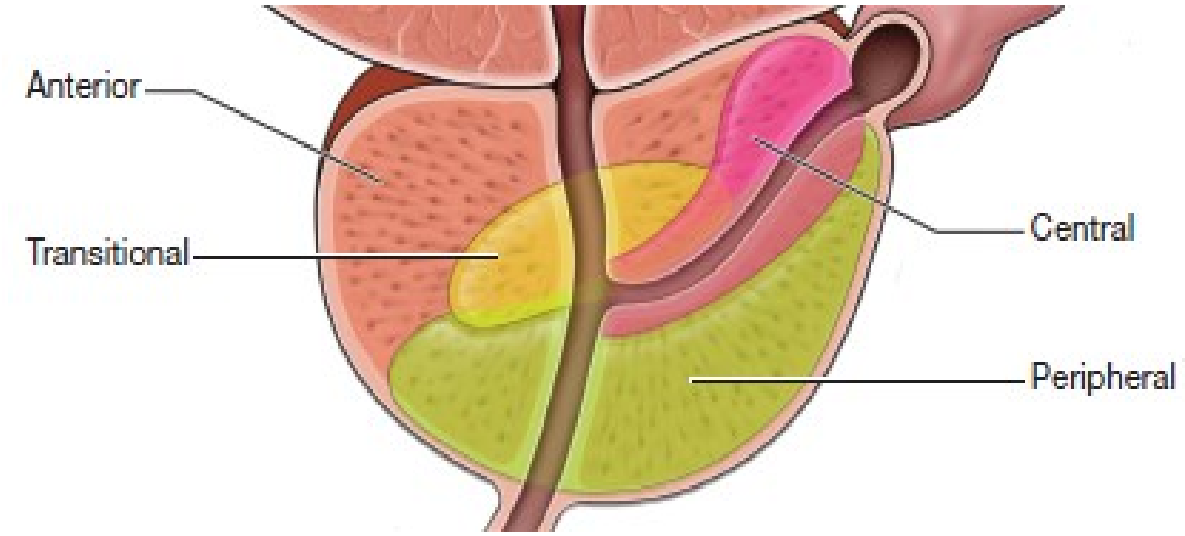


Figure 11 Zonal anatomy of prostate gland
Adapted from http://wholelifeprostate.com/prostate.html

1.7.2 Prostate cancer incidence

Prostate cancer (PC) is a major international health problem at present. It is one of the commonest life threatening malignancies afflicting men the world over [122]. In the year 2012, more than a million men were globally estimated to have been diagnosed with PC, with variable incidence rates across different geographical regions [123]. PC accounts for about 10% of all male cancers worldwide, with this figure being ~5% in less developed countries and ~15% in developed countries [124].

PC is the commonest malignancy and second most common cause of cancer death affecting men in the western world [125]. In the USA alone, the incidence rate for PC is nearly

a quarter of a million diagnoses each year and almost 30,000 deaths. PC is the second most common cancer after skin cancer and second most common cause of cancer mortality after lung cancer in the USA. About 1 in every 7 American men is likely to be diagnosed with PC and 1 in 38 will die from it [120]. Almost half a million men are diagnosed with PC in Europe each year and a tenth of a million die from it. In the UK, PC is the commonest male cancer, accounting for 24% of all new male cancer diagnoses. Around 42,000 British men were diagnosed with PC in the year 2011-2012 and more than 10,000 died from it [123].

1.7.3 Risk factors

PC is a multifactorial disease and exact aetiology is not clear yet. It has been suggested that genetic causes as well as environmental and life style factors contribute to the development of PC. In addition to these genetic and life style factors, variation in PC incidence and mortality rates across the different parts of the world can be attributed to healthcare differences in cancer screening and registration [124].

1.7.3.1 Age

PC is a considered a disease of the elderly as ageing is associated with an increased risk [120, 126]. PC is very rare in men below the age of 40 years, but more than two-thirds of all PC diagnoses are in men older than 65 years. Autopsy studies have revealed a 50% prevalence of PC in men between 70-80 years of age [124].

1.7.3.2 Family history

Increased incidence of PC has been observed in families suggesting hereditary tendency [126]. It has been reported that 10-15% of patients with PC have at least one family member who is also affected. First-degree relatives of patients have a two to three-fold increased relative risk for developing PC [127]. The clustering of PC in families can be due to

shared gene pool, exposure to common environmental factors and/or diet or perhaps due to chance alone considering the high prevalence of PC.

1.7.3.3 Racial predisposition

Race is an important risk factor for PC. The risk of developing PC and its associated mortality is highest among African-Americans, Caucasians have an intermediate risk while Asian men carry the lowest risk. This racial predisposition has been attributed to common genetics and exposure to similar environmental and/or life-style influences [120, 126].

1.7.3.4 Genetic susceptibility

Genetic factors have been estimated to account for half of the risk of PC. Genome-wide association studies (GWAS), which scan the genome for genetic polymorphisms occurring frequently in a particular disease than in the normal population, have identified almost one hundred such variants which have a multiplicative effect on increasing the risk of PC development. These genetic variations are suggested to be a major reason of familial aggregation of PC. Genetic mutations of the tumour suppressor genes breast cancer antigen 1 (BRCA1) and breast cancer antigen 2 (BRCA2) have also been linked with a 10% and 25% increased risk of PC respectively [127, 128].

1.7.3.5 Diet and nutrition

The role of dietary factors in development of PC has been studied since long but results are conflicting and no clear evidence has become available as yet [129]. Excessive consumption of red meat, fats, dairy products and alcohol have been associated with an increased risk of PC. Fresh fruits and vegetables are thought to decrease the risk [120, 124, 129]. Obesity is linked to PC development and regular exercise is associated with a reduced risk. A strong correlation between BMI at the time of PC diagnosis and PC-specific mortality

has been shown recently and this is more marked in overweight or obese patients with aggressive disease [130].

1.7.3.6 Other influences

Results from a recent study have shown male pattern baldness to be associated with an increased risk of aggressive PC [131]. It has been indicated that childhood height is associated with increased risk of PC and PC-specific mortality [132]. Sexually transmitted diseases and vasectomy have also been implicated as risk factors for PC [120]. Medications including aspirin, statins and anti-diabetics were previously suggested to reduce the risk of PC but a recent study did not find any such protective effect [133].

1.7.4 Clinical presentation

PC has no specific clinical features. An enlargement of the prostate can lead to development of the symptoms of urinary outflow obstruction such as frequency, nocturia, urgency, hesitancy and weak urinary stream. These symptoms are commonly seen in patients with benign prostatic hyperplasia (BPH). Haematuria, erectile dysfunction and perineal pain are some other symptoms associated with PC and patients with metastatic disease may also present with bone pain [120].

1.7.5 Diagnostic tools

PC diagnosis is based on a detailed clinical history followed by a digital rectal examination (DRE), a laboratory test for measuring serum levels of prostate-specific antigen (PSA) and imaging. A definitive diagnosis is established on histopathologic evidence of cancer following transrectal ultrasonography (TRUS)-guided prostate biopsy.

1.7.5.1 Digital rectal examination

A DRE is done to assess the shape, size, symmetry, nodularity and degree of firmness of the prostate gland. PC may be detected by DRE if the tumour is located in the peripheral zone of the prostate gland and its volume is ≥ 0.2 ml. A suspicious DRE indicates a need for biopsy [119, 134].

1.7.5.2 Prostate-specific antigen

PSA is a glycoprotein and functions as a serine protease. PSA is secreted by the secretory epithelial cells of prostate gland and is a major seminal protein where it helps to liquefy semen and facilitate fertilisation (Figure 13) [135]. Disruption of the normal glandular architecture in malignancy allows PSA increased access to peripheral circulation. It has been suggested that PSA also plays a role in PC disease progression by enhancing tumour proliferation, invasion and metastasis [136]. PSA is currently the best available and most commonly used serum marker to identify men with a higher probability of having PC. An elevated PSA level > 2-3ng/ml, particularly at a younger age, is considered high enough to warrant further investigation [134]. Non-cancerous conditions such as BPH, prostatitis, ejaculation and recent urologic procedures including DRE and biopsy can also result in an elevated serum PSA [120].

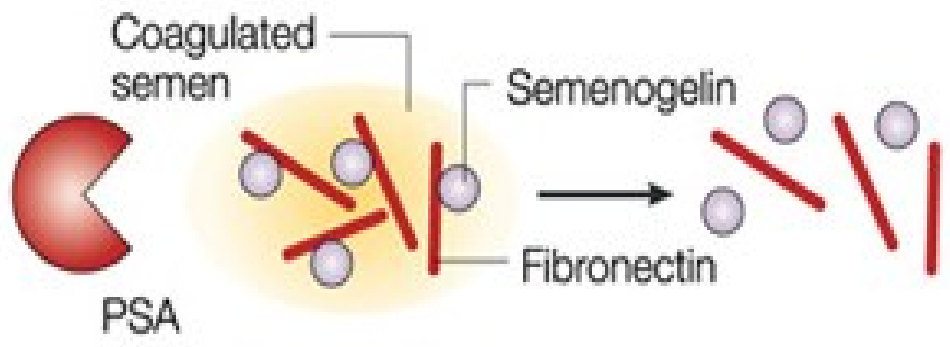


Figure 12 PSA cleaves proteins involved in coagulation of semen [136]

1.7.5.3 Imaging

Advancements in imaging technology have led to their increasing use in evaluating prostate cancer. TRUS, being inexpensive and easily available, is employed most frequently for PC detection. Magnetic resonance imaging (MRI) is useful in detection of PC particularly by enabling biopsy targeted at suspected regions. Other imaging techniques including computed tomography (CT), positron emission tomography (PET) and radionuclide bone scanning have no role in PC detection. The use of CT and PET imaging is limited to assessment of nodal involvement and/or distant metastases while bone scanning is solely employed for assessing the skeletal spread of cancer [119, 137, 138].

1.7.5.4 Biopsy

The decision to perform prostate biopsy is made on the basis of clinical history, DRE and PSA result. Histological assessment of suspected specimens obtained usually with TRUS biopsy is considered the benchmark for diagnosing PC. Multiple needle biopsies (up to 12 cores) are performed to seek histologic proof of cancer Possible complications following biopsy procedures include haematuria, haematospermia, rectal bleeding, prostatitis, epididymitis and fever [134].

1.7.5.5 Staging

Staging is a measure of the size and spread of tumour. Staging of PC is usually done by DRE, serum PSA measurement and radionuclide bone scan. CT, PET or MR imaging may also be employed for staging in certain clinical situations e.g. assessment of pelvic lymph nodes. Tumour-Node-Metastasis (TNM) system of staging is clinically useful for stratifying newly diagnosed PC into localised, advanced or metastatic disease (Table 4) [120, 134].

Primary tumour (T)	
TX	Primary tumour cannot be assessed
T0	No evidence of primary tumour
T1	Clinically inapparent tumour, not palpable or visible by imaging
T1a	Tumour incidental histologic finding in ≤5% of tissue resected
T1b	Tumour incidental histologic finding in >5% of tissue resected
T1c	Tumour identified by biopsy (because of elevated PSA level)
T2	Tumour confined within prostate; tumours found in 1 or both lobes on biopsy but not palpable or visible by imaging
T2a	Tumour involves one-half of 1 lobe or less
T2b	Tumour involves more than one-half of 1 lobe but not both lobes
T2c	Tumour involves both lobes
T3	Tumour extends through the prostatic capsule; invasion into the prostatic apex, or the prostatic capsule is classified not as T3 but as T2
T3a	Extracapsular extension (unilateral or bilateral)
T3b	Tumour invading seminal vesicle(s)
T4	Tumour invades adjacent structures other than seminal vesicles
Regional lymph nodes (N)	
NX	Regional lymph nodes not assessed
N0	No regional lymph node metastasis
N1	Metastasis in regional lymph node(s)
Distant metastasis (M)	
M0	No distant metastasis
M1	Distant metastasis
M1a	Non-regional lymph nodes(s)
M1b	Bone(s)
M1c	Other site(s) with or without bone disease

Table 4 TNM staging for prostate cancer

1.7.5.6 Grading

Grading refers to the histological appearance of cancer cells. Gleason scoring system is employed for grading PC and is useful in predicting the clinical behaviour in terms of disease

progression and treatment outcomes. The two predominant tumour histologies are each given a score from 1-5, depending on the degree of differentiation. The two scores are added to assign a Gleason grade (low, moderate, high) to the tumour (Figure 13). Thus a high grade represents a poorly differentiated cancer that it is more likely to grow and spread aggressively [120].

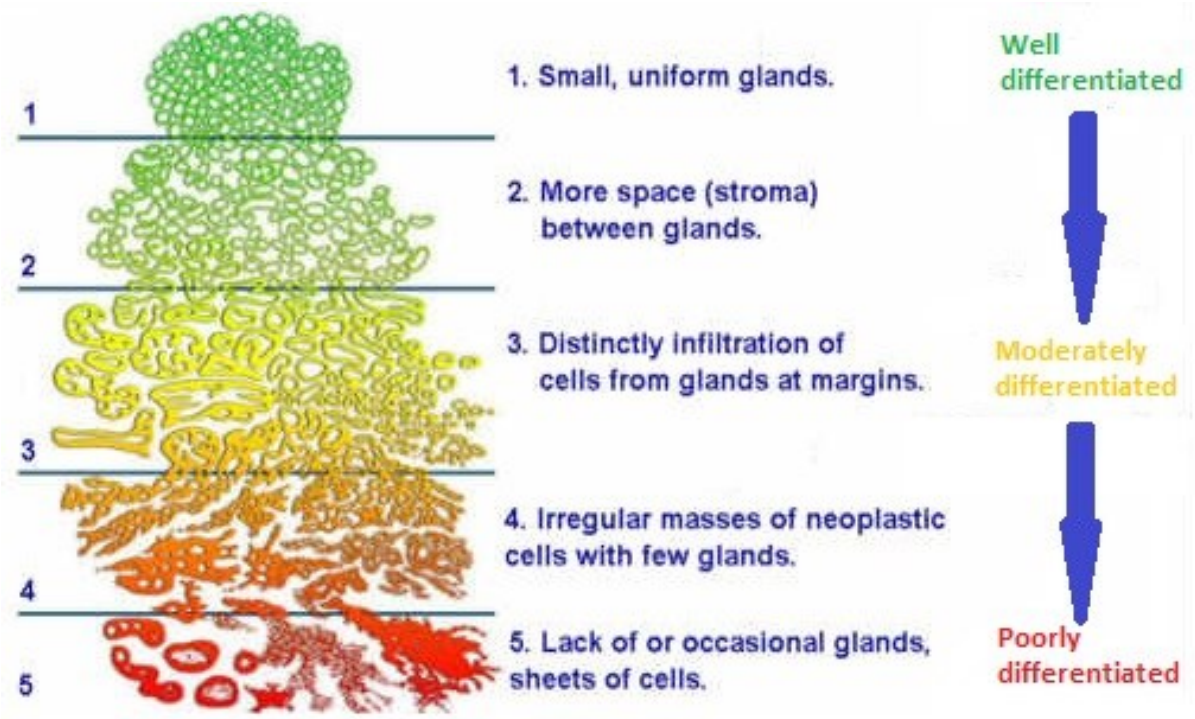


Figure 13 Gleason grading for prostate cancer

Adapted from http://www.prostatehealth.org.au/home/about-prostate-cancer/staging-grading/

1.7.6 Treatment options for prostate cancer

Several options are available for treating PC, offered and administered depending upon the stage and grade of the disease, patient preference and availability as well as the anticipated clinical outcomes. The current treatment strategies for PC include expectant management (watchful waiting or active surveillance), radical prostatectomy, radiation therapy, cryotherapy, androgen deprivation therapy (ADT) and chemotherapy.

1.7.6.1 Watchful Waiting

Watchful waiting is a conservative approach of observing the patient until symptomatic progression after which palliative treatment is started to relieve symptoms due to tumour enlargement (urinary outflow obstruction) or metastasis (bone pain, vertebral fractures and spinal cord compression). Watchful waiting is considered for treating patients with localised disease having a limited life expectancy due to co-morbidity or for elderly patients with less aggressive cancers [120, 134, 139].

1.7.6.2 Active surveillance

Active surveillance involves frequent follow-ups and close monitoring of patients with DRE, PSA testing, TRUS and repeat biopsy. Active surveillance is reserved for localised low-risk PC and helps treat only those patients who are more likely to get maximum benefit from radical treatment [140, 141]. Initiation of curative treatment (surgery or radiation therapy) is recommended in case of early indication of progression based on clinical examination and/or biochemical, ultrasonographic or histologic evidence [120, 134, 142].

1.7.6.3 Radical prostatectomy

Radical prostatectomy (RP), an established curative option for low to intermediate risk localised disease [143], is a surgical intervention which involves complete excision of the prostate gland along with seminal vesicles. Almost half of the men diagnosed with localised PC undergo radical prostatectomy [140]. Retropubic or perineal RP are the traditional surgical approaches while newer minimally invasive techniques include laparoscopic or robot-assisted laparoscopic RP [144]. Complications of RP include excessive bleeding, infection, rectal/ureteral injuries, incontinence and impotence. Nerve-sparing RP aims to preserve the neurovascular bundle supplying the corpus cavernosum, thereby minimising the risk of developing erectile dysfunction following surgery [144, 145].

1.7.6.4 Radiation therapy

Radiation therapy (RT), aimed at causing DNA damage to malignant cells, is a standard curative treatment for high risk localised PC administered commonly as either external beam radiation therapy (EBRT) or brachytherapy [143, 146]. About a quarter of all patients diagnosed with localised disease receive RT [140]. In EBRT, targeted radiation from an external source is given to the tumour over several weeks. Brachytherapy involves ultrasound-guided intra-prostatic placement of radiation pellets containing radioactive isotopes (Palladium-103 or Iodine-125). The possible complications of RT include obstructive urinary symptoms, rectal bleeding, diarrhoea and impotence [147].

1.7.6.5 Cryotherapy

Cryotherapy is a minimally invasive modality employed in the treatment of primary localised and recurrent PC [148]. Though not used routinely due to lack of long-term comparative data with other local treatments, recent advancements have improved clinical outcomes from cryotherapy for localised disease [149]. Cryotherapy involves TRUS-guided transperineal insertion of hollow probes into the prostate to deliver gases (argon-helium mixture) at extremely low temperatures (up to -180^{o}C) [148]. This leads to tissue ablation as a consequence of direct cellular injury and microcirculatory failure resulting from exposure to such extreme below freezing temperatures [150].

1.7.6.6 Androgen deprivation therapy

PC was historically considered an autonomous untreatable disease and a diagnosis of PC was thought to be synonymous with imminent death. Not much was known about the biology of PC until 1941, when ground-breaking findings from the experiments of Charles Huggins (Figure 14) and co-workers revealed the androgen-dependent nature of PC. It was demonstrated that reduction in serum androgen levels of patients with metastatic PC by castration with surgical orchidectomy or administration of oral oestrogen 'diethylstilboestrol' (DES) induced retardation of tumour growth and improvement of symptoms. In the year 1966, Huggins was awarded the Nobel Prize in Physiology and Medicine for his revolutionary work which signalled the commencement of an era of development of therapies aimed at depleting androgens for the treatment of advanced disease [113, 151, 152].

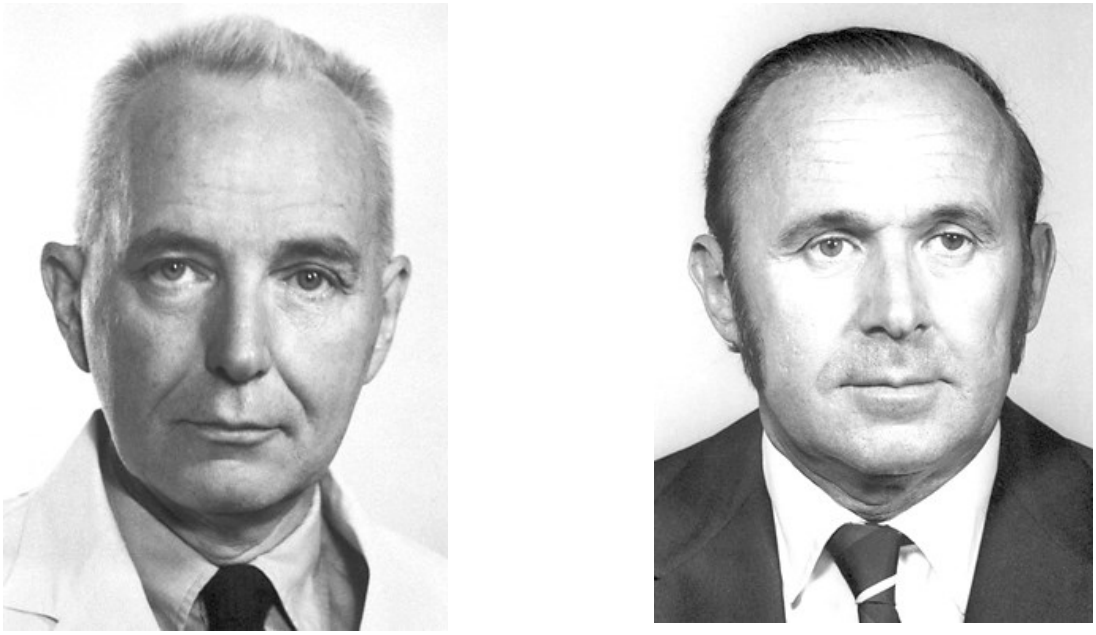


Figure 14 Charles B Huggins (left) and Andrew V Schally (right) [152, 153]

It is now known that PC cells, normal or malignant, require androgens for their growth and proliferation via activation of ARs. Testosterone, the major circulating androgen, is converted into DHT by the action of the enzyme 5α-reductase within the prostate cells. Intracellular DHT is almost 10 times more potent than testosterone and promotes androgen

signalling responsible for tumour progression. Withdrawal of androgens by ADT leads to PC regression [154, 155]. To-date, ADT remains the mainstay for treating advanced PC and it has undergone substantial evolution over the last five decades [156]. Table 5 lists the different modalities used as ADT for PC with their mechanisms of action.

Table 5 ADT modalities and their mechanisms of action

ADT Modality	Mechanism of Action
Surgical orchidectomy	Removal of testes which are the principal source of testosterone, contributing > 90% to the circulating testosterone pool
Diethylstilboestrol (DES; oral oestrogen)	Negative feedback inhibition of the HPG-axis
LHRH agonists (leuprolide, goserlin, triptorelin)	Downregulation of pituitary LHRH receptors, decrease LH release which subsequently suppresses testosterone production
LHRH antagonists (Degarelix)	Inhibition of pituitary LHRH receptors directly
Non-steroidal anti-androgens (bicalutamide, flutamide, nilutamide)	Competitive inhibition of AR activation by direct binding to AR ligand-binding sites
Steroidal anti-androgens (cyproterone acetate)	Blockade of enzyme CYP17 in adrenal steroid biosynthetic pathway, reduction in adrenal androgen production

1.7.6.6.1 Surgical orchidectomy

Surgical orchidectomy (bilateral removal of testes) was one of the first used ADT modalities [151], resulting in a rapid decline in serum testosterone to castrate levels (≤ 50 ng/dL or 1.7 nmol/L) as the testes are the principal source of circulating androgens (producing nearly 95% of total) [8, 157]. Despite being a cost-effective means of ADT for PC, surgical orchidectomy is rarely performed these days particularly in the western world owing to the psychological trauma associated with it [158, 159].

1.7.6.6.2 Oral oestrogen

Around half a century ago, a synthetic oral oestrogen DES was used for treating metastatic PC, thus making it the first pharmacological agent for ADT. Oestrogens, sharing the same steroid nucleus as testosterone, suppress testicular production of androgens via a negative feedback loop inhibiting the HPG axis [6].

DES was studied extensively by the 'Veterans Administration Cooperative Urological Research Group' (VACURG) who conducted a series of randomised clinical trials comparing DES to surgical orchidectomy for the treatment of newly diagnosed PC (8). Despite showing equivalent efficacy to surgical orchidectomy, DES was withdrawn from routine clinical use as the 5mg dose was shown to cause CVS toxicity in a third of treated patients with 15% experiencing a serious thromboembolic event [160]. However, subsequent trials showed cardiovascular mortality to be lower with low dose DES (1mg) as compared to high dose DES (5mg) without any change in the beneficial oncological effects [161].

1.7.6.6.3 Luteinising hormone releasing hormone agonists

The molecular structure of hypothalamic hormone LHRH, also called GnRH, was characterised by Andrew Schally's (Figure 14) laboratory in 1971. Following the remarkable discovery, Schally and co-workers developed synthetic decapeptides that acted as agonists of LHRH. Unlike the pulsatile hypothalamic release, short half-life and moderate receptor

binding affinity of LHRH, chronic administration of synthetic LHRHa or gonadotropin releasing hormone analogues (GnRHa) results in a continuous and prolonged action causing downregulation of pituitary receptors. The net effect is suppression of FSH and LH secretion from the anterior pituitary causing cessation of androgen production in the testicular Leydig cells and subsequent reduction of circulating testosterone [162]. Castrate levels of testosterone are achieved within a month of starting LHRHa therapy [157, 163]. Andrew Schally was awarded the Nobel Prize in Physiology and Medicine in 1977 for his pioneering work which led to LHRHa becoming a standard ADT modality [153].

LHRHa were shown to have a similar survival outcome to surgical orchidectomy or DES [164]. Due to their better psychological tolerability than surgical orchidectomy and improved CVS safety profile than high dose DES, LHRHa gained worldwide acceptance in the 1980s and have since remained the treatment of choice for androgen sensitive advanced PC [156]. The commonly employed LHRHa agents include goserelin, leuprolide and triptorelin, given usually as subcutaneous depot injections on a monthly or three monthly basis [165].

LHRHa are typically offered for long term therapy following a diagnosis of advanced (incurable) disease either at presentation and following failure of radical therapy with curative intent [165]. LHRHa are now also given for short term as adjuvant or neo-adjuvant to RT for localised disease after they were shown to improve clinical and survival outcomes [166]. LHRHa not only suppress serum concentration of testosterone to <5% of normal (castrate levels) but also result in an acute decline in oestrogen levels to <20% of normal (aromatisation of testosterone yields oestrogen in males) [6, 167]. As a consequence of diminished sex hormones, long term ADT with LHRHa has been associated with serious complications such as sarcopaenia, anaemia, sexual dysfunction and osteoporosis [168]. Intermittent ADT with LHRHa has been considered to overcome such toxicity. This involves cycling ADT with off-treatment periods, allowing testosterone to recover above castrate levels during the treatment cessation phase. Survival outcomes similar to continuous ADT have been shown with intermittent ADT in metastatic PC [169] and potential benefits relating to body composition

changes have been suggested [170]. However current evidence appears inadequate in establishing intermittent ADT for routine clinical use [171, 172].

Initial exposure to LHRHa leads to a 'testosterone flare reaction', due to preliminary transient activation of HPG-axis which leads to a surge in the production of testosterone. In a few patients, this can cause complications such as exacerbation of bone pain from skeletal metastasis and worsening of urinary obstructive symptoms. The flare phenomenon is blocked by administering anti-androgens a week before starting LHRHa and continuing for 2-3 weeks afterward [163].

1.7.6.6.4 Anti-androgens

Anti-androgens, also called androgen antagonists, are oral agents that inhibit AR signalling by competitively blocking the AR ligand-binding sites. There are two distinct classes of anti-androgens; steroidal (cyproterone acetate) and non-steroidal (bicalutamide, flutamide and nilutamide). In addition to AR blockade, steroidal anti-androgens also exhibit progestogen-like activity that results in central HPG inhibition and decrease in serum testosterone [173].

Anti-androgens are commonly employed for preventing the flare reaction from LHRHa therapy [163] and they have also been used in combination with LHRHa (combined androgen blockade; CAB). CAB was the first method of ADT shown to improve survival in patients with advanced disease as compared to surgical orchidectomy or DES [174].

Suppression of testosterone synthesis in the testes does not completely eliminate serum androgens as adrenal production accounts for 5% of the circulating androgen pool [8]. Further, the potent androgen DHT is also synthesized locally in the prostate from sex hormone precursor DHEA produced in the adrenal glands. The residual androgens from a non-testicular origin stimulate PC growth and CAB has been used to enhance the efficacy of ADT by countering this effect [8, 175].

1.7.6.6.5 Luteinising hormone releasing hormone antagonists

LHRH antagonist Degarelix is a newer ADT agent that binds to pituitary receptors and blocks the release of LH and FSH, without causing the flare reaction seen with LHRHa. Degarelix, currently available as a monthly depot injection, has been shown to induce a rapid decline in serum testosterone levels (96% patients achieve castrate levels within 3 days) and maintain castrate levels effectively [176]. Recent findings from analysis of pooled data of prospective randomised controlled trials (RCT) comparing degarelix to LHRHa suggest improvements in survival and musculoskeletal toxicity outcomes with degarelix [177].

1.7.6.7 Castration-resistant prostate cancer and its treatment

Following initial response to ADT, PC invariably progresses to a state of resistance called castration-resistant prostate cancer (CRPC) which is associated with a poor prognosis and reduced survival. Continued AR signalling due to intratumoral androgen synthesis, AR mutations and AR overexpression has been suggested to propel disease progression despite castrate levels of testosterone achieved with conventional ADT [156, 178]. A number of novel treatments offering survival benefit for CRPC have recently been introduced [179, 180]. These include cytotoxic chemotherapy (docetaxel, cabazitaxel) [181], new ADT agents (abiraterone, enzalutamide) [182-184] and immunotherapy (sipuleucel-T) [185]. Low-dose oral DES (1-3 mg) has also been demonstrated to be effective and safe as a CRPC treatment with a 5-10% rate of thromboembolic events [186, 187]. Table 6 lists the treatment options for CRPC and their mechanisms of action. The management of CRPC has been transformed with the introduction of these new agents but questions regarding their optimum timing, combination therapy and toxicity profile still need to be answered.

Table 6 Novel treatment options for castration-resistant prostate cancer

Treatment	Type	Mechanism of Action
Docetaxel, Cabazitaxel	Cytotoxic chemotherapy	Arrest tumour growth by binding to tubulin protein and causing cell cycle arrest
Abiraterone	Androgen deprivation therapy	Suppresses intratumoral androgen synthesis by inhibiting both 17-α-hydroxylase and 17,20 lyase activities of microsomal enzyme cytochrome P450 isoform-17
Enzalutamide	Anti-androgen	Multi-step inhibition of androgen signalling cascade (competitive binding to AR, reduced nuclear translocation, impaired DNA binding)
Sipuleucel-T	Immunotherapy	Activated autologous cytotoxic T-cell vaccine targets prostatic acid phosphatase expressed by tumour cells
DES	Synthetic oral oestrogen	Inhibits gonadal, extragonadal and intratumoral androgen synthesis

1.7.6.8 Toxicities of contemporary androgen deprivation therapy

Contemporary ADT, usually achieved with LHRHa, is associated with multiple debilitating adverse effects including compromised bone health (osteoporosis and fragility fractures), vasomotor disturbances (hot flushes, night sweats), metabolic imbalances (dyslipidaemia, impairments in glucose homeostasis), sexual dysfunction (diminished libido, impotence), neuropsychological issues (depression, cognitive decline), changes in body composition (loss of musculature, central adiposity, weight gain), changes in sleep pattern, anaemia and various other systemic problems (increased susceptibility to CVS events, renal damage, decreased physical strength) [168, 188-193]. The constellation of ADT induced serious toxicities is labelled as 'castration syndrome' or 'androgen deprivation syndrome', and it has a huge negative impact upon the quality of life (QoL) of the patients [168, 194, 195]. It has been suggested that partners and carers of men receiving ADT with LHRHa are also affected and often experience more distress than the patients themselves [196].

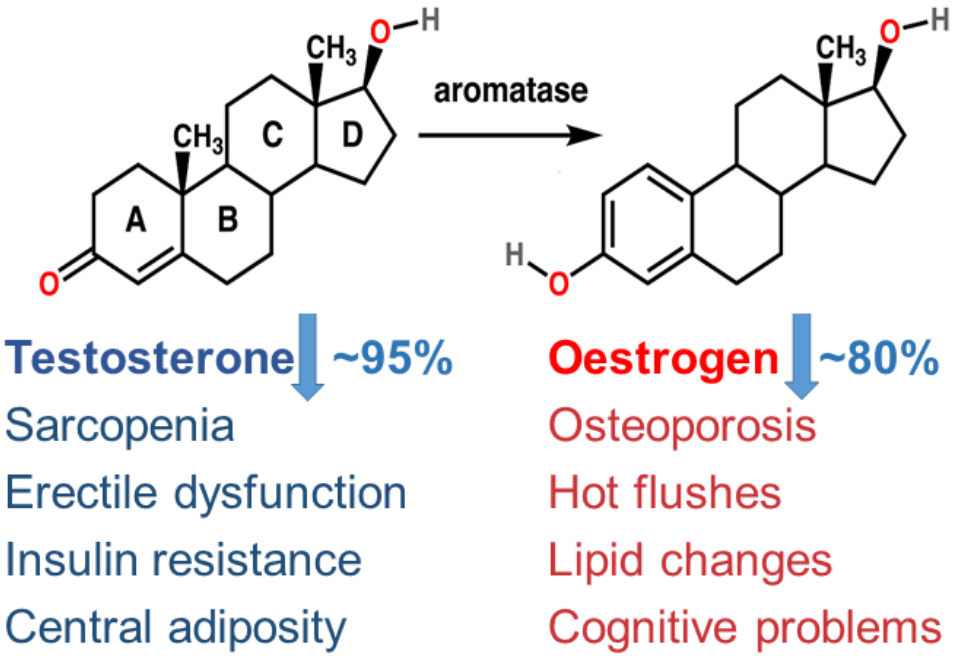


Figure 15 ADT toxicities due to reduction in sex hormone levels

ADT with LHRHa delivers up to a 95% reduction in serum testosterone levels, which in turn results in suppression of oestrogen (by about 80%) as testosterone is the substrate for the enzyme aromatase which converts testosterone into oestradiol [6, 164, 167]. This iatrogenic hypogonadism leads to the development of complications some of which (erectile dysfunction, loss of libido and decreased muscle mass) can be attributed to low testosterone levels while others (osteoporosis, fracture risk, hot flushes and dyslipidaemia) are thought to be due to the accompanying oestradiol deficiency (Figure 15) [155, 197].

There is a growing need to fully understand the nature, extent and impact of the toxicities resulting from long term contemporary ADT with LHRHa. This becomes even more relevant considering the widespread and increasing use of LHRHa. In North America, more than half a million men diagnosed with PC are on ADT with LHRHa at any given time. PC incidence has also risen with improvements in detection rates and overall survival rates have increased as well, resulting in more men than ever before being diagnosed with PC and receiving ADT for longer periods [198]. This has a multiplicative effect on the toxicity burden of ADT with LHRHa. The current UK costs for treating PC and its treatment-related toxicities exceed £100 million per year, with the total annual global expenditure estimated to be more than £2 billion [199].

1.7.6.8.1 Cognitive Impairment

Several studies using neuropsychological assessments done over the last decade or so have linked ADT to cognitive impairment. One of the first studies examining cognitive function randomised men with advanced PC (n=82) to receive active treatment with LHRHa / anti-androgen or conservative management. Cognitive assessments were conducted at baseline and 6 months and ADT was shown to be associated with impairments in memory, attention and executive functions [200]. Men undergoing ADT have also been shown to have slower processing speed and diminished verbal memory (immediate and delayed) as compared to age matched healthy controls [201]. In a small qualitative pilot study, men

receiving ADT following radical treatment for localised PC were surveyed for cognitive problems. Results from the study showed that 8 of the 11 men reported decreases in attention, verbal fluency, executive functions, memory and information processing (particularly visual information) [202].

A meta-analysis of 9 studies assessing cognitive function in patients receiving ADT for PC revealed that half of the men showed decline in at least one cognitive domain following ADT for 6 to 9 months. Executive functions and visuospatial abilities were the domains affected commonly [203]. A recent review of 11 clinical studies on the cognitive effects of ADT suggested a possible deleterious influence of ADT on cognitive domains including spatial memory and verbal memory [204]. A recent study compared cognitive performance in patients receiving ADT for PC (n=58) with age and education-matched PC patients treated with RP for localised disease (n=84) and healthy men (n=88). Groups did not differ in cognitive scores at baseline. Men on ADT showed higher rates of cognitive impairment at 6 months and one year after starting ADT compared to the two control groups. Exploratory genotyping done on samples obtained at baseline revealed association of guanine nucleotide binding protein (G protein), beta polypeptide 3 (GNB3) single-nucleotide polymorphism (SNP) rs1047776 with impaired cognitive performance in ADT recipients [205]. Another recent cross-sectional study showed decreased performance on cognitive tests of attention, memory and information processing in patients who received ADT for PC (n=33) as compared to patients who did not receive ADT (n=32) as well as age- and education-matched healthy controls (n=35) [206].

A few studies have yielded contradictory results. In one such study, lower than expected cognitive performance was seen among hormone naïve patients with PC who were to start ADT (n=32), suggesting a need to assess cognitive function in such men before starting ADT. However, no change in cognitive function was seen in those men after 6 months of ADT [207]. A prospective study assessing cognitive function in men (n=25) on ADT (neoadjuvant and adjuvant to radical RT) for 12 months showed improvement in measures of memory and no cognitive decline [208] One study compared cognitive performance in men with non-metastatic PC starting ADT (n=77) with demographically matched patients with PC

not undergoing ADT (n=82) and healthy controls (n=82). Cognitive assessments were done at baseline, 6 months and one year. Results from the study showed no consistent evidence of cognitive decline in ADT recipients over a period of one year as compared to the other two groups (P >0.05) but the type of ADT was not specified [209].

These conflicting findings suggest a need for continued evaluation of the impact of ADT on cognitive function. Brain imaging studies have recently been employed to investigate this further. In one such study, functional MRI (fMRI) was performed using a test of visuospatial memory on patients with PC (n=5) at baseline and 9 months after starting ADT and comparison was made with healthy controls (n=7). Results showed decreased task related blood oxygen level dependent (BOLD) activation from baseline in the parieto-occipital region of ADT users [210]. Another longitudinal fMRI study compared ADT users (n=15) with non-exposed controls (n=15). Results showed reduced BOLD activation in medial pre-frontal cortex on a test of cognitive control in ADT users after 6 months of treatment as compared to controls [211]. One study using structural MRI in PC patients receiving ADT (n=12) and non-users (n=12) revealed decreased cortical gray matter volumes in ADT users after 6 months of ADT [212].

1.7.6.8.2 Skeletal morbidity

The iatrogenic hypogonadism as a consequence of present day ADT with LHRHa shifts the balance between osteoblast and osteoclast activity toward bone resorption, leading to reduction in bone mass, osteoporosis and heightened fracture risk [48, 213]. The detrimental effects of ADT on bone health are now widely recognised and this skeletal morbidity is becoming an important clinical concern in PC treatment [214-216]. The use of anti-resorptive agents (bisphosphonates, denusomab, toremifene) has recently been shown to impart osteoprotective benefit in patients undergoing ADT for PC [217-223]. Bisphosphonates (pamidronate, alendronate, zoledronic acid) prevent resorption of bone matrix by osteoclasts. Denosumab is a monoclonal anti-RANKL antibody that inhibits

RANK/RANKL signalling thus impairing osteoclastogenesis. Toremifene, a selective SERM, offsets the effects of ADT on bone as a result of lack of oestrogen [224, 225].

Several studies have looked at the impact of ADT on skeletal health, mostly using BMD and T-scores as measures of bone loss. BMD, the current gold standard for fracture risk prediction, refers to the bone mass per unit area while T-score is the comparison of the BMD value of a subject to that of a young healthy adult. A decline in BMD of 2-3% has been reported after initiation of LHRHa treatment for PC and this bone loss predisposes to fractures [226].

A cross-sectional study comparing men receiving ADT for non-metastatic PC (n=133), hormone-naïve patients (n=43) and healthy controls (n=170) showed lowered BMD in ADT users than non-users. More than two-thirds of the patients on ADT were osteopaenic (T-score between -1 to -2.5) or osteoporotic (T-score less than -2.5) [227]. In a Canadian study of patients receiving ADT (n=395) with a mean follow-up of five and a half years, 23% of the patients became osteoporotic and 7% developed fractures. The duration of ADT was also associated with both osteoporosis and fractures [228]. Another study of patients with non-metastatic PC showed a higher prevalence of osteoporosis according to the duration of ADT. Osteoporosis rate was 35% in hormone-naive patients as compared to 80% in men receiving ADT for 10 years or longer [229]. A population based cohort study of patients with PC (n=961) showed that men receiving long term ADT had higher rates of fracture than men receiving short term ADT and untreated men [230]. A Swedish study showed an increased mortality risk following a hip fracture in patients who underwent ADT for PC as compared to patients who did not receive ADT [231]. A recent large study of Chinese patients with newly diagnosed PC (n=17359) linked ADT to a high fracture risk and the increased risk was associated with the number of LHRHa doses given in the first year following PC diagnosis [232].

A study of men diagnosed with PC (n=50,613) from the Surveillance, Epidemiology and End Results (SEER) - Medicare database showed ADT to be associated with an increased risk of fracture. 19% of the ADT recipients had a fracture, as compared with 12% percent of those who did not receive ADT ($p<0.001$). The number of doses of LHRHa received during the first year of treatment was also associated with fracture risk [233]. A recent study

of patients with localised PC (n=75,994) using the SEER - Medicare database showed that men assessed to have high baseline risk of skeletal complications had higher probability of receiving ADT than those with low risk (52.1 % versus 38.2 %; p<0.001). During the 12-year follow-up, nearly two-thirds of the patients with high fracture risk and one-third with low risk experienced at least one fracture after ADT, resulting in a 38% higher mortality risk [234].

A few studies utilising bone biomarkers have demonstrated negative skeletal changes following ADT for PC. One such study measured serum levels of bone alkaline phosphatase (BALP), a marker of osteoblast activity, in patients with non-metastatic PC receiving CAB (n=35). Comparison was made with patients undergoing RP for localised disease (n=36). Serum BALP levels were measured at baseline and annually for five years. The pre-treatment levels in both groups were similar but increased (> 60%) in the CAB group after 5 years with sharp rise (> 30%) seen at year 1 assessment [235]. Another cross-sectional study compared serum levels of BALP and N-terminal telopeptide of type I collagen (NTX), a marker of osteoclast activity, in hormone-naïve patients with nonmetastatic PC (n=20), patients with nonmetastatic disease receiving ADT (n=19) and patients with metastasic disease undergoing ADT (n=28). Men on ADT were shown to have elevated BALP and NTX levels as compared to hormone-naïve men (P < 0.01). Higher BALP levels were seen in patients on ADT for metastatic PC as compared to men on ADT for nonmetastatic disease (P = 0.01) but serum NTX levels were similar (P = 0.33) [236].

Novel imaging techniques have also been used to examine ADT induced skeletal changes. Using high-resolution peripheral CT, a prospective observational study demonstrated microstructural deterioration in distal radius and tibia of men with non-metastatic PC (n=26) after 12 months ADT [237]. High-resolution micro-MRI imaging (HR-MRI) has also shown vertebral microarchitectural decay in patients receiving ADT [238].

1.7.6.8.3 Sexual dysfunction

Sexual dysfunction and inactivity are common in PC patients on ADT with up to 90% of the patients reporting sexual side effects. This decline in sexual function and activity is a consequence of not only the sexual issues such as diminished libido, loss of sexual fantasies and erectile problems but adverse emotional and physical changes (decreased strength, fatigue, adiposity) also contribute to it [239, 240].

Sexual and vitality measures of health-related QoL (HRQoL), assessed using the validated PC specific instrument 'Expanded Prostate Cancer Index Composite' (EPIC), have been shown to be impaired in patients receiving neo-adjuvant ADT for localised PC before starting radical RT [241]. A recent study examined the effects of ADT on sexual function and sexual bother in patients (n=80) undergoing ADT for advanced PC. Findings revealed a high prevalence of sexual problems in these men. Higher sexual bother was associated with younger age and earlier phase of treatment [242]. Another recent study retrospectively compared ADT users who reported sexual activity with sexually inactive men to identify factors causing sexual dysfunction in men undergoing ADT for PC. Results showed sexual activity to be related to maintenance of quadriceps muscle strength and overall health, suggesting potential beneficial impact of exercise on ADT induced sexual problems [243].

1.7.6.8.4 Patient-partner relations

ADT negatively impacts upon intimacy and patient-partner relationships. Following a PC diagnosis, the couple dynamics change to that of a patient and carer. ADT further compromises dyadic relationships due to its adverse effects on patient's sexuality, vitality and psychological health [240]. In a small interview based study of men receiving ADT for PC (n=15), nearly half of the treated men were shown to experience an erosion of spousal relations which was not only limited to intimate sexual contact but also affected social interaction [244].

1.7.6.8.5 Cardiovascular complications

The relationship between ADT use and CVS complications appears to be complex as studies examining impact of ADT on CVS have yielded conflicting results. Previously, an observational study suggested that men receiving ADT with LHRHa were at an increased risk of CVD, myocardial infarction (MI) and sudden death [245]. However, a randomised trial of patients with PC (n=945, 9 years follow-up) comparing RT with adjuvant LHRHa to RT alone showed no difference in the rates of CVS events between the two groups (8.4% vs. 11.4%, p=0.17). Analysis of pooled data from 8 randomised trials showed no difference in cardiovascular mortality between ADT users and non-users (11% vs. 11.2%, p=0.41) [246].

Results from a pooled analysis of 6 randomised studies comparing ADT with LHRHa to degarelix (LHRH antagonist) showed LHRHa to be associated with a higher risk of CVS events in men having pre-existing CVD ($p < 0.002$) [247]. A recent meta-analysis of 6 studies has also shown LHRHa to be associated with increased CVS morbidity and mortality [248]. Results from a study of Swedish men with PC on ADT have shown LHRHa to be associated with an increased risk of CVD, with highest risk during the first 6 months of initiation of therapy in patients with a prior history of CVD [249]. A recent large population-based cohort study of men with newly diagnosed PC (n=21,729) showed current use of ADT to be associated with an 84% higher risk of venous thromboembolism [250]. Careful monitoring for CVS health needs to be exercised in patients receiving ADT with LHRHa, particularly in high risk men till future findings clarify the role of ADT in development of CVS complications [251].

1.7.6.8.6 Physical changes

ADT with LHRHa leads to physical changes including adiposity, sarcopaenia and weight gain [252, 253]. Diminution of muscle mass and excessive weight gain also contribute to fatigue which affects 50% of men on ADT [254]. A prospective study of men receiving ADT for PC (n=252) measured lean body mass (LBM) using whole body dual energy x-ray absorptiometry (DEXA) at baseline and then annually for three years. Findings showed a

progressive decline in LBM from baseline (1% decrease at year 1; p<0.01; 2.1% decrease at year 2, p<0.001; 2.4% decrease at year 3, p<0.001) [255]. A longitudinal study of men receiving ADT after RP (n=132) showed that every 2 of 3 men studied gained weight following ADT and the weight gain occurred in the first year of treatment [256].

1.7.6.8.7 Metabolic syndrome

ADT with LHRHa has been linked to development of metabolic complications including dyslipidemia, insulin resistance and diabetes [245, 257, 258]. Weight gain, obesity and adiposity are also seen with ADT [258, 259]. All these features constitute metabolic syndrome which is seen in half of the men receiving ADT. Metabolic syndrome predisposes these men receiving ADT for PC to an increased risk of CVS complications [260].

1.7.6.8.8 Anaemia

The development of normochromic normocytic anaemia is a commonly encountered hematological consequence of ADT which may also be one of the main factors causing fatigue in these patients. Reduction in the stimulatory effect of testosterone on the differentiation of erythroid stem cells in the bone marrow and renal erythropoietin production have been proposed as possible mechanisms of ADT associated anaemia [261, 262]. In a study of patients with metastatic PC (n=135), haemoglobin (Hb) levels were significantly reduced after 3 to 9 months of LHRHa therapy as compared to baseline (Mean Hb. decline -1.66 g/dL, p<0.001) [263]. A drop in Hb. levels following 3 months of ADT has been associated with reduced overall and progression-free survival [264].

1.7.6.8.9 Vasomotor symptoms

Vasomotor symptoms including hot flushes and night sweats are frequently reported by patients undergoing ADT for PC [188, 192, 193, 265]. Sharp decline in circulating sex hormone levels brought about by ADT is thought to induce re-setting of the thermostat in pre-

optic area of hypothalamus to a lower point, which causes hyperactivation of peripheral thermoregulatory mechanisms resulting in development of hot flushes and night sweats [168]. Findings from a questionnaire based study showed that more than 90% of men experience female climacteric-like side effects such as hot flushes and night sweats following ADT with LHRHa [266]. LHRHa cause vasomotor symptoms shortly after initiation of therapy. These symptoms persist beyond cessation of treatment in some men and are a cause of substantial psychological distress [265].

1.7.6.8.10 Sleep disturbances

Sleep problems occur commonly in men undergoing ADT for PC [266, 267]. In one study using wrist actigraphy and subjective measures for assessment of sleep pattern, patients receiving ADT (n=60) were shown to have reduced and poor night time sleep, and increased sleepiness during the day [268]. A recent questionnaire based study showed higher insomnia scores in patients receiving adjuvant ADT with RT compared to RT alone. Night sweats seemed to be associated with sleep disturbance [269].

1.7.6.8.11 Renal problems

Acute renal problems in patients undergoing ADT have recently been highlighted. Findings from an observational study of patients with newly diagnosed non-metastatic PC suggest ADT use to be associated with an increased risk of acute renal injury. In this study, 232 new cases of acute renal injury were identified from a cohort of men treated with ADT (n=10,250) during a mean follow-up of 4.1 years [270]. Results from another large retrospective study of patients with non-metastatic PC (n=69,292) have also linked administration of ADT to an increased risk of acute kidney injury. The 10 year rates of acute kidney injury were shown to be higher in men receiving LHRHa than bilateral orchidectomy (31% vs. 26%, $p< 0.001$) [271].

1.7.6.9 Parenteral oestrogen as androgen deprivation therapy

Oral oestrogen DES was previously used as ADT but its use was curtailed owing to concerns over CVS and thromboembolic toxicity [160]. It is now evident that oral administration exposes the liver to very high concentrations of oestrogen via portal circulation. This first pass through the liver upregulates hepatic synthesis of pro-coagulant proteins and induces a hypercoagulable state, thereby escalating the risk of serious thromboembolic and CVS events such as myocardial infarction and stroke [272].

Parenteral oestrogen administration (intramuscular, transdermal) not only results in central suppression of androgen production but also mitigates the thromboembolic consequences of oral therapy by avoiding first-pass effect through the liver. Castrate levels of testosterone for PC growth arrest can be achieved by this strategy, with little effect on haemostatic profile [273-275]. By replacing endogenous oestrogen lost otherwise as a result of contemporary ADT with LHRHa, parenteral oestrogen may potentially mitigate the oestrogen deficiency related serious adverse events such as osteoporosis [276, 277]. Previous data from studies using parenteral oestrogen as ADT for PC have highlighted the bone-sparing potential of this treatment. A study of patients with advanced PC (n=20) treated with transdermal oestradiol as primary ADT reported increases in total hip and lumbar spine BMD after a year of starting therapy [278]. In another study of men with advanced PC (n=910) with 9 years follow-up, none of the patients on intramuscular oestrogen (polyoestradiol phosphate) developed any serious skeletal event compared to 18 on CAB [275].

The PATCH (Prostate Adenocarcinoma TransCutaneous Hormones) study is an ongoing randomised clinical trial, now in Phase III, comparing transdermal oestradiol with LHRHa in locally advanced and metastatic PC. In the first stage (n=254) of the phase II study, similar rates of significant CVS events (the primary outcome) were reported in both trial arms. Serum glucose and cholesterol profiles were also shown to be more favourable in the oestradiol group than in the LHRHa group (Table 7) [279]. Results from a sub-study of the phase II trial evaluating bone health showed decreased lumbar spine BMD with LHRHa

compared to baseline while it increased with oestrogen patches (Table 6) [280]. Parenteral oestrogen appears to be an effective and safe therapeutic option for the treatment of PC. Future data from trials such as PATCH will contribute to the evidence-base required to establish parenteral oestrogen as an alternative to contemporary ADT with LHRHa.

Table 7 Comparison of CVS, metabolic and skeletal parameters from the PATCH study [279, 280]

Outcomes	LHRHa	Transdermal oestradiol
Proportion of patients with CVS events (Median follow-up 19 months)	7.1% (n=84)	10.1% (n=169)
Proportion of patients with castrate testosterone (≤1.7 nmol/L) at 3 months	93% (n=75)	92% (n=121)
Percentage change in fasting glucose at 12 months from baseline	+5.5% (n=54)	-2.4% (n=95)
	p-value 0.004	
Percentage change in fasting cholesterol at 12 months from baseline	+4.1% (n=55)	-3.3% (n=101)
	p-value <0.0001	
Percentage change in lumbar spine BMD at 12 months from baseline	-2.11% (n=21)	+6.43% (n=39)
	p-value <0.001	
Percentage change in lumbar spine BMD at 24 months from baseline	-6.09%, (n=10)	+4.58%, (n=20)
	p-value <0.001	

2. Statement of research problem

This project aimed to define the influence of alterations in sex hormones on certain important aspects of systemic function in a population of patients with PC receiving ADT. Usually achieved using LHRHa, contemporary ADT causes marked suppression of sex hormones (both androgens and oestrogens) resulting in 'castration syndrome', a constellation of adverse events including skeletal complications, cognitive problems and potential psychosocial issues arising from changes in odour signalling which are some of the least well studied yet important factors affecting morbidity and QoL of patients undergoing therapeutic sex steroid changes.

2.1 Scientific rationale

2.1.1 Cognitive study

Cognitive abilities differ between males and females [210], possibly due to the varying sex hormone levels and their receptor distribution in brain. Studies in menopausal women suggest that women who use HRT have better cognitive skills and memory compared with age-matched non-users [38, 42]. Cognitive decline in men has higher prevalence in the elderly and testosterone replacement in older men has been shown to improve cognitive functioning [281, 282]. At present, work on potentially deleterious cognitive effects of reduced sex hormone levels on cognitive function in men (such as that seen with ADT for PC) is inconsistent, limited to a few studies as compared to work in females, generally poorly controlled and uses vastly differing individual, often non-validated and subjective approaches which make interpretation difficult. It is not even firmly established that cognitive decline is always a consequence of ADT [203, 204]. Further work is required to objectively quantify cognitive dysfunction due to ADT, understand the mechanism, predict which patients will be affected and determine ways of reducing this side effect.

Establishing the presence of pathophysiology in ADT induced cognitive dysfunction first is important due to the lack of understanding of the underlying mechanism. A plausible scientific approach in this regard appears to be the use of the brain's immunological response to pathology using a PET imaging ligand for activated microglia. Brain microglia have been demonstrated to be highly responsive to brain injury and are rapidly activated in an attempt to envelope/ contain the focal pathology. When activated, the microglia express an 18 kDa protein called TSPO, formerly known as the peripheral benzodiazepine receptor (PBR), which has been proposed as a neuroinflammatory marker [283]. PET imaging ligands have been developed for TSPO and used successfully for researching a range of brain disorders such as neurodegenerative diseases, stroke, physical trauma and multiple sclerosis [284]. One such radio-ligand targeting TSPO is [11C]PBR28 (Carbon-11 Peripheral Benzodiazepine Receptor Ligand) which has a 20-minute half-life, thus making it feasible to perform PET without concern for residual radioactivity [285]. Although such imaging does not provide mechanistic information of the underlying pathology, except for neuroinflammation, it does offer a generic sensitive biomarker for demonstrating the presence of an on-going active pathology.

The non-invasive MRI technique has recently been used for assessing brain structure and function in patients receiving ADT for PC [210-212]. To better gauge the cognitive changes and pathology in settings of altered sex hormones, further studies employing imaging modalities and specific validated neuropsychological assessments are warranted.

2.1.2 Bone study

Osteoporosis, a disease characterised by compromised bone strength and increased risk of fractures, is a serious public health concern and estimates suggest that over 200 million people are affected worldwide [286]. A quarter of all men over the age of 50 years are likely to suffer an osteoporotic fracture at some point in their lifetime and the mortality risk one year post hip fracture is double in men as compared to women [287, 288]. Long-term glucocorticoid therapy, chronic alcoholism and hypogonadism account for nearly 50% of all cases of osteoporosis in men. ADT for PC is a major cause of iatrogenic male hypogonadism [226].

PC has a strong tendency toward bone metastases which disrupt skeletal homeostasis, resulting in elevated serum levels of biochemical markers of bone metabolism (e.g. NTX, alkaline phosphatase; ALP). These markers are associated with higher mortality and fracture rates in patients with metastatic disease [286, 287]. ADT induced decline in circulating sex hormone levels accelerates bone turnover, a process characterised by increased bone resorption and formation, which leads to a rise in the levels of biomarkers of osteoblastic and osteolytic activity [235, 236]. ALP is one such marker which is derived from several sources including liver, bone, kidneys, spleen, intestines and placenta. The two predominant serum isoforms of ALP are of hepatic and skeletal origin [289]. Serum BALP, the bone-specific isoform, is a measure of osteoblastic activity that can potentially be utilised as an early indicator of development of osteoporosis and associated fragility fractures [290]. In postmenopausal women, serum BALP is associated with fracture risk irrespective of BMD [291].

Progressive increase in the prevalence of osteoporosis has been reported in patients undergoing ADT, reaching up to 80% after 10 years of ADT [229]. The negative skeletal effects of contemporary ADT with LHRHa are increasingly recognised and an important consideration in overall management of these men [214, 233, 234]. At present, osteoporosis and associated fracture risk are mostly gauged clinically in terms of BMD assessment performed by DEXA [238, 292]. BMD refers to the bone mass per unit area and is employed as an indirect indicator

of fracture risk. However, BMD measurements are rarely employed clinically in patients receiving ADT; of 28,960 men with loco-regional PC receiving ADT for more than 1 year, only 10% had BMD testing during an 18-month period, starting from 6 months before beginning ADT [293].

Importantly, BMD does not reflect bone quality which is an integration of several features influencing skeletal resistance to fractures. Bone quality depends upon material and structural factors including but not limited to mineralisation, quality of collagen, rate of bone turnover, cortical geometry and trabecular micro-architecture (including trabecular number, thickness, orientation and connectivity) [294, 295]. The ability of bone to resist fracture depends not only on the amount of bone (as measured at present by BMD testing) but also the spatial distribution of bone mass and the intrinsic properties of the materials that constitute bone which come under the wide ranging concept of bone quality. Bone strength reflects the combination of bone mass and bone quality [296]. BMD testing only measures the areal bone mass and does not capture any structural information because images are two dimensional (2D). Thus BMD is not an adequate surrogate marker of bone strength; it only explains about 50% of *ex vivo* strength [297].

Development of improved measures of bone strength is required for identification and assessment of rapidly occurring skeletal changes seen with therapeutic interventions like ADT as well as other pathological or age-related deterioration in bone health. Three dimensional (3D) imaging techniques including micro-computed tomography (micro-CT), are employed for assessing bone strength *ex-vivo* but are of limited clinical use at present owing to radiation hazards as well as technical issues, high costs and accessibility [298, 299]. Micro-CT enables *ex vivo* evaluation of various parameters such as volumetric bone mass (expressed as bone volume fraction 'BVF') which has been identified as a strong determinant of bone strength (R^2 > 0.8) [300, 301].

Low-resolution clinical-CT is a non-invasive and accessible imaging technique available to most hospitals. 3D measures such as BVF derived from clinical-CT scans are potentially a useful option for estimating bone strength. Clinical-CT derived measures of hip

morphology may be especially useful because the hip (a key fracture site in PC patients associated with high mortality) is routinely imaged as part of a pelvic CT scan for staging PC and planning radiation therapy, potentially offering low cost, easily accessible and safe technology for predicting bone strength. Bone markers reveal variations in bone homeostasis and early changes in bone markers such as BALP, which precede measurable changes in bone strength, need to be studied in the context of ADT for PC. The importance of measuring bone markers for monitoring early response to treatment with anti-resorptive or bone forming agents has been highlighted in some studies [302-304]. Such measurements can potentially allow for non-invasive and convenient monitoring of bone health in patients receiving ADT for PC and help clinicians to target CT based skeletal assessments at high-risk patients for diagnosing, treating and/or preventing osteoporosis and fractures.

2.1.3 Odour study

Every individual has a unique body odour that can be discerned by others. Axillary odour, readily identifiable due to its pungent nature, is generally referred to as body odour [73]. Body odour is affected by several factors and gender is one of the major ones. Axillary secretions are known to contain various odoriferous volatile chemicals, some of which are metabolic derivatives of sex hormones [74]. Body odour is thought to provide cues about a potential sexual partner's health, reproductive status and genetic quality. Body odour is considered a signal of physical and sexual appeal in humans and it has also been associated with masculinity and dominance [65-67]. Odour of men has been shown to change with age [72] but it has not yet been studied whether that is due to age-related variations in their sex hormone profile.

Sexual activity has been shown to decline considerably in PC patients on ADT with LHRHa. This sexual inactivity is a consequence of low testosterone, diminished libido, decreased physical strength and erectile dysfunction [239, 240]. Loss of masculine traits in PC patients negatively impacts their social and sexual life. These side effects of ADT also affect the intimate partners of patients and it has been indicated that partners often experience more distress than the patients themselves [196]. Results from an earlier study suggested that nearly half of all men on ADT experienced an erosion of dyadic relations and causal evidence for these relationship changes should be sought in factors affected by treatment [244]. Body odour changes following ADT have not been documented but they may have a significant psychosocial impact on the QoL of patients with PC and their partners.

2.2 Hypotheses

- Increased cerebral binding of [11C]PBR28 is seen on PET imaging in patients with PC having LHRHa induced cognitive impairment
- PC patients reporting cognitive problems since starting ADT with LHRHa show reduced cognitive performance on neuropsychological testing using specific validated instruments.
- BVF metrics obtained from CT scans provide improved measures of bone mass and strength.
- ADT with LHRHa results in elevation of serum BALP levels in patients at three months following commencement of treatment.
- ADT with LHRHa induces perceptible changes in body odour of PC patients PC after three months of therapy.

2.3 Objectives

- To demonstrate, using a PET imaging biomarker of activated microglia (TSPO), the presence of global and regional pathological changes in the brain that relate to LHRHa induced cognitive impairment in patients with PC
- To objectively determine cognitive changes in patients with PC undergoing ADT with LHRHa using validated paper-based neuropsychological assessments and computerised cognitive testing
- To provide insight into possible mechanisms of LHRHa induced cognitive decline i.e. association of a specific cognitive dysfunction with regional neuroinflammatory response.
- To develop a method for measuring BVF of the femoral head trabecular bone at a low resolution (550μm voxel size; i.e. comparable to clinical-CT resolution)
- To validate the devised BVF metrics against high-resolution (30μm voxel size) micro-CT data
- To determine whether the BVF metrics calculated at low resolution relate to bone strength.
- To determine early changes in serum BALP following ADT with LHRHa
- To relate serum BALP changes in patients undergoing ADT to serum sex hormone concentrations
- To determine changes in odour signalling following ADT with LHRHa
- To relate the odour changes in patients on ADT with LHRHa to the serum levels of sex hormones

3. Research methodology

3.1 Cognitive Study

3.1.1 Regulatory approvals

The study received ethical approval from the Queen Square Research Ethics Committee (REC), London (Ref 13/LO/0731). Local research and development (R&D) approval and sponsorship was granted by the Joint Research Compliance Office (JRCO) at Imperial College Healthcare NHS Trust (ICHT), London (JRCO Ref 13HH0558). Insurance cover was provided by Imperial College London's Arthur J. Gallagher negligent and non-negligent harm policy (B1262FI0103012). The study received peer review certification from Imperial College London Peer Review Committee (Ref 0057/IC0031). The study protocol was subsequently amended twice to attain the final form and each amendment was duly approved by the REC and JRCO (Appendix II).

3.1.2 Study design

A cross-sectional analytical study design was employed for this study to compare quantitative [11C]PBR28 PET imaging in patients with clinically noticeable ADT associated cognitive impairment with an age and treatment matched cohort with no such observable change. Neuropsychology testing was done to assess cognitive function.

3.1.3 Sampling

Non-probability consecutive sampling technique was used for recruiting subjects in this study. All accessible subjects fulfilling the eligibility criteria were approached as they presented to the clinics for potential enrolment in the study.

3.1.4 Study population

Target population included patients with PC receiving LHRHa for either newly diagnosed advanced disease, as adjuvant or neoadjuvant to RT or for biochemical relapse with a rising PSA following definitive treatment with RP or RT (without adjuvant or neoadjuvant LHRHa).

3.1.5 Recruitment

Patients were recruited from the uro-oncology follow-up clinics of ICHT. The potential participants were identified and approached in the clinics. Written informed consent was obtained before participants entered into the study. When obtaining consent from a patient, the study and the information sheet were introduced in full by the researcher. Patients were allowed at least 24 hours to consider participation but they were free to take as long as they wished before making a decision and then consent taken. Consent forms, regulatory approval documents and non-anonymised patient data were kept at secure ICHT location.

As advised by the REC, the participants were asked about any previous radiotherapy and scans they have had in the last year and their medical notes were also reviewed for the same to ensure the participants did not get excessive radiation exposure. The radiation dose from this study was less than 3mSv which is a little more than the average annual background radiation dose of 2.5mSv in the UK. The radiation exposure from a CT scan of the chest, abdomen and pelvis on the other hand is of the magnitude of 25 mSv. UK residents in general have a 1 in 4 risk of developing fatal cancer in their lifetime. The additional risk of developing fatal cancer as a result of the radiation exposure from this study was about 1 in 6500 for healthy middle aged adults, which would change the estimated risk of developing fatal cancer from approximately 1 in 4, to 1 in 3.998. Thus, the radiation dose in this study had a negligible impact on the risk of future cancer development.

The eligibility criteria employed for this study is given below:

3.1.5.1 Inclusion Criteria

- Patients between the ages 50 to 80 years.
- Currently on ADT with LHRHa for at least 3 months and up to a year.
- Able to give written informed consent.
- Able to lie still for up to 90 minutes for a PET scan.
- Not claustrophobic and so able to undergo an MRI scan.

3.1.5.2 Exclusion Criteria

- Known history of neurodegenerative disorders and associated dementia or delirium.
- Known history of neuropsychiatric conditions, stroke or traumatic brain injury (TBI).
- Clinically assessed as having MCI prior to starting ADT with LHRHa.
- History of receiving ADT with LHRHa prior to current treatment
- A medical survival prognosis of less than 3 months.
- Patients who were claustrophobic.
- Patients having any metal implanted in their body e.g. heart pacemaker, cochlear implant or any other electronic device.

3.1.6 Study procedures

The study procedures were conducted in two stages (Figure 16). Details of each step are described below.

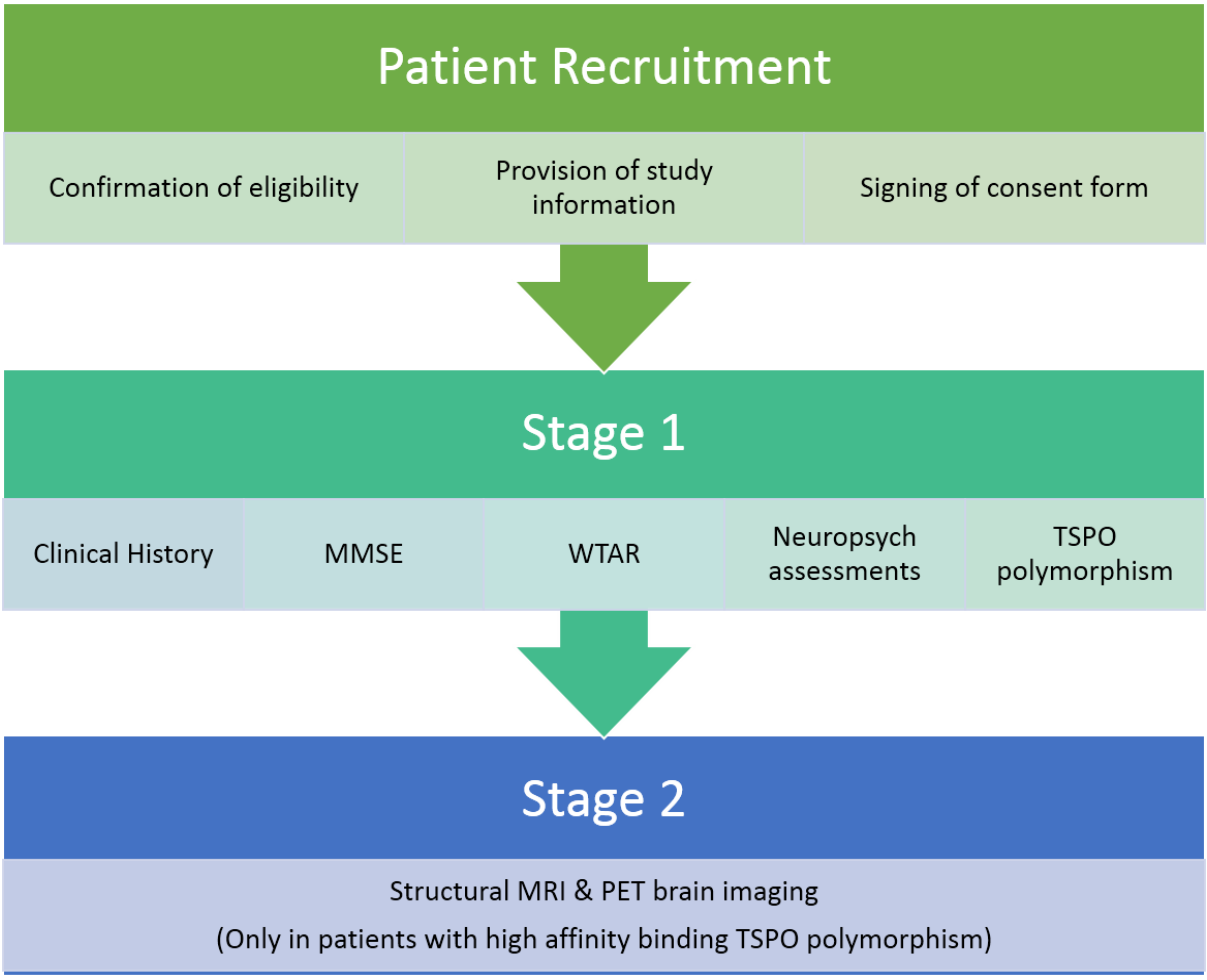


Figure 16 Stage wise conduct of study procedures for the cognitive study

3.1.6.1 Stage 1

All the recruited participants underwent Stage 1 procedures which included detailed clinical history, mini-mental state examination (MMSE), Wechsler's test of adult reading (WTAR), blood test for determining TSPO genetic polymorphism and detailed neuropsychological assessment (paper-based and computerised). All stage 1 procedures were carried out at the uro-oncology clinics of ICHT on routine follow-up visits. For neuropsychological testing, it was ensured that each participant was wearing his routine hearing and/or visual aid if any, as testing required adequate hearing and vision.

3.1.6.1.1 Clinical history

Recruited patients were grouped into two based on a detailed clinical history taken by the researcher. History was taken from the patient and/or their partners/carers where applicable. The study group comprised of patients who reported or were clinically assessed to have noticeable impairment in cognitive ability since starting on treatment with LHRHa. The control group comprised of patients who did not notice and were not clinically assessed to have any change in cognition.

3.1.6.1.2 Mini-mental state examination

MMSE is a short clinical screening tool (11 items, maximum score 30) that tests cognitive areas including orientation, registration, attention, calculation, recall, and language (Appendix III) [305]. MMSE was used in this study to substantiate clinical history and screen out patients with overt cognitive dysfunction that might possibly be related to a pathology or event other than LHRHa. Patients with MMSE score of 24 or above were included.

3.1.6.1.3 Wechsler's test of adult reading

WTAR is a neuropsychological test used to estimate the pre-morbid intelligence quotient (IQ) [306]. WTAR was administered to the study participants to provide a baseline measure of their intellectual capacity before starting ADT with LHRHa. A list of fifty words was presented to the patient. The list contained irregularly spelled words with an increasing complexity (Appendix III). The participants were asked to pronounce each word aloud. One mark was given for each word pronounced correctly for a maximum raw score of 50. The raw score was then standardised by age using a standardisation chart.

3.1.6.1.4 TSPO genetic polymorphism testing

For determining the TSPO genetic polymorphism, 5ml venous blood was collected from each participant in ethylenediaminetetraacetic acid (EDTA) vacutainer tubes (purple cap). The sample was sent for TSPO mutation analysis to Dr. Letizia Foroni at the Imperial Molecular Pathology Laboratory, Hammersmith hospital within 4 hours of collection. A standard analysis was requested with a turnaround time of 10 working days.

Rationale, analysis and interpretation

TSPO genotype at position 439 (SNP rs6971) predicts the binding affinity of TSPO receptor to [11C]PBR28. For analysis, DNA was extracted from each peripheral blood sample using the Qiagen QIAamp DNA blood mini kit. TSPO genotyping of the SNP rs6971 was performed using a TaqMan allelic discrimination assay [307]. The analysis of each sample yielded one of the following three results:

- The homozygous G variant allele; predictive of high affinity
- The heterozygous A/G allele; predictive of medium affinity
- The homozygous A allele; predictive of low affinity

Some patients were excluded at this stage and only those participants who were shown to have the homozygous G variant allele (incidence in general population is 25-50%) which demonstrates expression of high affinity binding of the TSPO receptor to the PET imaging marker [11C]PBR28 proceeded to stage 2 of the study.

3.1.6.1.5 Detailed neuropsychological assessment

Cognitive functions of the study participants were assessed using a comprehensive battery of standard validated paper-based neuropsychological questionnaires and computer-based tests (www2.CBSTrials.com) [308, 309].

3.1.6.1.5.1 Paper-based tests

Administering the paper-based tests took one and a half hours on average per participant. The individual test descriptions are given below:

3.1.6.1.5.1.1 Trail making test

This task was used to assess cognitive processing speed and working memory [310-312]. The task had two conditions (Appendix III). In condition A, the participants were asked to connect circled numbers by drawing lines in an ascending numerical sequence as quickly as possible without lifting the pen from the paper. In condition B, the participants were asked to connect circled numbers and letters in an alternating numeric and alphabetic sequence by drawing lines as quickly as possible without lifting the pen from the paper. The participants were timed as they connected the trail. Scores were reported as seconds required to complete each condition (higher scores meant greater impairment).

3.1.6.1.5.1.2 People test

This sub-set of the doors and people test was employed to measure associative learning and verbal recall memory [313]. In this task, the participants were instructed to remember the names of four people. The participants were then shown papers containing photographs of those four people with their name (forename and surname) and job written underneath (Appendix III). Then they were asked to recall the names. The procedure was repeated two more times. For each forename or surname recalled correctly, 1 mark was given and an extra mark was given for a correct pairing. Sum of the scores from the three trials gave the total score for immediate recall. The participants were asked to recall the names once again after 10 minutes for delayed recall.

3.1.6.1.5.1.3 Colour-Word interference test

The colour-word interference test (CWIT), taken from the Delis-Kaplan executive function system (D-KEFS), was used to assess executive functions including cognitive flexibility and processing speed [314]. The test involved four trials (Appendix III). In the 'colour naming' trial, the participants were asked to identify colours of the printed squares. In the 'word reading' trial, the participants were asked to read colour names (red, blue, green) printed in black ink. In the 'inhibition' trial, the participants were required to name the colour of the inks in which the words were printed and not read the printed word. In the 'inhibition switching' trial, the participants were asked do the same as they did in inhibition trial for words not in a box but for words inside a box, they were required to read the word and not name the colour of the ink. Each trial was timed and scores were reported as seconds required to complete each condition (higher scores meant greater impairment).

3.1.6.1.5.1.4 Letter fluency

Letter fluency is a form of verbal fluency (ability to produce words under specific instructions and fixed time) which involves working memory, cognitive flexibility and strategy making. Letter fluency subtest of D-KEFS was used [315, 316]. The participants were asked to retrieve as many words as possible starting with letter F in one minute and the procedure was repeated with letters A and S. The participants were instructed to avoid repetition and names of places, people and numbers. One mark was given for each correct word and the sum of all gave the total score.

3.1.6.1.5.1.5 Logical memory

Logical Memory subtest of the Wechsler Memory Scale, third edition (WMS-III) was used as a measure of immediate and delayed verbal recall [317]. The test, involving two short stories, was administered as immediate and delayed trials (Appendix III). Story A was read out once to the participants who were then asked to recall the information as word for word as

possible. Story B was read out twice and after each reading, the participants were asked to recall the information in as much detail as possible. One mark was awarded for each specific information recalled. The participants were instructed to try to remember the stories. After a lapse of half an hour, the participants were asked to provide any information recalled from each of the two stories and scores were recorded again. For each story, the participants were also asked fifteen yes/no type questions for recognition memory and scores were recorded.

3.1.6.1.5.1.6 Similarities

This subtest of the Wechsler Abbreviated Scale of Intelligence (WASI) was used to measure semantic verbal reasoning ability [318]. Pairs of words were read out to the participants who were asked to tell how the two words in each pair were similar. Scoring was done according to the general scoring principles and sample responses provided in the manual (e.g. for the word pair 'grapes-strawberry', 2 marks were awarded if the response was 'fruits', 1 mark was awarded if the response was 'sweet' and no mark was awarded for an irrelevant response such as 'minerals').

3.1.6.1.5.1.7 Matrix Reasoning

This WASI subtest was used to assess visuospatial and non-verbal logical reasoning ability [318]. The participants were shown incomplete matrices and they were asked to select the correct response options that completed the matrices (Figure 17). Five response options were provided for each matrix shown. 1 mark was given for each correct response and the sum gave the total score.

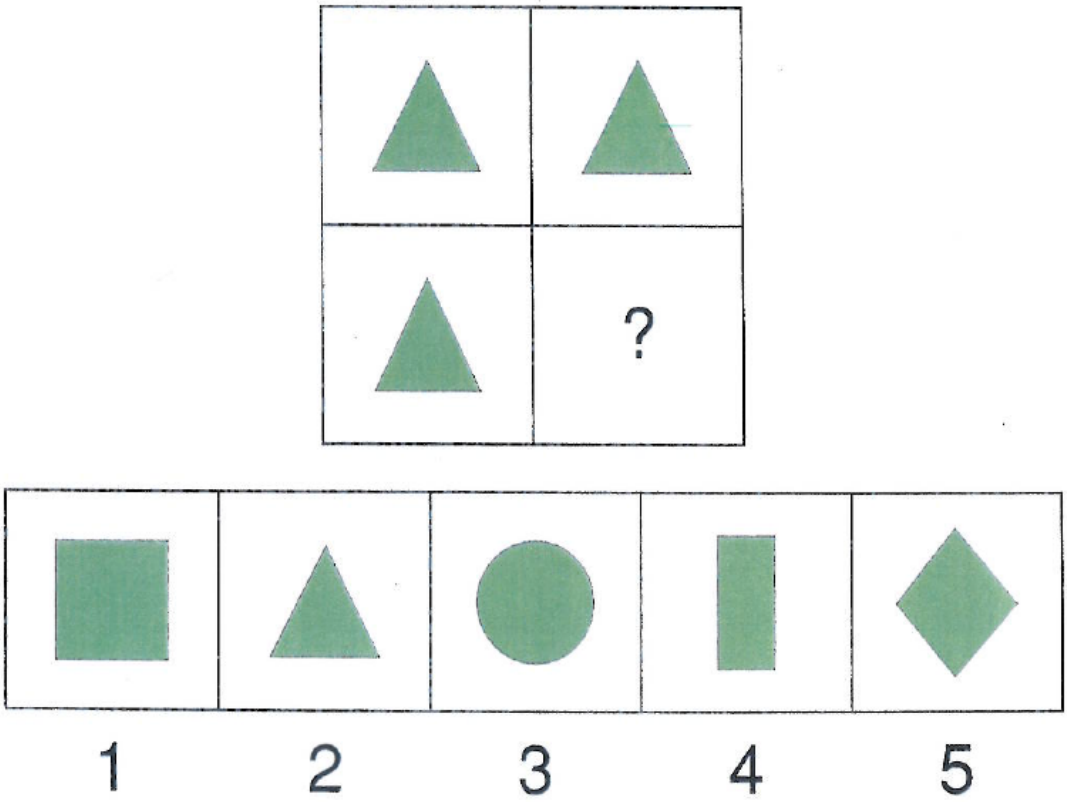


Figure 17 Administered item from the matrix reasoning test

3.1.6.1.5.1.8 Digit Span

This WMS-III subtest was used for the assessment of short-term memory and attention span. It has two components, forward and backward digit spans [319]. Digit sequences were read out to the participants who were asked to recall the digits in the same sequence for forward and in reverse order for backward test. The sequences were presented beginning with a set of two trials of two digits length which increased by one digit for the next set of trials. Testing was stopped when the participants failed to recall correctly a set of two trials for a particular digit length. 1 mark was awarded for each accurate recall and the sum of the scores on forward and backward tests gave the total score.

3.1.6.1.5.2 Computer-based tests

All computer-based tests were administered on a laptop computer (HP ProBook 6570b, Intel CORE i5 processor, Windows 7) using the application CBS App V2 running on Adobe AIR software (Version 15.0.0.293). Administering the computer-based tests took one hour on average per participant. The individual test descriptions are given below. Each test was preceded by a set of instructions displayed on the computer screen. Verbal instructions and explanation were avoided to ensure consistency.

3.1.6.1.5.2.1 Digit span

This test was used to assess short-term working memory [320]. A sequence of digits appeared on the screen one after another and participants were asked to repeat the sequence by pressing the respective number keys on the keyboard (Figure 18). The number of digits in the subsequent trial increased or decreased by 1 subject to the participant repeating the sequence correctly or incorrectly. The test finished after 3 errors.

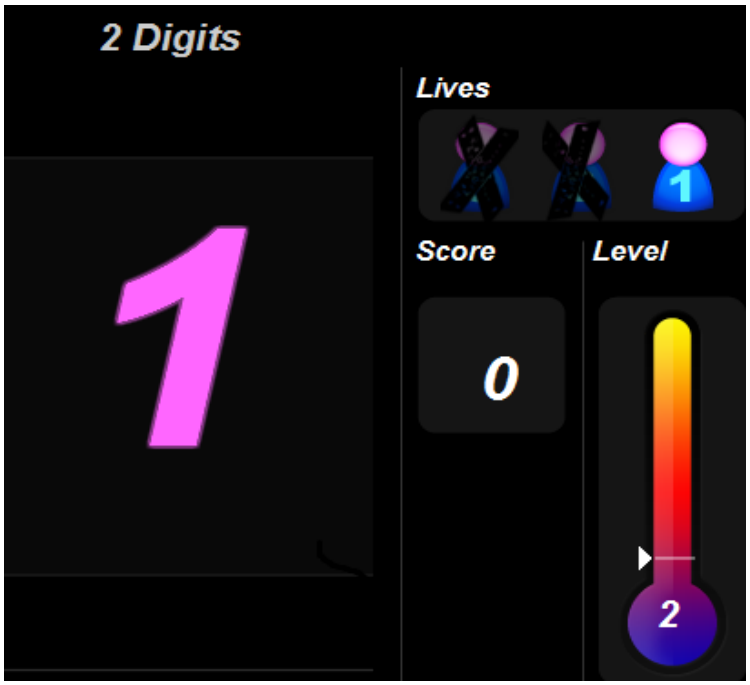


Figure 18 Screenshot of the digit span test

3.1.6.1.5.2.2 Spatial span

Spatial span task was employed for measuring short-term spatial memory [321]. Sixteen squares were displayed on the screen. A sub-set of the squares flashed in a random order and the participants were then asked to click on the squares in the same sequence in which they flashed (Figure 19). The test started with four flashes and with a correct response, the length of the following sequence increased by one flash. An incorrect response resulted in the length of the subsequent sequence being one flash shorter. The test finished after 3 incorrect responses.

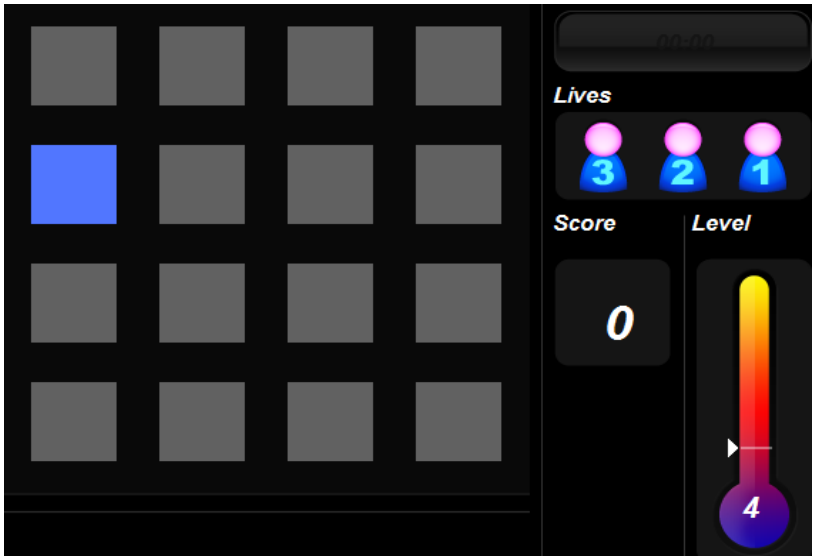


Figure 19 Screenshot of the spatial span task

3.1.6.1.5.2.3 Paired associates

This task was used to assess associative memory performance [322]. In each trial, boxes were displayed on the screen at random positions. The boxes opened one after another to reveal an object which then disappeared (Figure 20). The revealed objects were then presented in the centre of the screen in a random sequence and the participants were asked to click on the respective boxes that contained them. If the participant correctly identified all object-box pairs in the trial, the next trial increased by 1 object-box pair and vice versa. The first trial started with two boxes. The test ended after 3 errors.

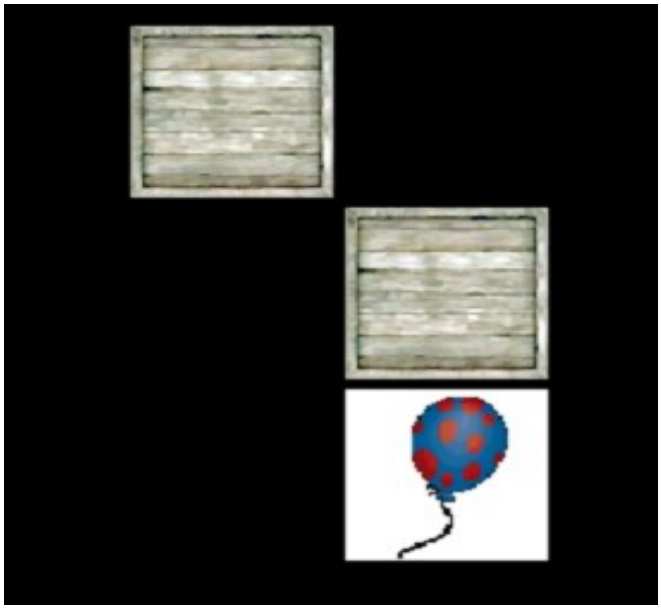


Figure 20 Screenshot of the paired associates test

3.1.6.1.5.2.4 Self-Ordered search

This test was employed to assess strategy making ability [323]. Sets of boxes were presented at random spots on the screen. The participants were required to search for hidden tokens in all the boxes by clicking on them one at a time (Figure 21). A token would not appear within the same box twice in a given trial. The trial ended if the participant clicked on an empty box twice or on a box in which a token had already been found and the subsequent trial had one box less to search. If the tokens were identified once in each box without any errors, the subsequent trial had one additional box to search. The first administered trial had four boxes. The test ended after 3 errors.

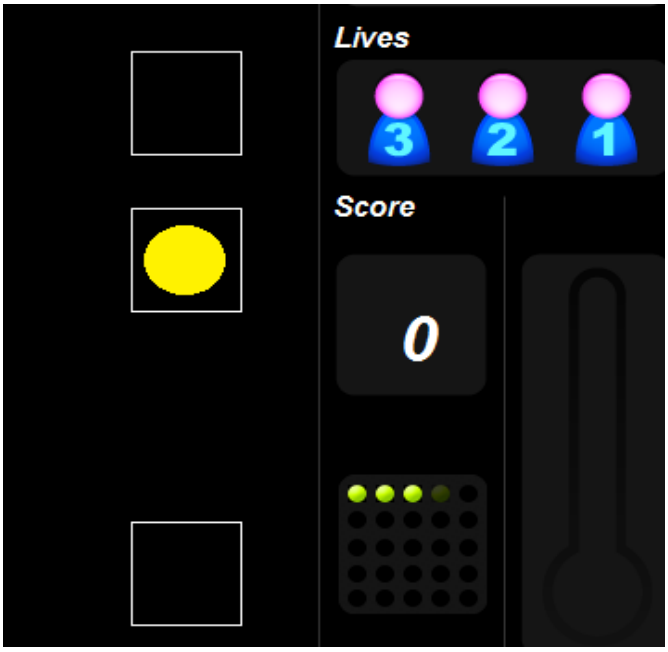


Figure 21 Screenshot of self-ordered search task

3.1.6.1.5.2.5 Spatial rotation

This task was used to measure visuospatial capacity (the ability to rotate an object in mind) [324]. In each trial of the task, two grids of coloured squares were presented at the same time with one of the grids rotated by either 90º, 180º or 360º. The grids when aligned after rotating in mind were either identical or different. The participants were required to indicate whether the grids were identical or not by clicking ‘match’ or ‘mismatch’ on the screen (Figure 22). The trials were presented one after the other for 90 seconds and the participant was asked to solve as many problems within that time. The first trial contained 4 coloured squares in each grid. Every correct response led to an increase in the total score by the number of squares in the grid and the next trial had more squares. Every incorrect response led to a decrease in the total score by the number of squares in the grid and the following trial had fewer squares.

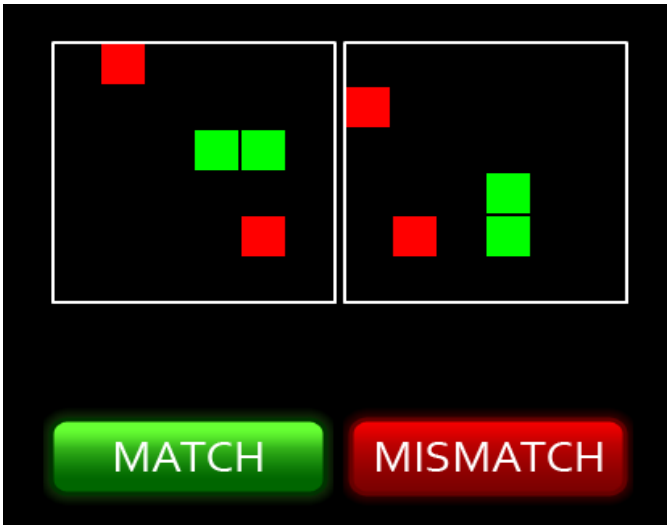


Figure 22 Screenshot of the spatial rotation task

3.1.6.1.5.2.6 Feature Match

This task was used to estimate attentional processing ability [325]. In each trial, two grids were shown on the screen each having a set of abstract shapes. The participants were asked to indicate whether contents in the two grids were matching or not (Figure 23). For a correct response, the total score increased by the number of shapes in the grid and next trial had more shapes. For an incorrect response, the total score decreased by the number of shapes in the grid and subsequent trial had fewer shapes. The first trial had two abstract shapes in each grid. The trials were presented for 90 seconds.

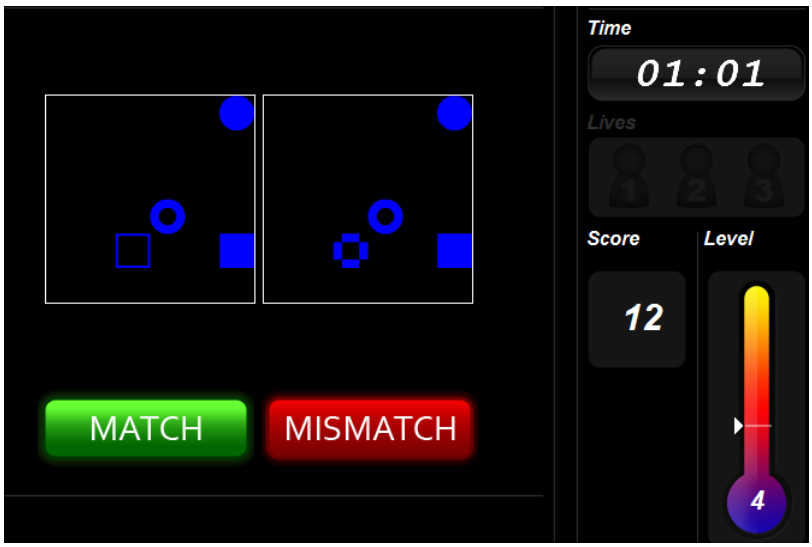


Figure 23 Screenshot of the feature match test

3.1.6.1.5.2.7 Polygons

Polygons test was used to assess visuospatial and constructional ability [305]. In each trial, a pair of interlocking polygons was presented on one side of the screen and a single polygon was displayed on the other side. The participants were required to point out if the single polygon exactly matched one of the overlapping polygons or not by clicking 'match' or 'mismatch' on the screen (Figure 24). The trials were presented for 90 seconds and the participants could attempt as many trials as possible within that time.

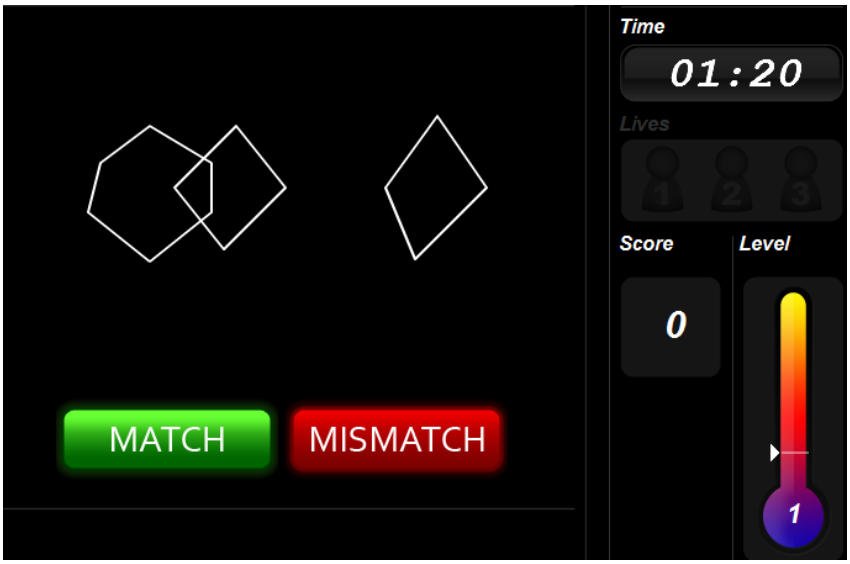


Figure 24 Screenshot of the polygons task

3.1.6.1.5.2.8 Monkey span

This task was used to assess visuospatial working memory [326]. Sets of numbered squares were shown simultaneously at random locations on the screen (Figure 25). The numbers were then removed from the squares and the participants were asked to click on the squares starting from number 1 onward in an ascending sequence. The first trial started with a set 2 numbered squares which then increased or decreased by 1 for a correct or incorrect response respectively. The test finished after 3 incorrect responses.

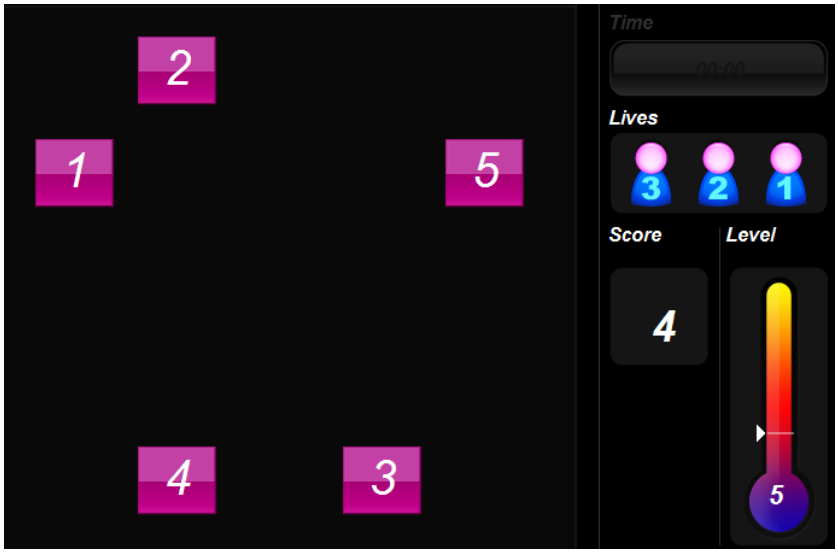


Figure 25 Screenshot of the monkey span test

3.1.6.1.5.2.9 Double trouble

Double trouble test, a colour-word mapping task, is devised from the colour-word test [327]. In each trial, one of the two words RED or BLUE was shown at the top of the screen in either red or blue font colour. The words RED and BLUE were also displayed at the bottom of the screen, again having either red or blue font colour (Figure 26). The participants were required to click on the word at the bottom of the screen that described the font colour of the word at the top of the screen. The participants were instructed to solve as many trials as possible within 90 seconds. The total score increased or decreased by 1 after each trial depending on the participant's answer.

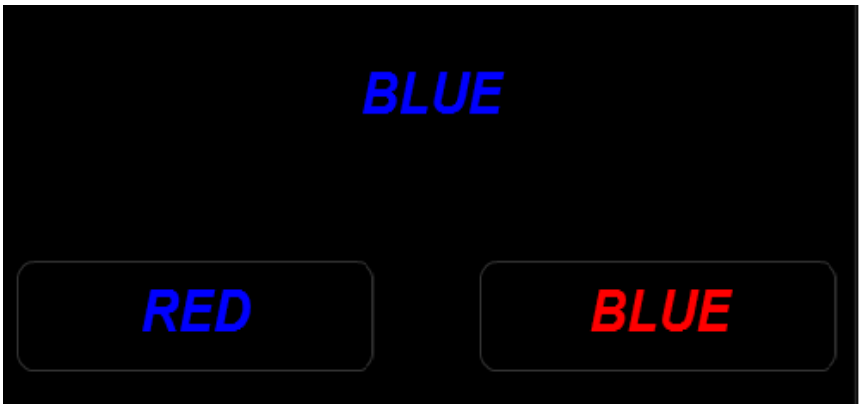


Figure 26 Screenshot of the double trouble task

3.1.6.1.5.2.10 Verbal Reasoning

This test was employed to assess verbal reasoning ability [328]. In each trial, two objects (e.g. circle and square) were displayed on the screen with a statement (e.g. square is bigger than circle, square is not encapsulated by circle etc.). The participants were asked to tell whether or not the statement accurately described the relation between the objects shown by clicking 'true' or 'false' on the screen (Figure 27). The participants were instructed to attempt as many trials as possible within 90 seconds. Total score increased by 1 for each correct response and decreased by 1 for each incorrect answer.

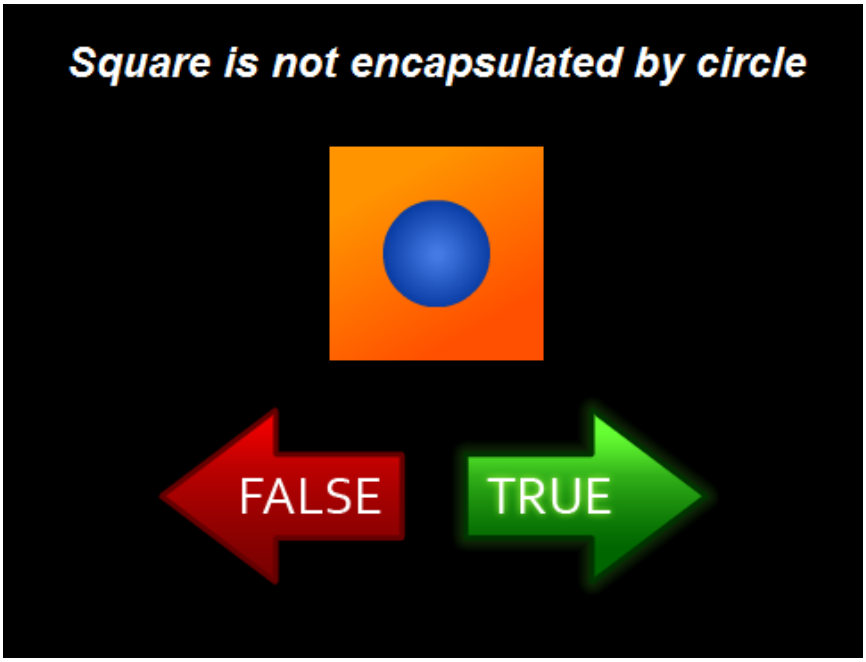


Figure 27 Screenshot of the verbal reasoning test

3.1.6.1.5.2.11 Odd one out

This task was employed to assess deductive reasoning ability [12]. Nine cells were shown on the screen, each containing patterns with features (shape, colour and number of copies) corresponding to each other according to some rules (Figure 28). The participants were asked to deduce those rules in order to identify the one cell whose contents did not relate to those rules. Total score increased by 1 and the following trial became more complex after

a correct response and vice versa. The trials were presented one after the other for three minutes.

Figure 28 Screenshot of the odd one out task

3.1.6.1.5.2.12 Hampshire tree test

This spatial planning task was used to measure executive function [329]. Numbered balls were positioned on a tree-like frame in each trial (Figure 29). The participants were required to reposition the balls, in as few moves as possible, in an ascending numerical order running from top to bottom and left to right of the frame. The trial was terminated if the participants made more than double the minimum number of moves required to correctly re-arrange the balls in that trial. The participants were given three minutes to complete as many trials as possible. Score for each trial was calculated by subtracting the number of moves taken by the participant from the minimum number of moves required x2. Sum of the scores in each trial gave the total test score.

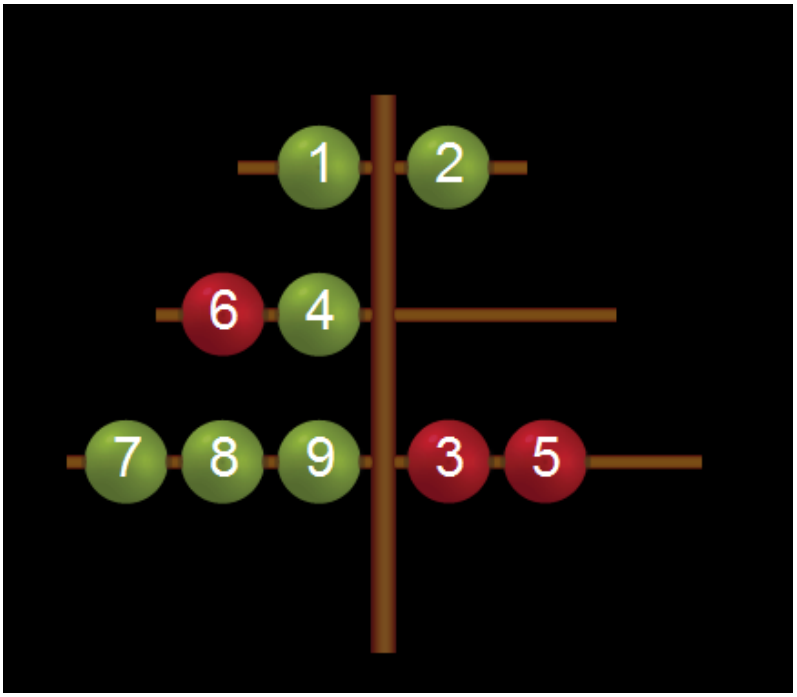
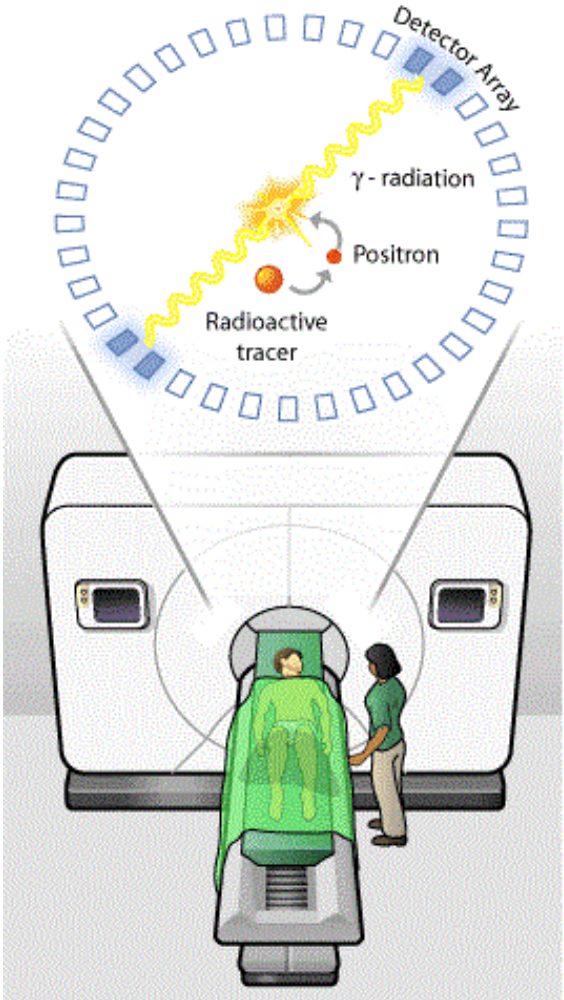


Figure 29 Screenshot of the Hampshire tree task

3.1.6.2 Stage 2

Stage 2 involved PET brain imaging which was undertaken at the Imanova Centre for Imaging Studies, London, UK. Figure 30 describes the basic principle of PET imaging. A structural MRI scan was also performed on the same day for neuroanatomical interpretation of the PET scan. Checks were made before MRI scanning to ensure that subject had no MRI-incompatible metallic object on or in his body. High resolution T1-weighted structural MRI images were acquired on a 3T Siemens Magnetom Verio Syngo MR B17 scanner (Siemens AG Healthcare, Germany) using 3D magnetisation-prepared rapid gradient-echo (MP-RAGE) sequence [330] (orientation = sagittal, repetition time (TR) = 2300ms, echo time (TE) = 2.98ms, flip angle = 9°, voxel size = 1mm x 1mm x 1mm).



The target receptor (e.g. TSPO) binds its ligand radio-tracer (e.g. [C11]PBR28). Decay of the radio-tracer results in emission of positrons (anti-electron particles) which annihilate with electrons present in the surrounding tissue, producing two gamma rays per annihilation event which move in opposite directions. The PET scanner detects the rays resulting from these interactions (co-incidence events) and images are produced by computer processing.

Figure 30 Basic principle of PET imaging

Adapted from http://www.scq.ubc.ca/looking-inside-the-human-body-using-positrons/

3.1.6.2.1 PET imaging and analysis

An intravenous cannula was inserted in the subject's arm before the PET scanning session for administering the radio-tracer [11C]PBR28. The PET imaging was performed on Siemens Biograph™ TruePoint™ (Siemens Healthcare GmbH, Germany) PET/CT scanner (Figure 31). Prior to the PET scan, an x-ray topogram was performed to localise the brain. This was followed by a low dose CT scan localised to brain for attenuation and scatter corrections. Dynamic PET imaging was performed for 90 minutes after intravenous bolus administration of [11C]PBR28 over 20 seconds with a target dose of 400MBq. PET images were reconstructed using the filtered back projection (FBP) and ordered subset expectation maximisation (OSEM) algorithms and data archived.

Figure 31 PET scanner at Imanova Centre for Imaging Sciences

Data analyses were performed by Dr. Christopher Coello at Imanova Centre for Imaging Studies using MIAKAT™ software (Imanova, London, UK). A modified version of the Harvard-Oxford cortical and sub-cortical structural atlas was non-linearly warped to the T1-weighted structural image to outline cortical anatomy [331, 332]. Reconstructed dynamic PET

images were realigned for motion correction and registered to the T1-weighted structural MRI images. Regions of interest (ROI) were defined (frontal, temporal, parietal and occipital cortices) using the co-registered atlas. Based on the mean voxel radioactivity in each ROI and each frame, time activity curves (TAC) were derived for the full duration of the scan. Time-averaged images of scan data were generated for a 30-min interval between 60-90 minutes. Whole brain, cerebellar and regional cortical standardised uptake values (SUV) were calculated after multiplying measured activity with the subject's body weight and dividing by injected activity. Global and regional standardised uptake value ratios (SUVR) were calculated by dividing the SUV of whole brain and individual cortices by cerebellar SUV [333].

3.1.6.3 Statistical analysis

Demographic and neuropsychological testing data were analysed using SPSS version 22.0 (SPSS Inc, Chicago, IL, USA). Mean $\pm$ SEM was calculated for normally distributed quantitative variables. Independent sample t-test was applied to observe group mean differences. A p-value of <0.05 was considered statistically significant.

3.2 Bone study

3.2.1 Bone volume fraction experiment

3.2.1.1 Study design

A cross-sectional analytical study design was employed to develop a measure for quantifying trabecular bone mass from femoral head CT scans with large voxels (up to 550μm) and determine whether the devised metric related to bone strength.

3.2.1.2 Regulatory approvals

Human femoral head samples used in this research project were obtained from the Imperial College Healthcare Tissue Bank (ICHTB). ICHTB is supported by the National Institute for Health Research (NIHR) Biomedical Research Centre (BRC) based at ICHT and Imperial College London. ICHTB is approved by the National Research Ethics Service (NRES) to release human material for research (12/WA/0196), and the samples for this project (Ref 13004) were issued from sub-collection MEDA.

3.2.1.3 Study sample

Femoral heads (n=20) were collected post-operatively from patients giving informed consent undergoing hip arthroplasty for osteoarthritis or osteoporosis at Department of Orthopaedic Surgery, ICHT.

3.2.1.4 Image acquisition

Femoral heads were micro-CT scanned by Dr. Richard Abel at the Natural History Museum, London using a Nikon (Metris X-Tek) HMX-ST 225 CT system (Nikon Metrology, UK) (Figure 32) [334]. The CT scanner has a cone beam projection system with a four megapixel tungsten detector panel (Perkin Elmer XRD 1621 AN3 HS). Specimens were

mounted on a styrofoam base and placed onto a turntable inside the scanner. Specimens were scanned at fixed parameters of 180kV and 165μA with a 0.1mm copper filter. A total of 6315 projections were collected at an angular interval of 0.057^{O}. The resulting scan for each femoral head had 2000 serial cross-sectional views with a cubic voxel size of 30μm. Scans were reconstructed using CT Pro 2.2 (Metris X-Tek, UK) and saved in the DICOM (Digital Imaging and Communications in Medicine) file format using VG Studio Max 2.0 (Volume Graphics, Heidelberg, Germany).

Figure 32 CT scanner at Natural History Museum, London

Adapted from http://www.nhm.ac.uk/research-curation/science-facilities/analytical-imaging/imaging/computed-tomography/micro-ct/instrument/index.html

3.2.1.5 Imaging data analysis

Data were collected using external hard drives (NTFS format) and analyses were performed on a high power workstation (HP Z800) at the Musculoskeletal Lab, Imperial College London using the BoneJ (version 1.3.1) plugin for bone image analysis in ImageJ (version 1.48d) software (Java image processing program) [335]. The micro-CT DICOM data files were imported into ImageJ and serially downgraded in resolution from 30μm to 50, 100, 200, 400 and 550μm voxel size (Figure 33).

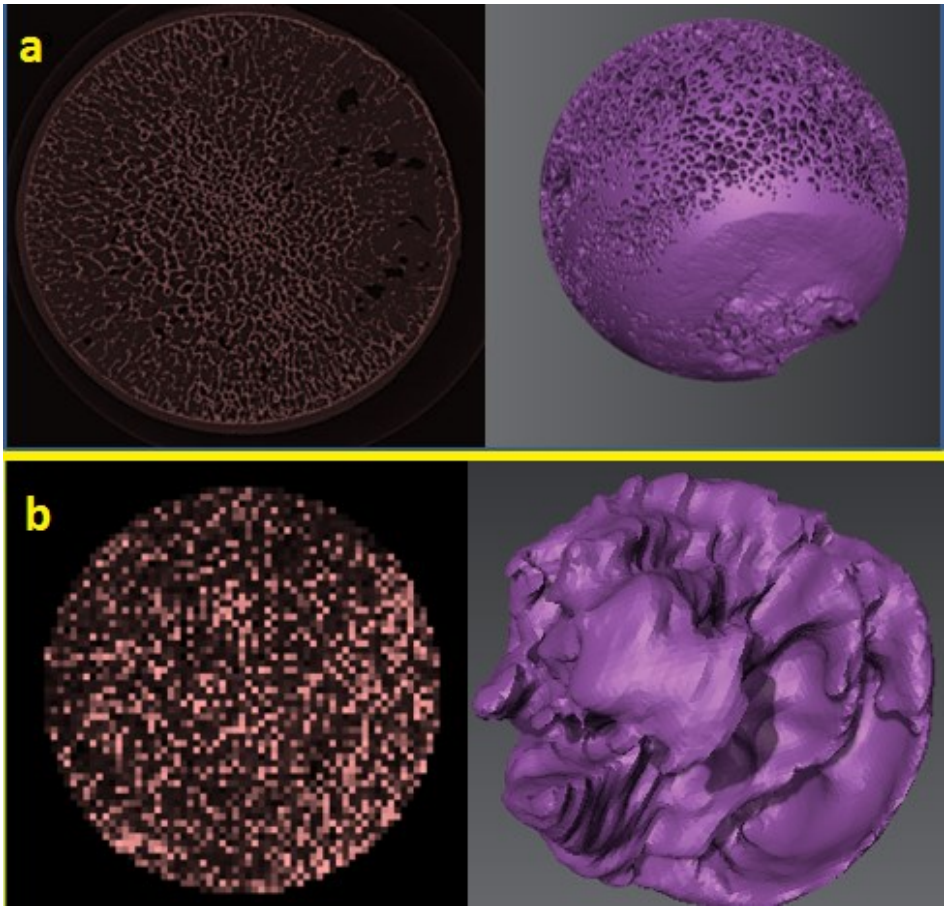
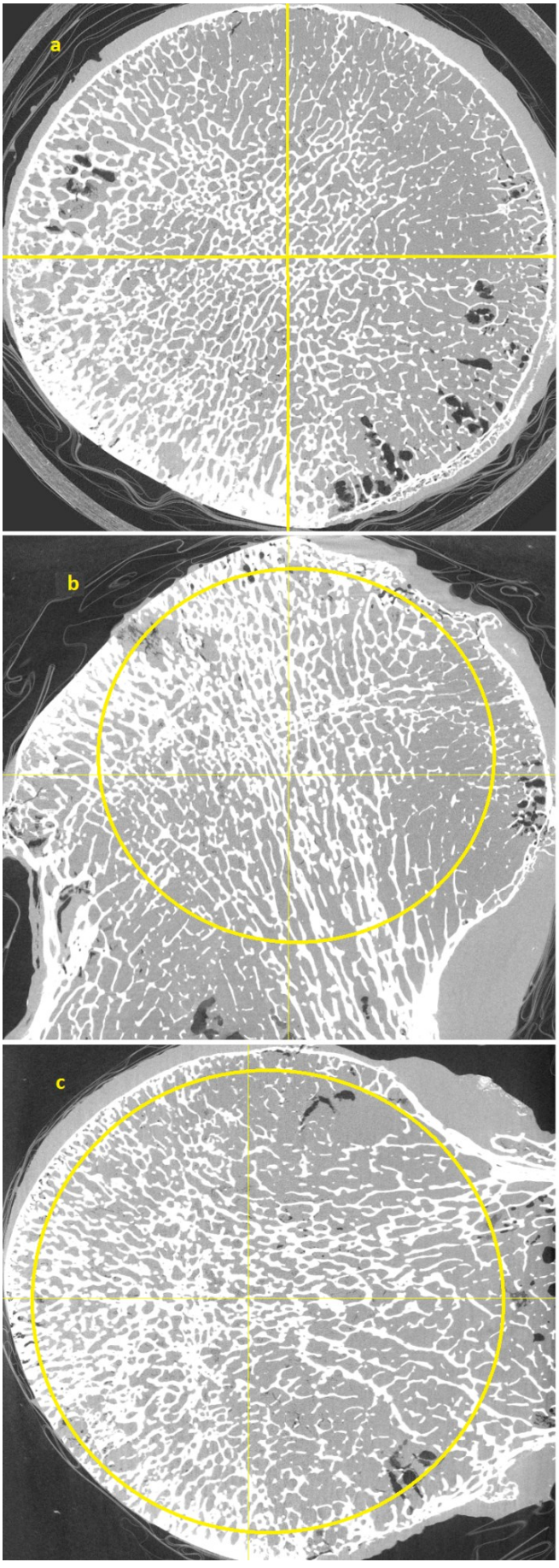


Figure 33 Downgradation of micro-CT data from high to low resolution
a. A slice of high resolution (30μm) micro-CT scan and 3D model of femoral head built from micro-CT scan b. A slice of low resolution (550μm) scan downgraded from 30μm 3D model of femoral head built from downgraded scan

Spheres of trabecular bone were virtually sectioned from all the high and low-resolution 3D scans of the femoral heads. This was done to ensure that only trabecular bone was analysed and cortical region was excluded. The largest sphere possible was collected about the centre of the femoral head. To do so, orthogonal views of the images were opened. The biggest slice of femoral head on the Z-axis view was located (Figure 34) and then corresponding slices on the X- and Y-axes were identified. Largest fitting circles that excluded cortical bone from the femoral head were then drawn on to the slices identified on the X- and Y-axes (Figure 34). ROI markers were placed one each on the bottom, top and lateral ends of the circles and entered into the ROI manager. As defined by the 8 ROI markers, a sphere was sectioned for each scan using the sphere fitting algorithm in BoneJ (Figure 4).



a) The largest slice of femoral head in the Z-axis (normal view seen in ImageJ), crosshair depict the corresponding slices seen in the X- and Y-axes. b) Corresponding slice of femoral head in the Y-axis with largest fitting circle drawn in trabecular bone without capturing cortical bone. c) Corresponding slice of femoral head in the X-axis with largest fitting circle drawn excluding cortical bone.

Figure 34 Orthogonal views used for sectioning a sphere

Bone J plugin required binary images for trabecular analyses. For this purpose, frequency distribution plots of the voxel grey values were plotted using the 'histogram' function in ImageJ. Threshold grey values were calculated by finding the trough that separated bone and non-bone peaks [336, 337]. Based on the threshold values, black and white binary images were generated using the 'threshold' function in ImageJ (Figure 35). Trabecular BVF was measured by counting voxels representing bone using the BVF function in BoneJ. Data were entered into Excel spreadsheet (Microsoft Excel 2013). The accuracy of lower-resolution metrics was validated against the high-resolution data by plotting scatter graphs and calculating correlation coefficients (R^2).

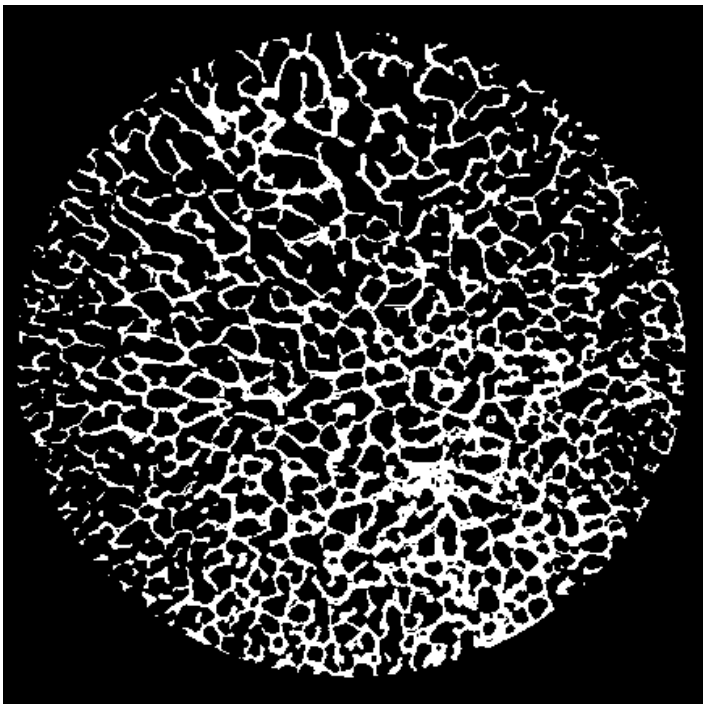


Figure 35 Binary image of a slice of sphere from a femoral head micro-CT scan
Trabecular microstructure clearly visible at high resolution

3.2.1.6 Bone strength testing

Mechanical testing of the femoral heads was employed to assess bone strength. Eight (out of the 20) femoral heads from patients who underwent hip replacement for osteoarthritis were mechanically tested. Testing was performed by Dr. Andi Jin at the Department of

Mechanical Engineering, Imperial College London. Femoral heads were sectioned and five cylindrical sub-samples (10mm in height and 7mm in diameter) were drilled out from the central trabecular part of each femoral head (Figure 36).

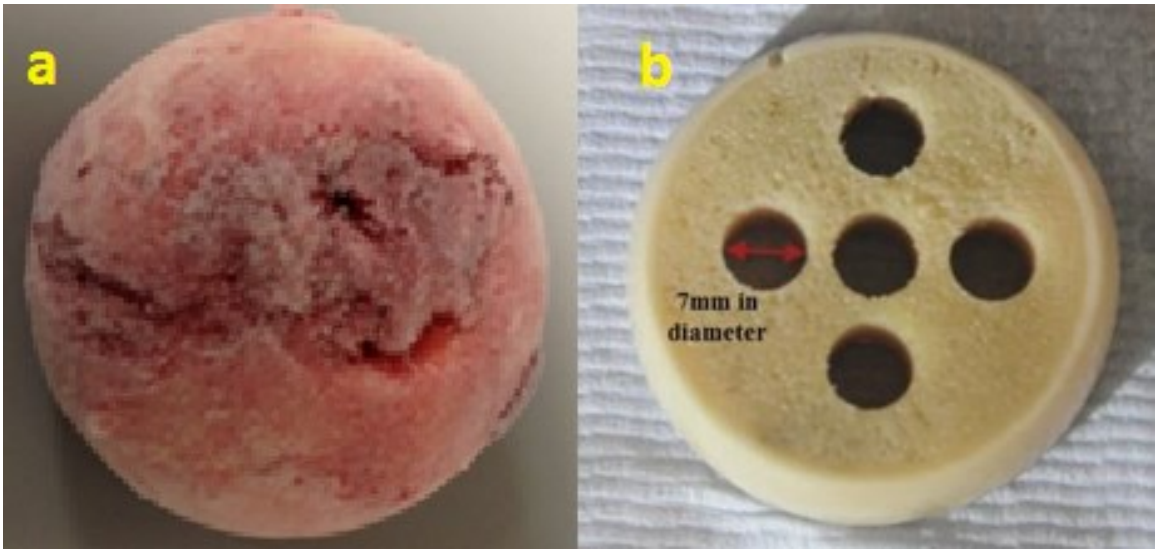


Figure 36 Sectioning and drilling of femoral heads in preparation for mechanical testing
a. Whole femoral head b.) Five cylindrical sub-samples (7mm diameter, 10mm height) drilled out from the femoral head

Two sub-samples from the five were selected randomly and mechanically tested until fracture under uniaxial compressive loading (Figure 37) using an Instron 5565 mechanical test machine (Instron Engineering Corporation, USA). The maximum load (the peak of the graph shown in Figure 37, which corresponded with fracture) divided by specimen cross-sectional area, was used to calculate the bone strength of each cylindrical specimen [338]. Mean strength of the two specimens for each femoral head was computed and the relationships between BVF values and measures of ex-vivo bone strength were determined by plotting scatter graphs and calculating R^2.

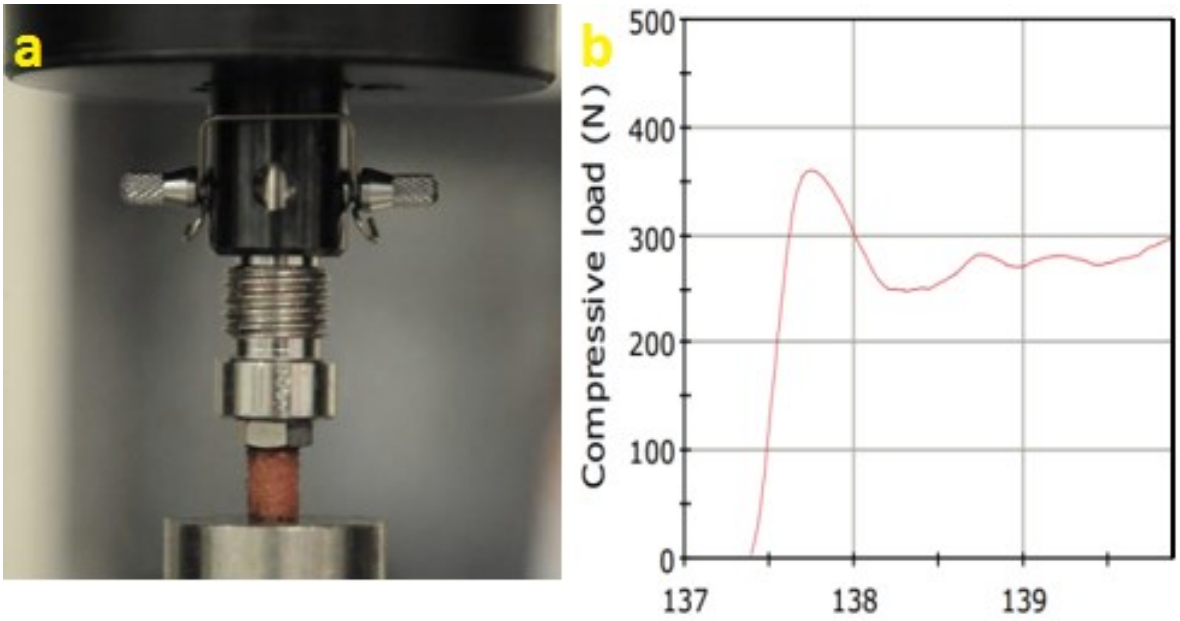


Figure 37 Mechanical testing to assess bone strength of femoral head samples

a. Cylindrical sub-samples of femoral heads were uniaxially compressed to fracture b. Mechanical behaviour of the tested specimen as seen on load-displacement graph. Peak of the graph represents the maximum load sustained

3.2.2 Bone alkaline phosphatase experiment

3.2.2.1 Study design

A longitudinal study design was employed for this study to determine changes in serum BALP in patients with non-metastatic PC following initiation of ADT with LHRHa and to relate those changes to serum testosterone and oestradiol levels.

3.2.2.2 Regulatory approvals

Blood samples were collected as ICHTB sub-collection Sur_PA_12_044 and were later issued for BALP analysis following ICHTB approval (Ref R14110) (Appendix II).

3.2.2.3 Study population

Patients with PC (n=9) between the ages 50 to 75 years who were to begin ADT with LHRHa for either newly diagnosed advanced non-metastatic disease or as adjuvant or neoadjuvant to RT for localised disease provided blood samples for the study. Written informed consent was obtained and blood samples were taken at baseline before starting treatment and three months afterward. Comparisons were made with blood samples taken at similar time points from age-matched controls without PC (n=9). Blood samples were centrifuged at 3000 rpm for 10 minutes and separated serum was stored at -80^{o}C until assayed.

3.2.2.4 Serum BALP and sex hormone assays

Serum BALP assays were performed by Dr. Alan Courtney at the Imperial Bone Marker Service, Charing Cross Hospital. Serum BALP was measured using the Metra® BALP enzyme immunoassay (EIA) kit (Quidel® Corporation, San Diego, California, USA) [339]. The assay utilised monoclonal anti-BALP antibodies which were coated on a micrititer strip to capture BALP in the serum samples. The enzyme activity of the captured BALP was detected with para-nitrophenylphosphate (pNPP) which is a chromogenic substrate of BALP. The enzyme

BALP catalysed the hydrolysis of pNPP to yellow coloured para-nitrophenol which was measured using a visible light spectrophotometer at a wavelength of 405 nm to provide a quantitative measure of serum BALP activity [339].

Serum samples were analysed at Imperial Clinical Chemistry Laboratory, Charing Cross Hospital for quantitative determination of testosterone and oestradiol using commercially available kits. Serum testosterone was measured by the single-step ARCHITECT® testosterone 56-4969/R1 B7K730 assay (Abbott Laboratories, Abbott Park, Illinois, USA) using Chemiluminescent Microparticle Immunoassay (CMIA) technology with Chemiflex™ protocol (Abbott Laboratories, Abbott Park, Illinois, USA). Serum sample, paramagnetic microparticles coated with murine monoclonal anti-testosterone antibody, acridinium-labeled testosterone conjugate and assay diluent were combined to create the reaction mixture. Testosterone present in the sample competed with the acridinium-labeled testosterone conjugate for binding with anti-testosterone coated microparticles to form an antigen-antibody complex. After washing, pre-trigger and trigger solutions were then added to the reaction mixture and the resulting chemiluminescent reaction was measured as relative light units (RLU) detected by the ARCHITECT® i optical system (Abbott Laboratories, Abbott Park, Illinois, USA). The amount of total testosterone in the assayed serum sample was determined from a calibration curve established with RLU calibrators of known testosterone concentrations. The lowest detection limit of the testosterone assay was 0.45 nmol/L [340].

Serum oestradiol was measured using the two-step ARCHITECT® oestradiol 48-9132/R3 B7K720 assay (Abbott Laboratories, Abbott Park, Illinois, USA) using CMIA technology with Chemiflex™ protocol (Abbott Laboratories, Abbott Park, Illinois, USA). In the first step, sample, specimen diluent, assay diluent, and paramagnetic microparticles coated with anti-oestradiol antibody (rabbit, monoclonal) were combined to prepare reaction mixture. Oestradiol present in the sample bound to the anti-oestradiol coated microparticles. In the second step, acridinium-labeled oestradiol conjugate was added to the reaction mixture. After washing, pre-trigger and trigger solutions were added and the chemiluminescence generated was measured as relative light units (RLU) detected by the ARCHITECT® i optical system

(Abbott Laboratories, Abbott Park, Illinois, USA). The concentration of total oestradiol in the assayed serum sample was determined from a calibration curve established with RLU calibrators of known oestradiol concentrations. The lowest detection limit of the oestradiol assay was 37 pmol/L [341].

3.2.2.5 Statistical analysis

The data were entered and analysed using SPSS version 22.0 (SPSS Inc, Chicago, Illinois, USA). Age data were presented as range and mean. Mean $\pm$ S.E.M was calculated for normally distributed quantitative variables. One-way ANOVA was applied to observe group mean differences and post-hoc Tukey test was applied to observe which group means differ. Pearson's correlation was applied to observe correlation between BALP and serum sex hormone levels. A p-value of <0.05 was considered statistically significant.

3.3 Odour study

3.3.1 Study design

A longitudinal study design was employed for this study to determine changes in social perception of odour samples of patients with PC receiving ADT with LHRHa. Study subjects provided samples at baseline before starting treatment and three months after initiation of ADT with LHRHa. Comparisons were made with age-matched controls.

3.3.2 Regulatory approvals

Human samples used in this study were collected as ICHTB sub-collection Sur_PA_12_044. Collected samples were stored in a -80^{o}C freezer at the Medical Oncology and Biochemistry Laboratory, Charing Cross Hospital, ICHT. Stored samples were later issued for analysis following ICHTB approval (Ref R14110) (Appendix II). ICHTB is funded under the tissue banking theme of NIHR Imperial BRC, and approved by the NRES to release human material for research (12/WA/0196).

3.3.3 Study population

Study samples were provided by two population groups; (i) patients with PC undergoing ADT with LHRHa (n=10) and (ii) age-matched male patients without PC as controls (n=10). Patients who were to commence ADT with LHRHa for PC treatment were approached at the uro-oncology clinics of ICHT to provide odour and blood samples for this study. Age matched male patients without PC who presented to the urology clinics with other urological complaints were approached to enrol as controls. Written informed consent was obtained from each participant as outlined by the ICHTB. The purpose of the study was introduced in full and then consent taken. All study documents were kept at a secure ICHT location.

3.3.4 Eligibility criteria

Male patients between the ages 50 to 75 years who were to begin ADT with LHRHa for either newly diagnosed advanced PC or as adjuvant or neoadjuvant to RT for localised disease were invited to co-operate with the study.

3.3.5 Odour sample collection

Axillary odour samples were collected on white T-shirts worn by the study participants. Experimental materials, including a white cotton T-shirt, a non-perfumed soap bar and a zip-lock plastic bag, were provided to the participants. The participants were required to wear each T-shirt overnight for two consecutive nights [71]. They were instructed to follow a strict hygiene regime commencing 24 hours before sampling. The instructions included abstinence from sexual activity, not sharing a bed, avoiding deodorants and other perfumed products, using the provided non-perfumed soap (Simple pure soap bar, UK), wearing only the provided cotton T-shirts (prewashed in non-perfumed detergent) while sleeping at night, avoidance of tobacco smoke, alcohol and strong smelling foods (e.g. chilli, garlic, pepperoni, curry, blue cheese, asparagus, yogurt and fried onion) [342]. Subjects were requested to shower just before going to bed on each of the two consecutive nights, wear the T-shirts in bed and to return them to the provided bags each morning. The T-shirts were collected on the second morning in their sealed plastic bags. The bottom half of each T-shirt, below the rib cage, was cut and discarded. The remaining part of each T-shirt was put in its zip-lock plastic bag which was labelled in an anonymised form with key codes indicating only the group (PC or control) and treatment stage (baseline or three month sample). The plastic bag containing the sample was frozen and stored at -80^{o}C on the same day until studied. Freezing avoids changes in subsequent odour perception [343]. Gloves were worn during the handling of T-shirts to avoid odour contamination.

3.3.6 Odour bioassays

Odour bioassays were performed at Dr. S Craig Robert's laboratory, Department of Psychology, University of Stirling, Scotland. The stored samples were transported overnight and were kept frozen in an insulated box containing dry ice during transport. The samples were immediately put into a freezer upon arrival at the site.

The zip-lock plastic bags containing the T-shirts were removed from the freezer 2 hours before the testing. The samples were left to thaw at room temperature. The bags were shaken thoroughly and inverted several times just before the smelling sessions. A group of 100 young healthy adult heterosexual university students (50 females, 50 males) were recruited to sniff and rate individual odour samples. Only those females who were using hormonal contraception were recruited to rate the odour samples. Hormonal contraceptive users were employed to avoid the potential effect of fluctuations in olfactory function during the natural menstrual cycle. Before recruitment, information about the study was given to the participants and written consent obtained. Participants were asked to rate individual samples for their masculinity, attractiveness and intensity, using a 7-point scale (from 1-very low to 7-very high) (Appendix III). They were also asked to complete a questionnaire containing some basic demographic items (e.g. age, sex). Participants then smelled and rated a series of 8 individual odours each (baseline and 3 month samples from 2 controls and 2 PC patients each). The raters were blinded to the identity of the samples and their responses were kept anonymous. Shirts were discarded on completion of the rating experiment.

3.3.7 Sex hormones assays

Venous blood samples for measurement of testosterone and oestradiol levels were obtained from the subjects in the study group on two occasions: (i) immediately before starting ADT with LHRHa and (ii) three months after initiation of ADT. Subjects in the control group also provided blood samples twice according to the same time schedule as described for subjects in the study group. Blood samples were spun down at 3000 rpm for 10 minutes to

separate serum from the cells and aliquoted serum specimens were stored at -80^{o}C until assayed.

Quantitative determination of serum testosterone and oestradiol was carried out at Imperial Clinical Chemistry Laboratory, Charing Cross Hospital using commercially available kits (detailed description given in section 3.2.2.4). Serum testosterone was measured by the single-step ARCHITECT® testosterone 56-4969/R1 B7K730 assay (Abbott Laboratories, Abbott Park, Illinois, USA) using CMIA technology with Chemiflex™ protocol (Abbott Laboratories, Abbott Park, Illinois, USA) [340]. Serum oestradiol was measured using the two-step ARCHITECT® oestradiol 48-9132/R3 B7K720 assay (Abbott Laboratories, Abbott Park, Illinois, USA) using CMIA technology with Chemiflex™ protocol (Abbott Laboratories, Abbott Park, Illinois, USA) [341].

3.3.8 Statistical Analysis

Data were statistically analysed in an anonymised form. Odour ratings data were entered into Excel spreadsheet (Microsoft Excel 2013). Averages were computed for attractiveness, masculinity and intensity ratings by male raters, female raters and all raters combined. The rating averages were then imported into and analysed using SPSS version 22.0 (SPSS Inc, Chicago, Illinois, USA). Serum sex hormones and demographic data were also entered into and analysed using SPSS version 22.0 (SPSS Inc, Chicago, Illinois, USA). Age data were presented as range and mean. Qualitative data were presented as percentages. Mean $\pm$ S.E.M was calculated for normally distributed quantitative variables. One-way ANOVA was applied to observe group mean differences and post-hoc Tukey test was applied to observe which group means differ. Pearson's correlation was applied to observe correlation between odour ratings and serum sex hormone levels. A p-value of <0.05 was considered statistically significant.

4. Results

4.1 Cognitive study

A total of 10 patients who consented for the study underwent stage 1 procedures (TSPO polymorphism test and neuropsychological testing). Figure 38 summarises recruitment for the study and Table 8 describes characteristics of the study participants.

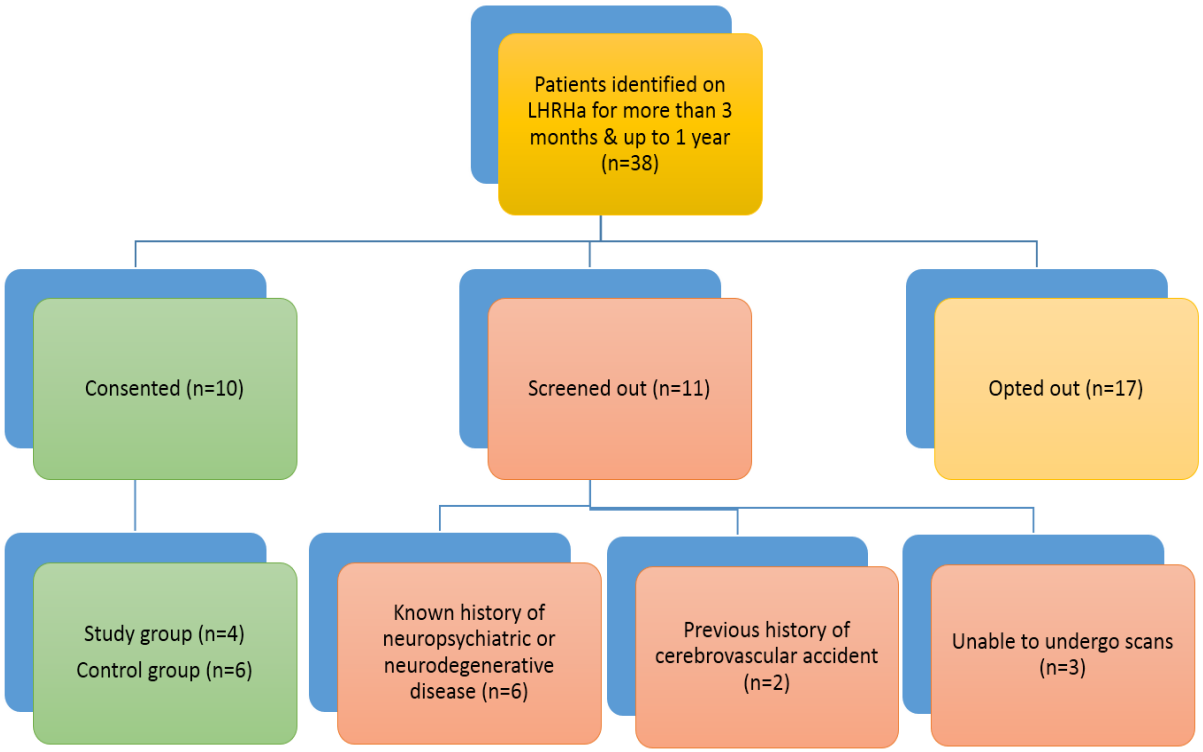


Figure 38 Recruitment summary for cognitive study

Table 8 Descriptive characteristics of study participants (n=10)

Age	Mean = 68 years
	Range= 57-77 years
ADT duration	Mean = 7 months
	Range = 4-11 months
Stage 1 groups	Cognitive impairment (n=4)
	Control (n=6)
TSPO Polymorphism	High binding affinity (n=4)
	Medium binding affinity (n=2)
	Low binding affinity (n=4)
PET Imaging	Cognitive impairment (n=2)
	Control (n=2)

The age range of 'cognitive impairment' (CI) group was 59-77 years with a mean age of 71.7 years. The age range of control group was 57-71 years with a mean age of 64.8 years. The range of ADT duration in CI group was 4-11 months with a mean duration of 7.2 months. The range of ADT duration in control group was 4-11 months with a mean duration of 7.3 months. The range of MMSE score in CI group was 24-29 with a mean of 26.2. The range of MMSE score in control group was 24-30 with a mean of 27.8. The range of WTAR score in CI group was 90-114 with a mean of 105. The range of WTAR score in control group was 97-123 with a mean of 114.There was no significant difference between the two groups in terms of age, ADT duration, MMSE score and WTAR score (Table 9).

Table 9 Group comparison of age, duration of ADT, MMSE and WTAR scores

	CI (n=4) Mean ± SEM	**Control (n=6)** Mean ± SEM	**p-value**
Age (years)	71.75 ± 4.3	64.83 ± 2.24	0.156
ADT duration (months)	7.25 + 1.43	7.33 ± 1.14	0.960
MMSE score	26.25 ± 1.31	27.83 ± 0.87	0.324
WTAR score	105.00 ± 5.44	114.00 ± 3.86	0.202

A general trend of poor performance on all the paper-based neuropsychological tests was observed in the CI group as compared to controls (Table 10). Significant differences were observed between test scores of CI group and control group on 'People Test Immediate Recall' (13.00±0.70 vs. 21.33±1.52; p=0.003), 'People Test Delayed Recall' (1.50±0.64 vs. 6.83±0.47; p=0.000), 'CWIT Word Reading' (26.75±1.49 vs. 22.83±0.70; p=0.029), 'CWIT Inhibition' (114.00±24.75 vs. 67.83±2.56; p=0.048) and 'Letter Fluency' (31.00±3.34 vs. 48.83±2.41; p=0.002) (Table 10). No significant differences were observed between the two groups on any of the computer-based tests (Table 11).

Table 10 Group comparison of paper-based test scores

	CI (n=4) Mean ± SEM	**Control (n=6)** Mean ± SEM	**p-value**
Trail Making A*	46.25 ± 10.07	39.17 ± 3.30	0.453
Trail Making B*	121.75 ± 13.36	106.67 ± 6.55	0.292
People Test *Immediate Recall*	13.00 ± 0.70	21.33 ± 1.52	0.003**
People Test *Delayed Recall*	1.50 ± 0.64	6.83 ± 0.47	0.000**
CWIT Colour Naming*	47.75 ± 6.96	35.33 ± 2.45	0.085
CWIT Word Reading*	26.75 ± 1.49	22.83 ± 0.70	0.029**
CWIT Inhibition*	114.00 ± 24.75	67.83 ± 2.56	0.048**
CWIT Inhibition Switching*	110.00 ± 26.19	75.33 ± 3.66	0.141
Letter Fluency	31.00 ± 3.34	48.83 ± 2.41	0.002**
Logical Memory *Immediate Recall*	31.50 ± 3.42	34.00 ± 2.51	0.564
Logical Memory Delayed Recall	13.00 ± 2.27	17.50 ± 1.38	0.109
Logical Memory *Recognition*	22.25 ± 1.88	23.50 ±0.61	0.478
Similarities	27.00 ± 8.40	35.67 ± 5.53	0.082
Matrix Reasoning	13.00 ± 6.37	19.33 ± 5.61	0.135
Digit Span	12.75 ± 2.98	15.67 ± 2.65	0.143

**Higher score implies lower performance on test*

*** Difference significant at p<0.05*

Table 11 Group comparison of computer-based test scores

	CI (n=4) Mean ± SEM	**Control (n=6)** Mean ± SEM	**p-value**
Digit Span	5.50 ± 0.28	5.50 ± 0.42	1.000
Spatial Span	3.75 ± 0.75	3.83 ± 0.47	0.924
Paired Associates	3.00 ± 0.40	3.16 ± 0.30	0.748
Self-Ordered Search	6.00 ± 0.91	6.00 ± 0.25	1.000
Spatial Rotation	49.75 ± 7.60	38.00 ± 7.00	0.301
Feature Match	60.75 ± 7.01	69.16 ± 13.38	0.646
Polygons	19.50 ± 7.13	18.50 ± 5.44	0.913
Monkey Span	5.50 ± 0.64	6.33 ± 0.42	0.289
Double Trouble	7.75 ± 2.13	9.00 ± 2.30	0.718
Verbal Reasoning	8.50 ± 2.32	7.16 ± 1.70	0.648
Odd One Out	12.25 ± 0.62	12.00 ± 0.81	0.831
Hampshire Tree Task	5.25 ± 0.75	7.66 ± 1.80	0.330

**Difference significant at p<0.05*

4 of the 10 patients who underwent stage 1 procedures were found to have a high affinity binding TSPO polymorphism (n=2 each in the control and CI groups). These patients continued to stage 2 and underwent TSPO PET imaging. TACs for the full duration of scan (0-90 minutes) were plotted for global and regional brain SUVs and SUVRs (Figures 39,40). Time-averaged images of PET scan SUVR data generated for a 30-min interval between 60-90 minutes showed a diffuse TSPO uptake pattern in the brain (Figure 41). The CI subjects showed higher time-averaged whole brain SUVR (mean SUVR between scan times of 60-90 minutes) as compared to controls (CI 0.985 vs. Controls 0.92). The frontal, parietal, temporal and occipital SUVRs were also higher in the CI subjects as compared to control subjects (Table 12).

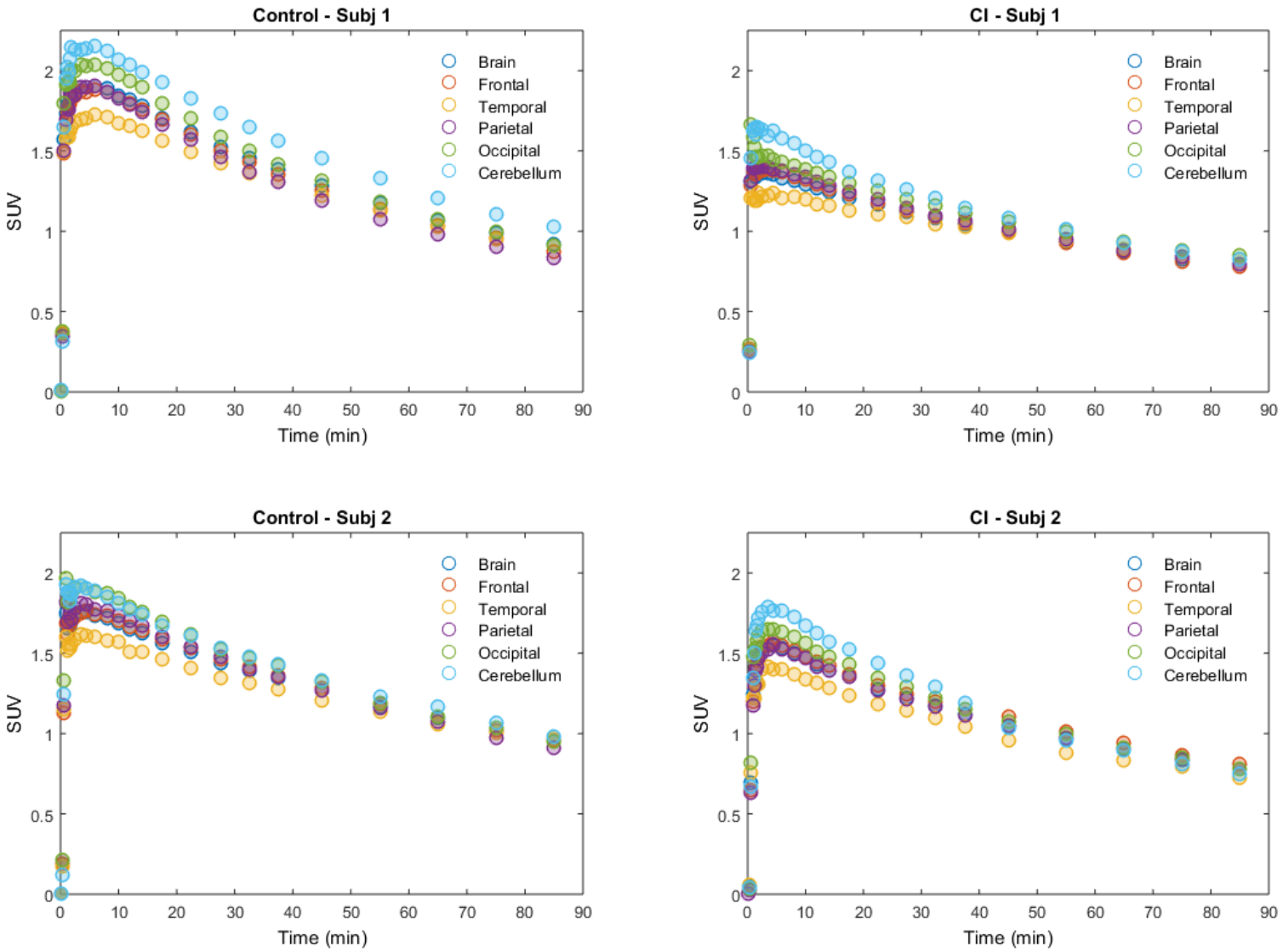


Figure 39 Time activity curves of SUV in the PET scanned study subjects

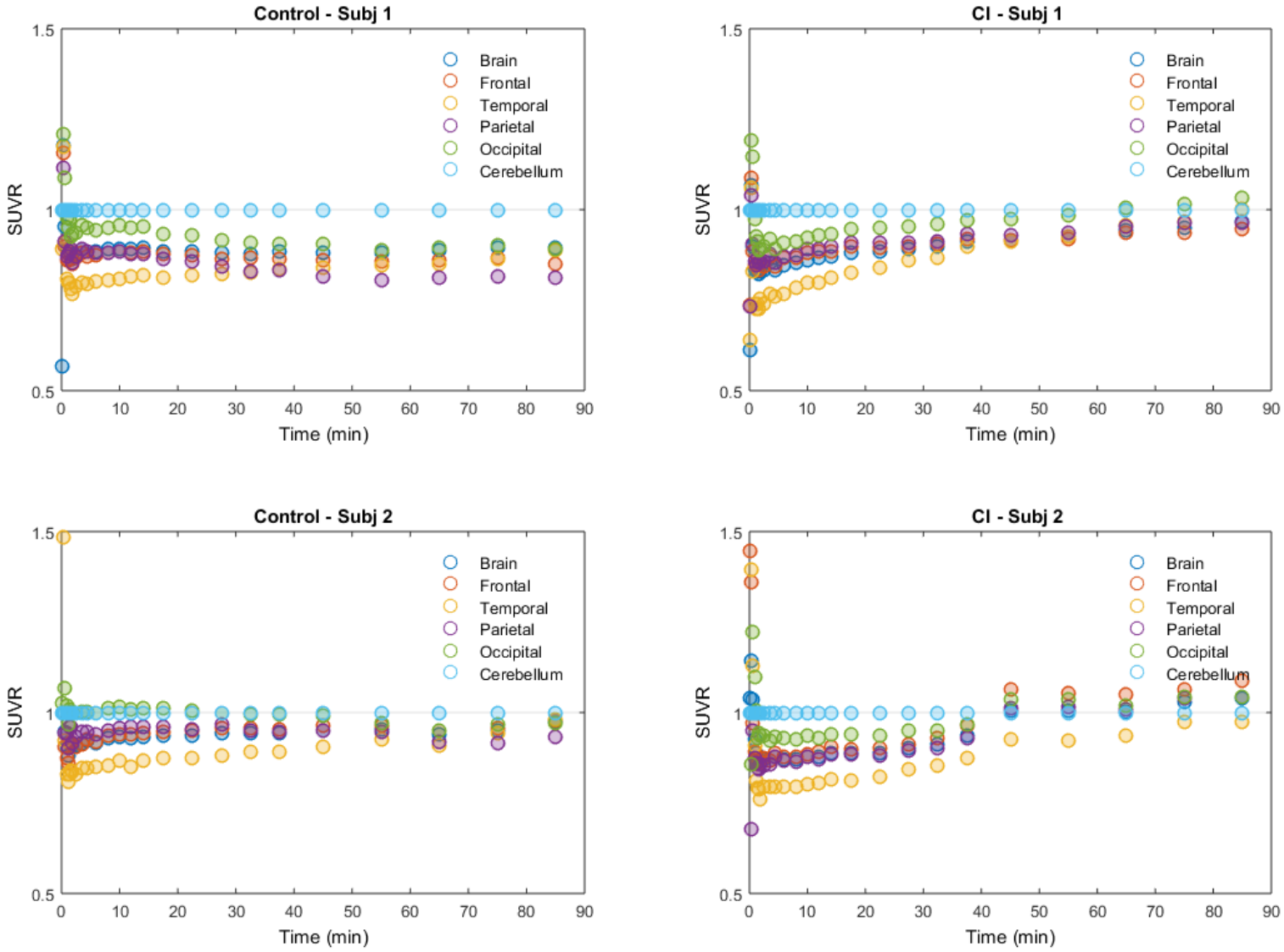


Figure 40 Time activity curves of SUVR in the PET scanned study subjects

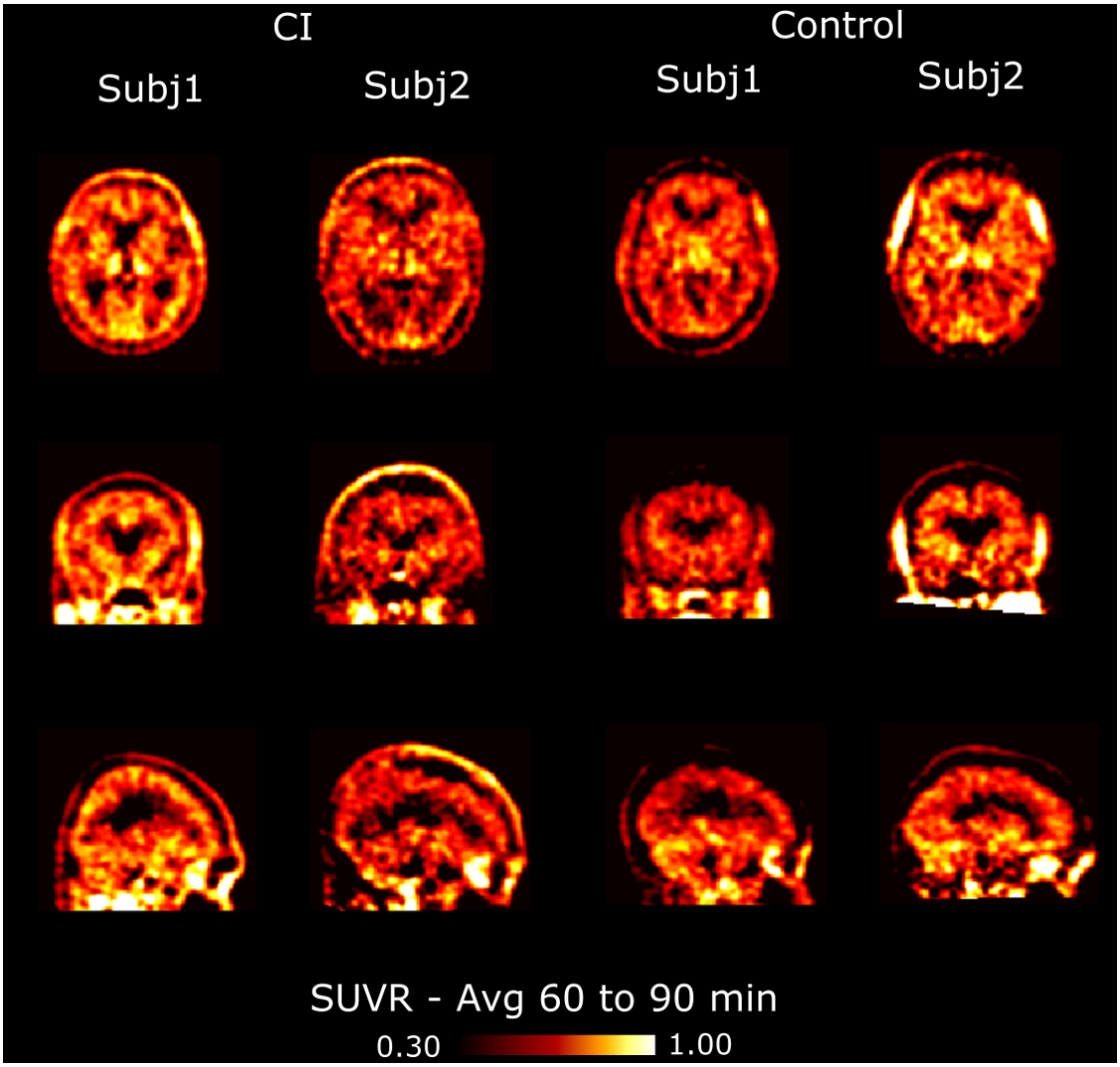


Figure 41 Time averaged PET images showing brain TSPO uptake pattern

Table 12 Time averaged global and regional brain SUVR (60-90 minutes)

	Control (n=2)	**CI (n=2)**
Brain	0.92	0.985
Frontal Lobe	0.91	1.005
Temporal Lobe	0.905	0.97
Parietal lobe	0.865	0.995
Occipital lobe	0.93	1.03
Cerebellum	1	1

4.2 Bone study

4.2.1 Bone volume fraction experiment

BVF data collected at high resolution (30μm voxel size) were highly correlated (R^2 > 0.9) with BVF data collected at serially downgraded lower resolutions (50, 100, 200, 400 and 550μm voxel sizes) (Figures 42-46). A high correlation (R^2 = 0.9032) was seen between BVF measures calculated from high resolution (30μm voxel size) micro-CT images and BVF values measured at the lowest downgraded resolution (550μm voxel size) (Figure 46).

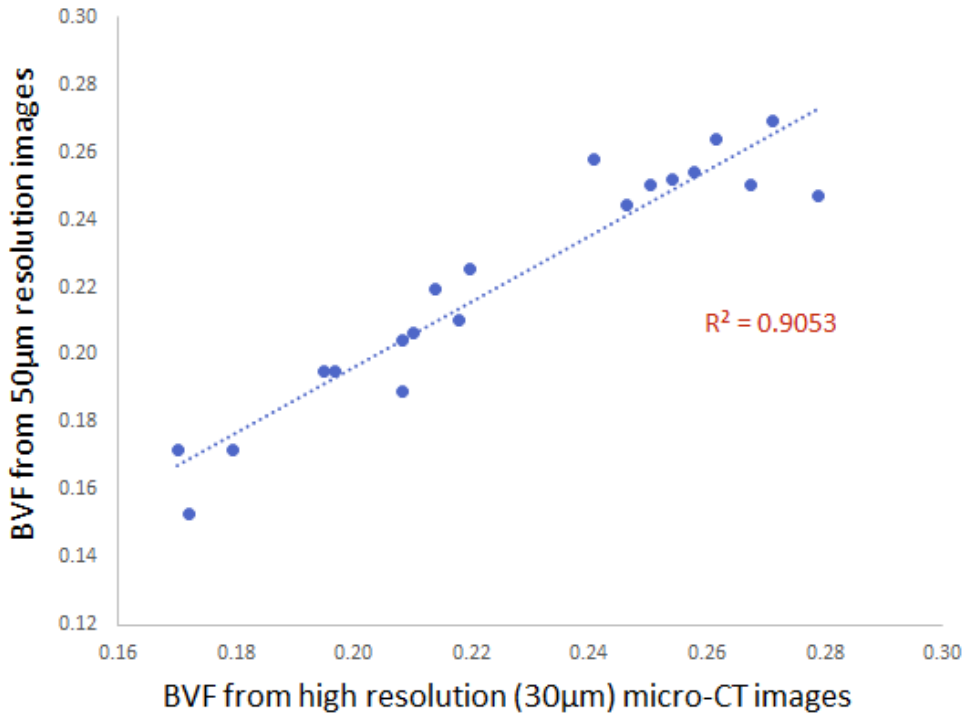


Figure 42 Scatter diagram of BVF measures at 30μm and 50μm resolutions

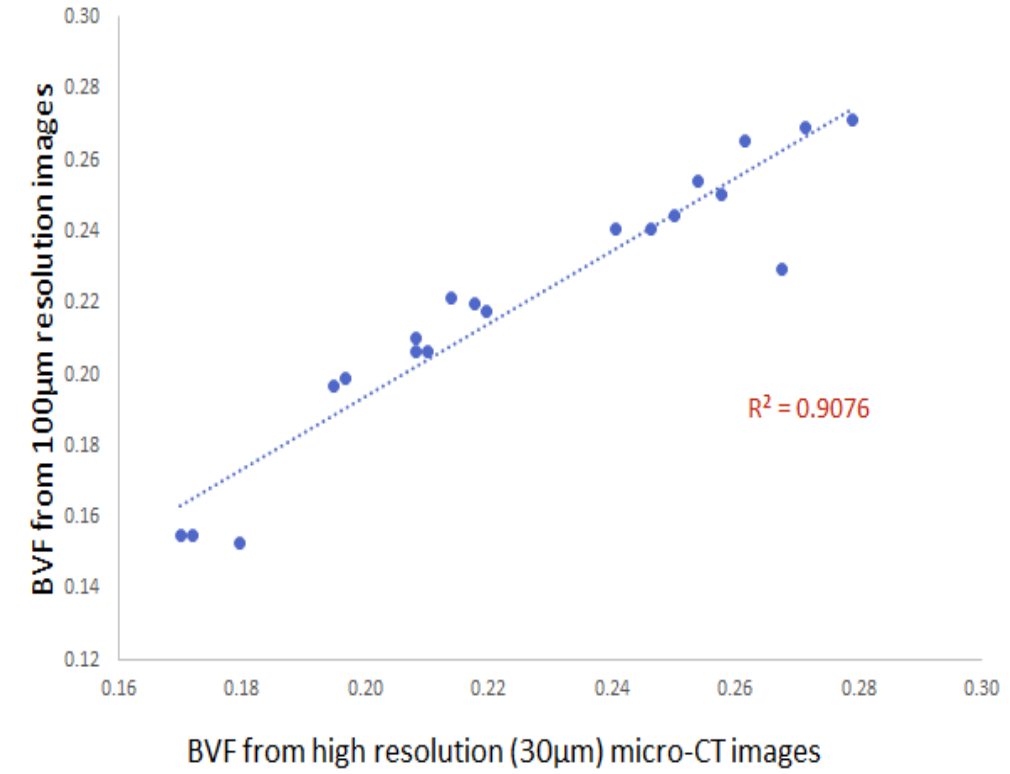


Figure 43 Scatter diagram of BVF measures at 30µm and 100µm resolutions

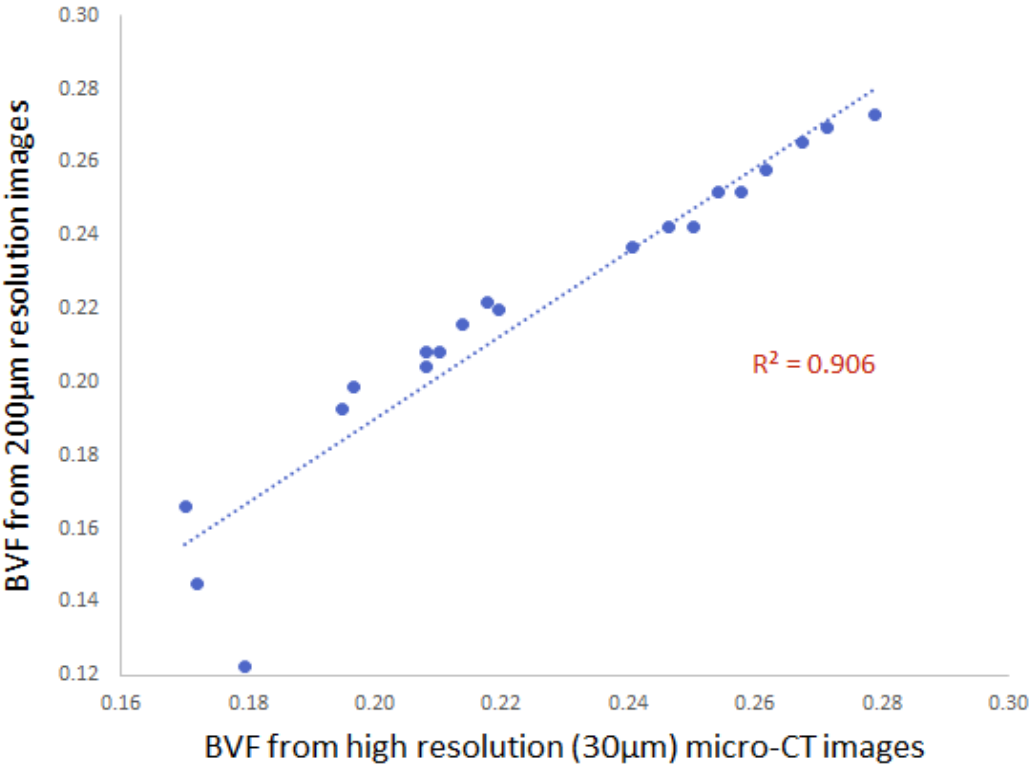


Figure 44 Scatter diagram of BVF measures at 30m and 200m resolutions

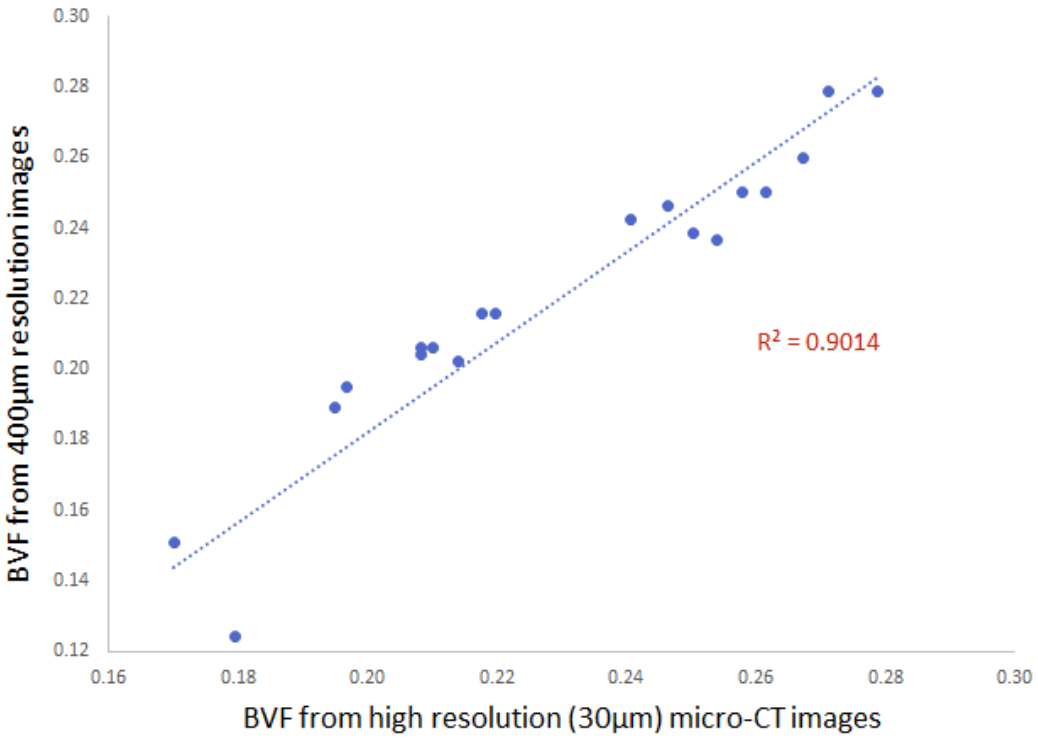


Figure 45 Scatter diagram of BVF measures at 30µm and 400µm resolutions

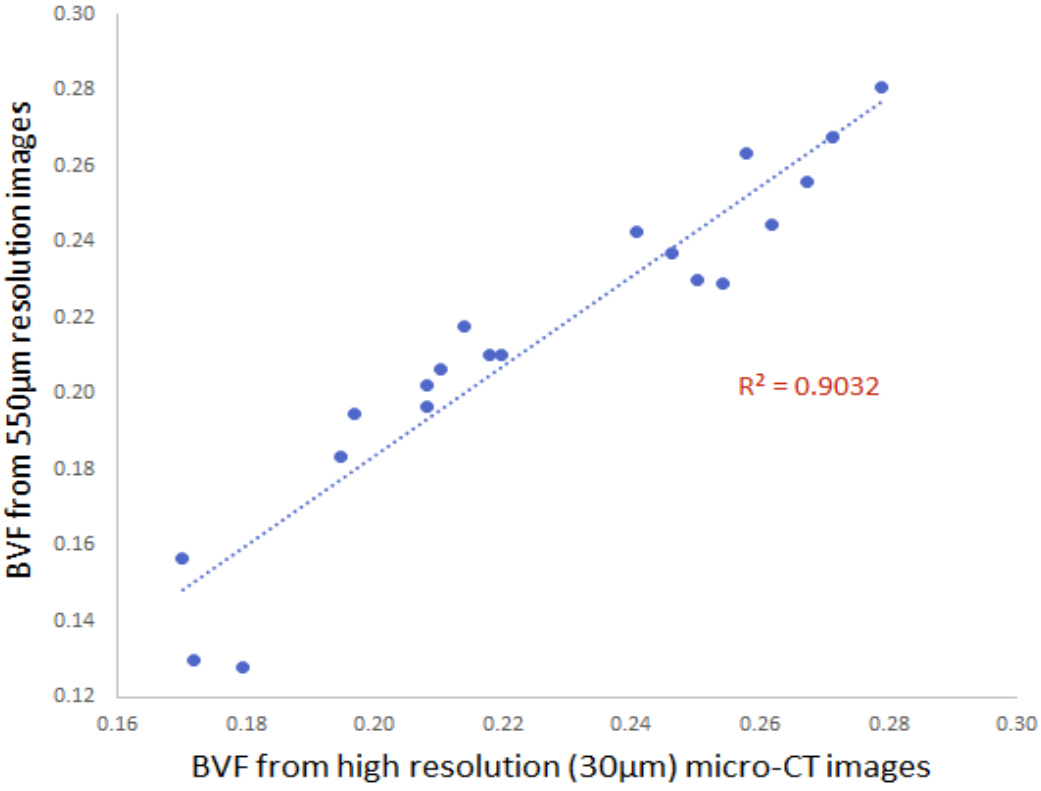


Figure 46 Scatter diagram of BVF measures at 30µm and 550m resolutions

The BVF metrics calculated from high resolution (30µm voxel size) micro-CT images were highly correlated (R^2=0.953) with the mean of the bone strengths calculated *ex vivo* for the two cylindrical specimens from each femoral head (Figure 47). A similar high positive correlation (R^2=0.959) was observed between BVF measures at the lowest downgraded resolution (550µm voxel size) and *ex-vivo* bone strength (Figure 48).

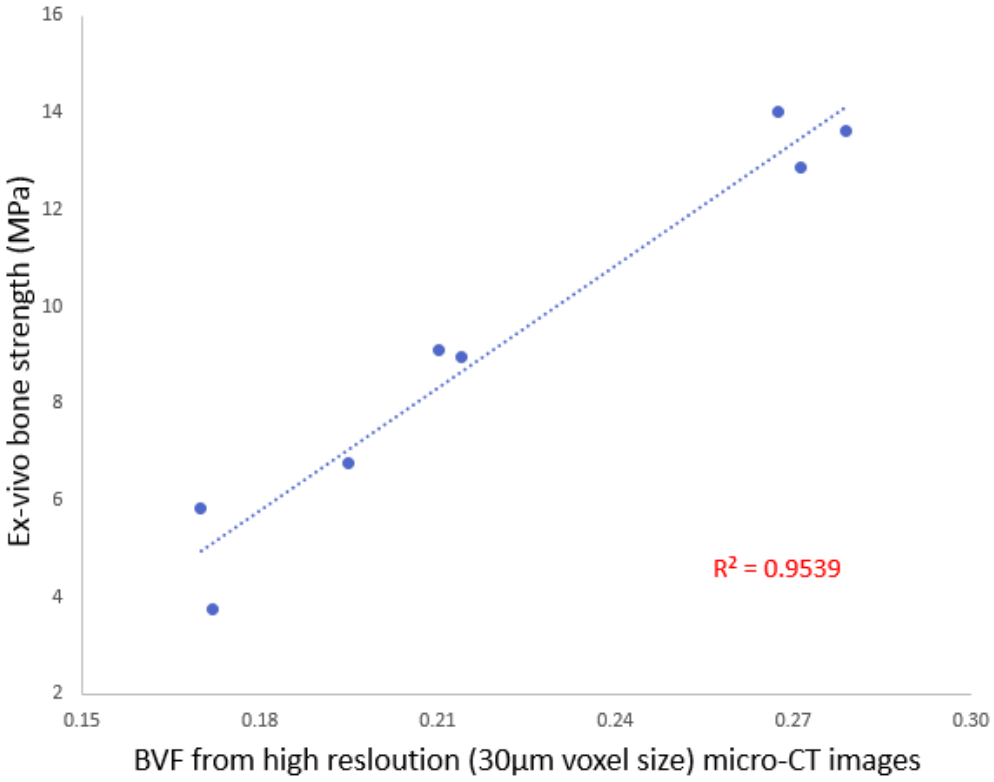


Figure 47 Scatter diagram of BVF values calculated from high resolution (30mµ) micro-CT images and ex-vivo bone strength measures

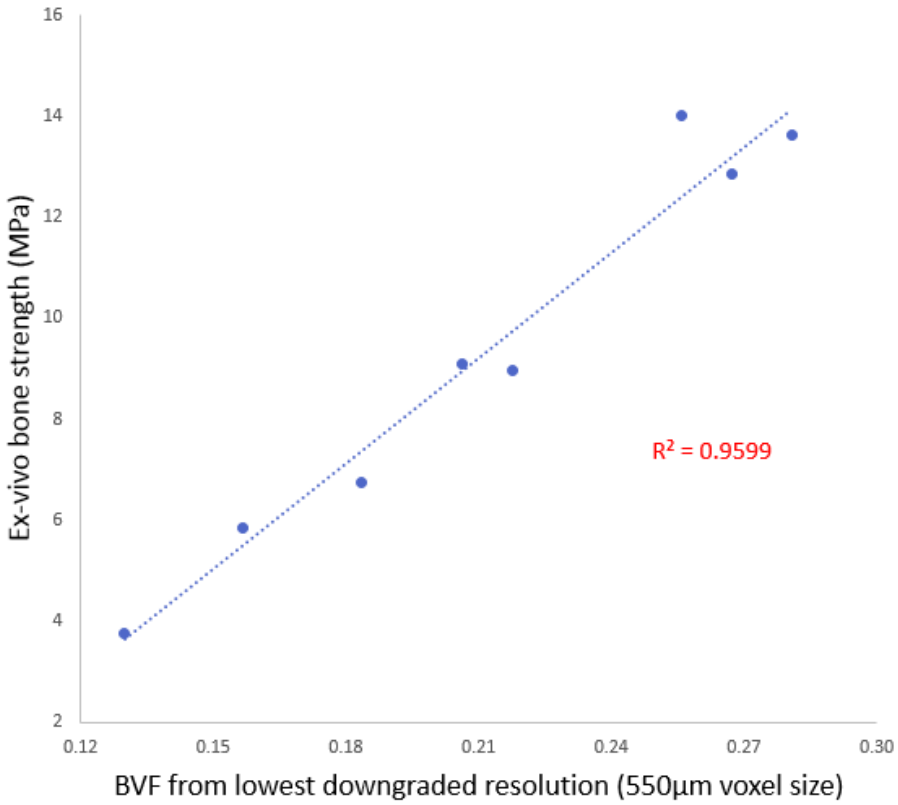


Figure 48 Scatter diagram of BVF values calculated from lowest downgraded resolution (550µm) images and ex-vivo bone strength measures

4.2.2 Bone alkaline phosphatase experiment

The age range of control group was 50-73 years with a mean age of 62.11 years. The age range of study group was 59-75 years with a mean age of 70.22 years.

Table 13 Group characteristics of BALP, testosterone and oestradiol

Parameter	Control group n=9 Mean ± S.E.M		Study group n=9 Mean ± S.E.M		p-value
	Baseline	3 months	Baseline	3 months	
BALP (U/L)	32.01 ± 4.69	36.14 ± 4.94	26.15 ± 2.22	26.17 ± 3.62	0.242
Testosterone (nmol/l)	15.72 ± 1.89	14.85 ± 2.05	17.04 ± 1.47	0.66 ± 0.14	0.000*
Oestradiol (pmol/l)	102.78 ± 13.41	93.78 ± 7.45	90.56 ± 9.35	37.00 + 0.00	0.000*

*Difference is significant at p<0.05

The BALP range of control group at baseline was 20.4-65 U/L with a mean of 32.01 U/L. The BALP range of control group at 3 months was 24.0-65.2 U/L with a mean of 36.14 U/L. The BALP range of study group at baseline was 16.7-34.4 U/L with a mean of 26.15 U/L. The BALP range of study group at 3 months after start of ADT was 15.7-45.6 U/L with a mean of 26.17 U/L (Figure 49). No significant difference in BALP levels was observed between groups (p=0.242) (Table 13). The testosterone range of control group at baseline was 9.19-27.70 nmol/L with a mean of 15.72 nmol/L. The testosterone range of control group at 3 months was 9.41-22.55 nmol/L with a mean of 14.85 nmol/L. The testosterone range of study group at baseline was 9.31-21.40 nmol/L with a mean of 17.04 nmol/L. The testosterone range of study group 3 months after start of ADT was 0.45-1.42 nmol/L with a mean of 0.66 nmol/L (Figure 49). Significant difference in testosterone levels was observed between groups (p=0.000) (Table 13). The oestradiol range of control group at baseline was 46-175 pmol/L

with a mean of 102.78 pmol/L. The oestradiol range of control group at 3 months was 56-131 pmol/L with a mean of 93.78 pmol/L. The oestradiol range of study group at baseline was 51-147 pmol/L with a mean of 90.56 pmol/L. The mean value of oestradiol in the study group at 3 months after start of ADT was 37 pmol/L (Figure 49). Significant difference in oestradiol levels was observed between groups (p=0.000) (Table 13).

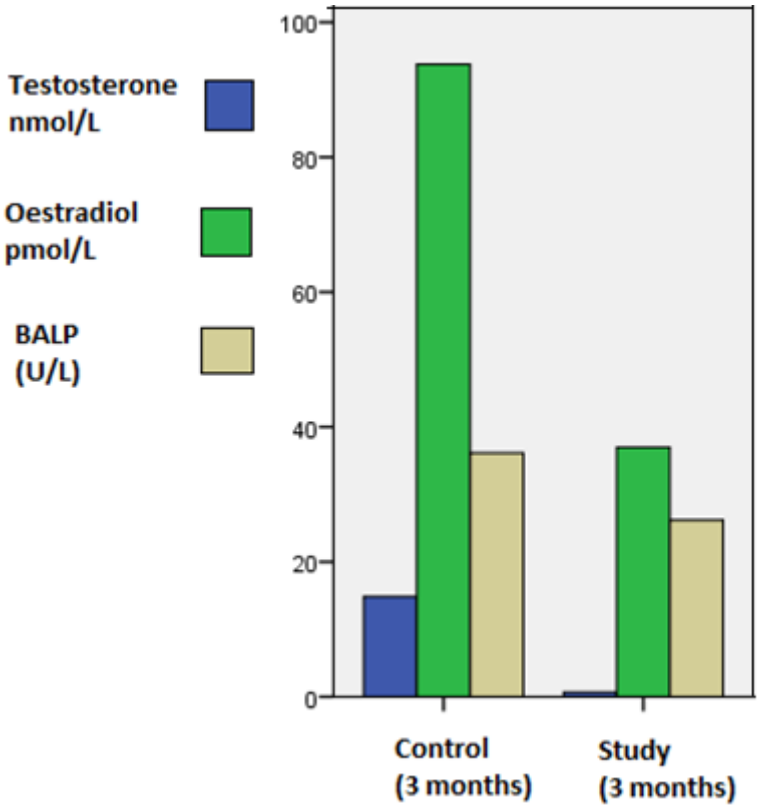


Figure 49 Mean BALP, testosterone and oestradiol levels in control and study groups at 3 months assessment

After applying post-hoc Tukey test, testosterone levels were significantly lower in the study group at 3 months than the study group at baseline as well as the control group at both assessments (baseline and 3 months) (p=0.001) (Table 14) (Figure 49). Oestradiol levels of study group at 3 months assessment were significantly lower than oestradiol levels of study group at baseline assessment and control group at both time points (baseline and 3 months) ($p \leq 0.001$) (Table 15) (Figure 49).

No significant correlation was observed between serum testosterone and serum BALP levels (R=0.187, p=0.276) (Figure 50). No significant correlation was observed between serum oestradiol and BALP levels (R=0.055, p=0.75) (Figure 51).

Table 144 Group comparisons of testosterone at baseline and 3 months

Group 1	Group 2	p-value
Control (Baseline)	Control (3 months)	0.980
	Study (Baseline)	0.934
	Study (3 months)	0.000*
Control (3 months)	Control (Baseline)	0.980
	Study (Baseline)	0.762
	Study (3 months)	0.000*
Study (Baseline)	Control (Baseline)	0.934
	Control (3 months)	0.762
	Study (3 months)	0.000*
Study (3 months)	Control (Baseline)	0.000*
	Control (3 months)	0.000*
	Study (Baseline)	0.000*

*Difference is significant at p<0.05.

Table 155 Group comparisons of oestradiol at baseline and 3 months

Group	Group	p-value
Control (Baseline)	Control (3 months)	0.893
	Study (Baseline)	0.772
	Study (3 months)	0.001*
Control (3 months)	Control (Baseline)	0.893
	Study (Baseline)	0.994
	Study (3 months)	0.001*
Study (Baseline)	Control (Baseline)	0.772
	Control (3 months)	0.994
	Study (3 months)	0.001*
Study (3 months)	Control (Baseline)	0.000*
	Control (3 months)	0.001*
	Study (Baseline)	0.001*

*Difference is significant at p<0.05.

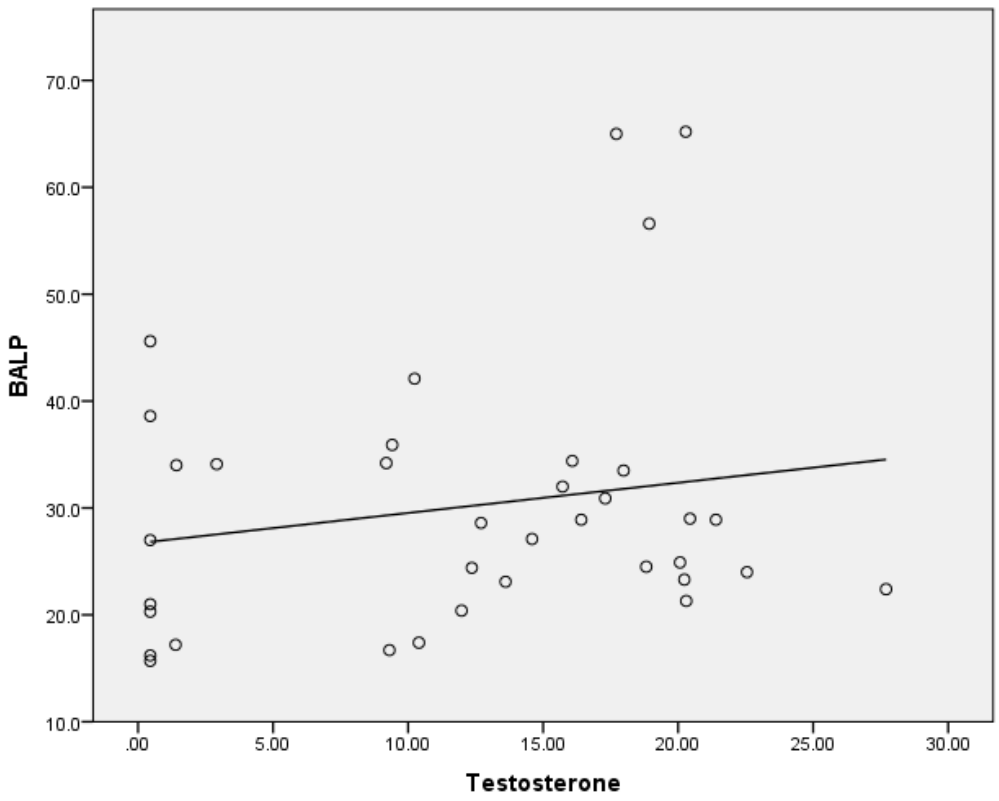


Figure 50 Scatter diagram of serum testosterone and BALP levels

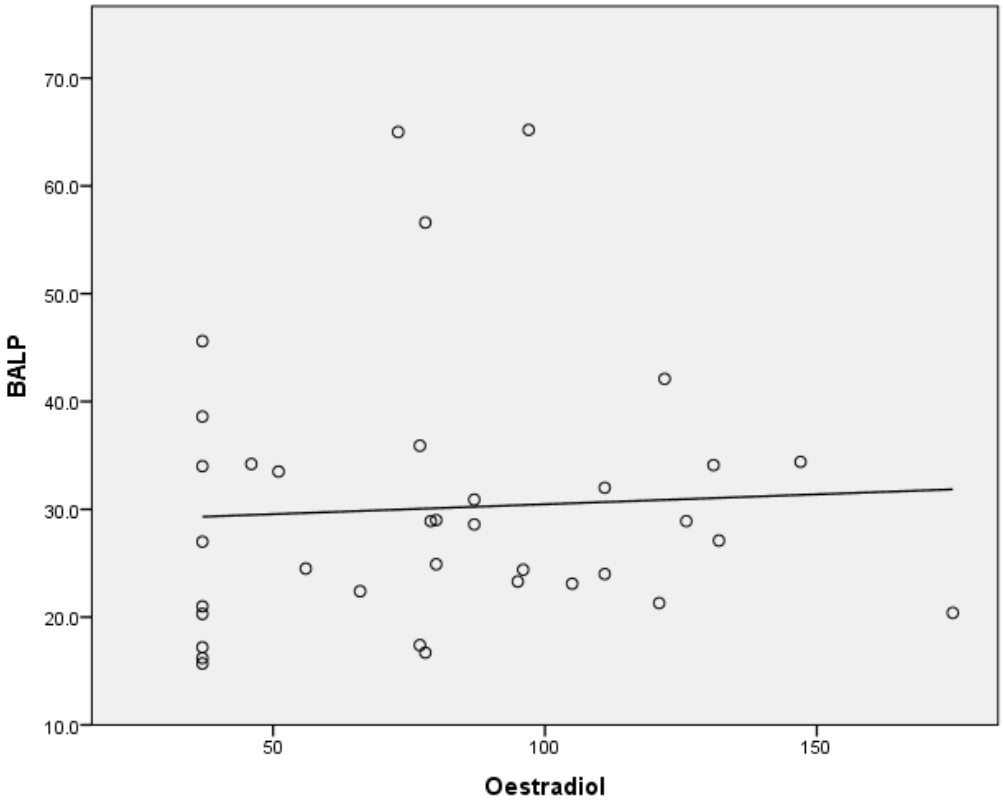


Figure 51 Scatter diagram of serum oestradiol and BALP levels

4.3 Odour study

The age range of study group (n=10) was 50-74 years with a mean age of 63.3 years. The age range of control group (n=10) was 59-75 years with a mean age of 69.6 years. Figure 52 describes the ethnic distribution of the study participants who provided odour samples.

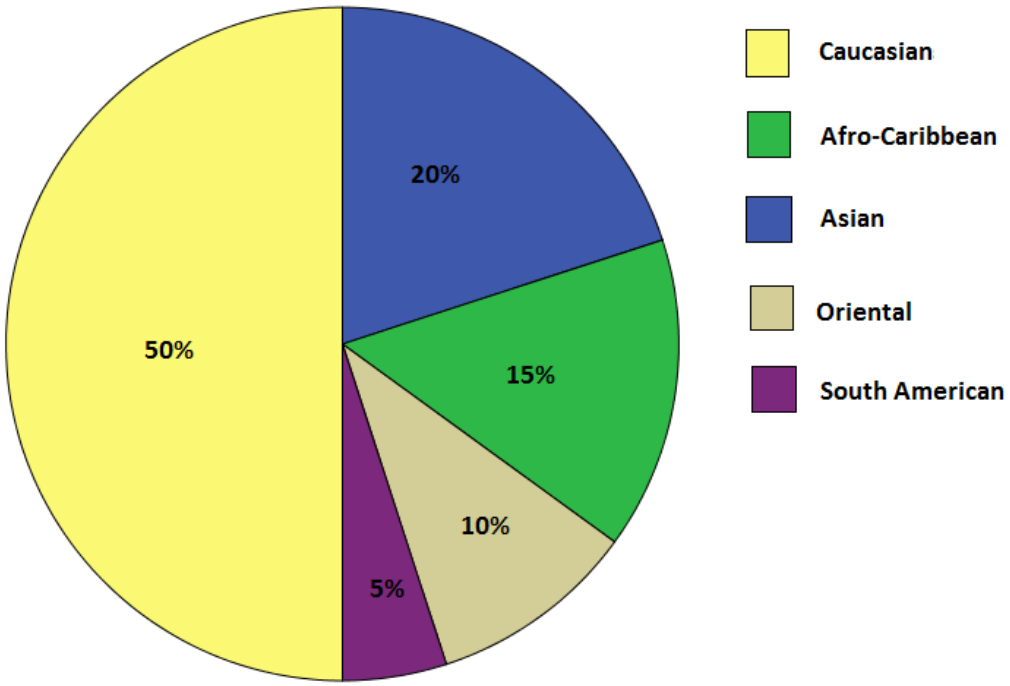


Figure 52 Ethnic distribution of study population

The age range of male raters (n=50) was 17-33 years with a mean age of 21.56 years. The age range of female raters (n=50) was 17-34 years with a mean age of 21.26 years. The age range of all raters (male and female, n=100) was 17-34 years with a mean age of 21.41 years.

Table 16 Group characteristics of odour ratings for attractiveness, masculinity and intensity by male raters

Parameter	Control group n=10 Mean ± S.E.M		Study group n=10 Mean ± S.E.M		p-value
	Baseline	3 months	Baseline	3 months	
Attractiveness	2.92 ± 0.16	2.92 ± 0.13	2.72 ± 0.18	2.82 ± 0.14	0.778
Masculinity	3.13 ± 0.15	2.78 ± 0.14	3.27 ± 0.10	3.21 ± 0.13	0.079
Intensity	3.26 ± 0.33	2.61 ± 0.17	3.16 ± 0.22	3.02 ± 0.18	0.241

Table 177 Group characteristics of odour ratings for attractiveness, masculinity and intensity by female raters

Parameter	Control group n=10 Mean ± S.E.M		Study group n=10 Mean ± S.E.M		p-value
	Baseline	3 months	Baseline	3 months	
Attractiveness	2.68 ± 0.22	2.72 ± 0.24	2.55 ± 0.11	2.61 ± 0.20	0.933
Masculinity	2.94 ± 0.13	2.82 ± 0.22	2.85 ± 0.17	2.74 ± 0.20	0.900
Intensity	3.03 ± 0.30	2.73 ± 0.18	2.87 ± 0.24	2.63 + 0.18	0.650

Table 188 Group characteristics of odour ratings for attractiveness, masculinity and intensity by all raters (males and females)

Parameter	Control group n=10 Mean ± S.E.M		Study group n=10 Mean ± S.E.M		p-value
	Baseline	3 months	Baseline	3 months	
Attractiveness	2.81 ± 0.17	2.83 ± 0.15	2.64 ± 0.12	2.72 ± 0.13	0.803
Masculinity	3.03 ± 0.09	2.80 ± 0.12	3.06 ± 0.07	2.98 ± 0.11	0.329
Intensity	3.14 ± 0.29	2.67 ± 0.14	3.02 ± 0.16	2.82 ± 0.15	0.374

One-way ANOVA test did not show any difference in the ratings for attractiveness, masculinity or intensity by male raters, female raters or both male and female raters combined (Tables 16,17,18).

Table 19 Group characteristics of serum sex hormone levels

Parameter	Control group n=10 Mean ± S.E.M		Study group n=10 Mean ± S.E.M		p-value
	Baseline	3 months	Baseline	3 months	
Testosterone nmol/L	16.55 ± 1.88	16.58 ± 1.45	16.56 ± 1.45	0.64 ± 0.12	0.000*
Oestradiol pmol/L	102.70 ± 12.00	95.10 ± 6.79	89.40 ± 8.44	37.00 ± 0.00	0.000*

*Difference is significant at $p<0.05$.

The testosterone range of control group at baseline was 9.19-27.70 nmol/L with a mean of 16.55 nmol/L. The testosterone range of control group at 3 months was 9.41-22.55 nmol/L with a mean of 16.58 nmol/L. The testosterone range of study group at baseline was 9.31-21.40 nmol/L with a mean of 16.56 nmol/L. The testosterone range of study group 3

months after start of ADT was 0.45-1.42 nmol/L with a mean of 0.64 nmol/L (Figure 53). Significant difference in testosterone levels was observed between groups (p=0.000) (Table 19). The oestradiol range of control group at baseline was 46-175 pmol/L with a mean of 102.70 pmol/L. The oestradiol range of control group at 3 months was 56-131 pmol/L with a mean of 95.10 pmol/L. The oestradiol range of study group at baseline was 51-147 pmol/L with a mean of 89.40 pmol/L. The mean value of oestradiol in the study group at 3 months after start of ADT was 37 pmol/L (Figure 53). Significant difference in oestradiol levels was observed between groups (p=0.000) (Table 19).

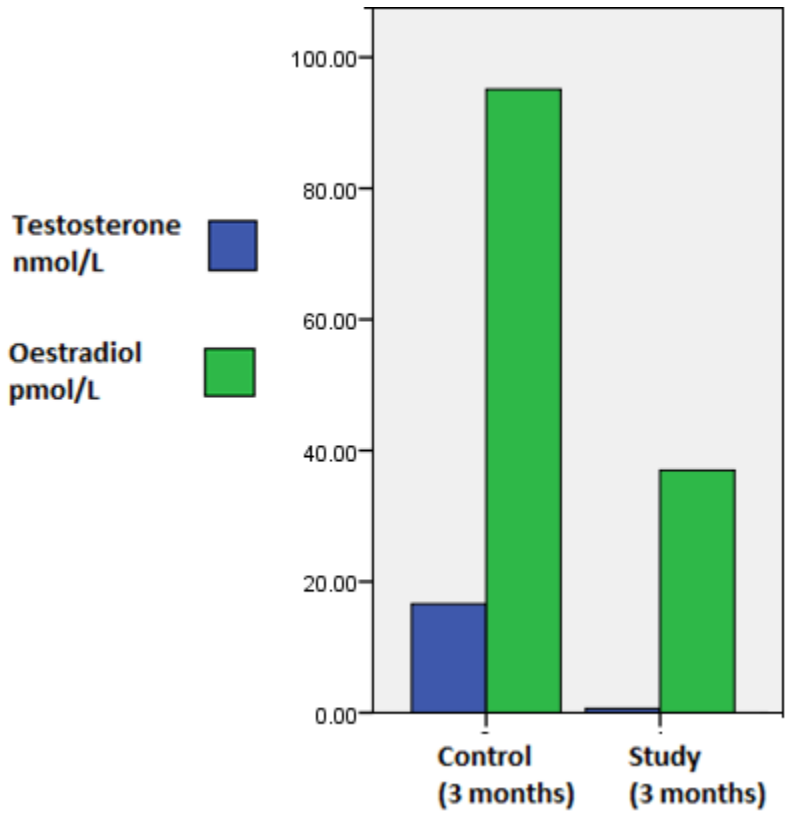


Figure 53 Mean serum testosterone and oestradiol levels in control and study groups at 3 months assessment

Table 190 Group comparisons of serum testosterone levels

Group 1	Group 2	p-value
Control (Baseline)	Control (3 months)	1.000
	Study (Baseline)	1.000
	Study (3 months)	0.000*
Control (3 months)	Control (Baseline)	1.000
	Study (Baseline)	1.000
	Study (3 months)	0.000*
Study (Baseline)	Control (Baseline)	1.000
	Control (3 months)	1.000
	Study (3 months)	0.000*
Study (3 months)	Control (Baseline)	0.000*
	Control (3 months)	0.000*
	Study (Baseline)	0.000*

*Difference is significant at p<0.05

Table 201 Group comparisons of serum oestradiol levels

Group 1	Group 2	p-value
Control (Baseline)	Control (3 months)	0.910
	Study (Baseline)	0.654
	Study (3 months)	0.000*
Control (3 months)	Control (Baseline)	0.910
	Study (Baseline)	0.959
	Study (3 months)	0.000*
Study (Baseline)	Control (Baseline)	0.654
	Control (3 months)	0.959
	Study (3 months)	0.000*
Study (3 months)	Control (Baseline)	0.000*
	Control (3 months)	0.000*
	Study (Baseline)	0.000*

*Difference is significant at p<0.05

After applying post-hoc Tukey test, testosterone levels were significantly lower in the study group at 3 months than the study group at baseline as well as the control group at both assessments (baseline and 3 months) (p=0.001) (Table 20) (Figure 53). Oestradiol levels of study group at 3 months assessment were significantly lower than oestradiol levels of study group at baseline assessment and control group at both time points (baseline and 3 months) (p=0.000) (Table 21) (Figure 53).

No significant correlation was observed between serum sex hormone levels and odour ratings by male or female raters and by all raters combined (Table 22-24) (Figures 54-59).

Variable 1	**Variable 2**	**Pearson's R**	**p-value**
Testosterone	Attractiveness	0.059	0.716
	Masculinity	-0.131	0.422
	Intensity	-0.01	0.995
Oestradiol	Attractiveness	0.015	0.926
	Masculinity	-0.296	0.064
	Intensity	-0.206	0.202

Table 21 Correlation between sex hormone levels and odour ratings by male raters

Variable 1	Variable 2	Pearson's R	p-value
Testosterone	Attractiveness	-0.042	0.799
	Masculinity	0.057	0.728
	Intensity	0.196	0.226
Oestradiol	Attractiveness	0.136	0.404
	Masculinity	0.008	0.962
	Intensity	0.038	0.818

Table 22 Correlation between sex hormone levels and odour ratings by female raters

Variable 1	Variable 2	Pearson's R	p-value
Testosterone	Attractiveness	0.010	0.952
	Masculinity	-0.046	0.777
	Intensity	0.114	0.483
Oestradiol	Attractiveness	0.103	0.528
	Masculinity	-0.190	0.239
	Intensity	-0.096	0.555

Table 23 Correlation between sex hormone levels and odour ratings by all raters

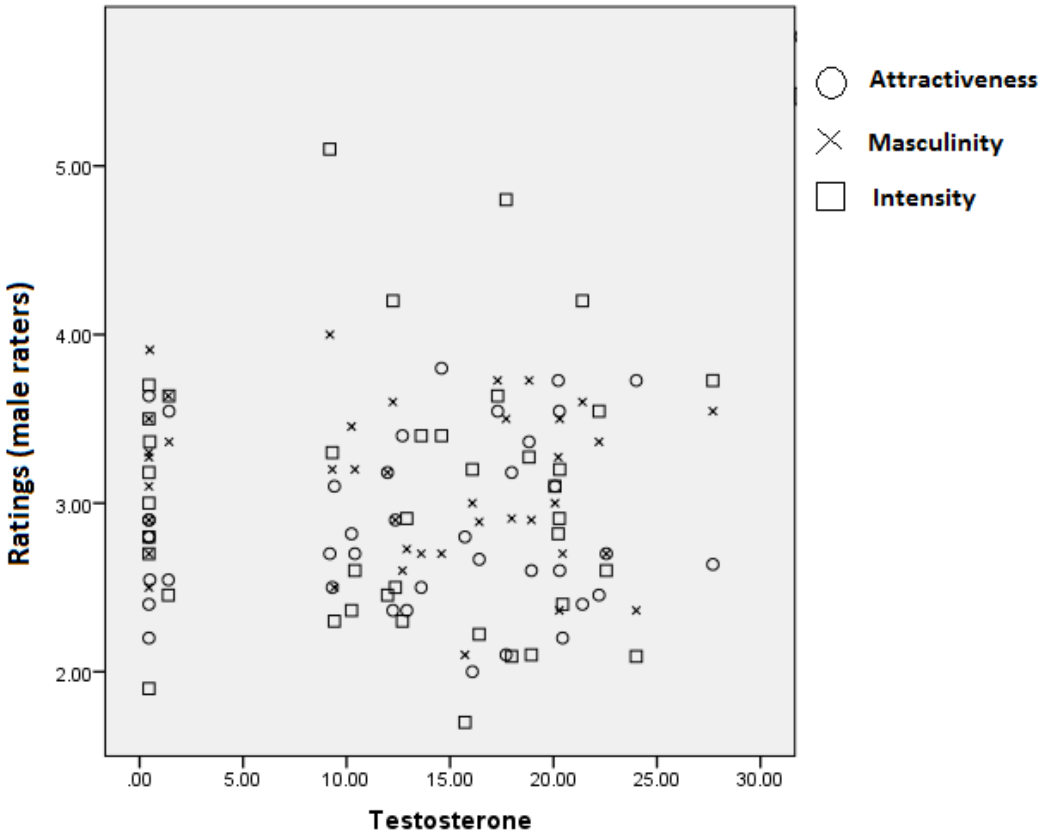


Figure 54 Scatter diagram of serum testosterone and odour ratings by male raters

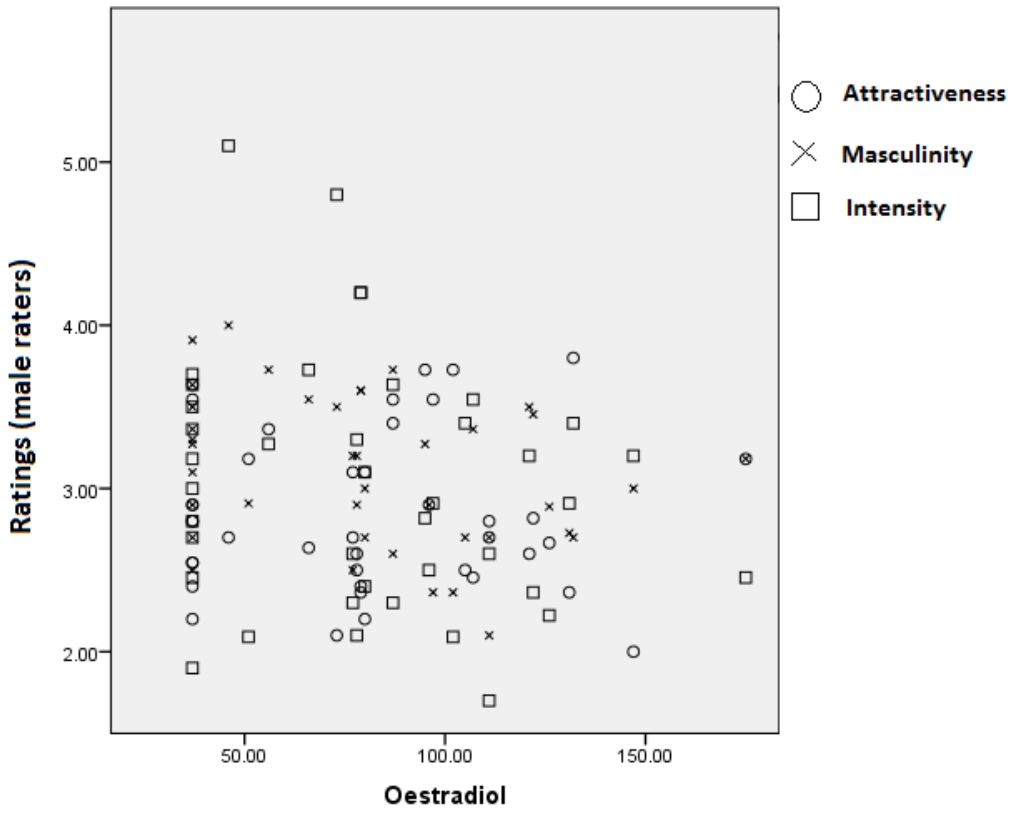


Figure 55 Scatter diagram of serum oestradiol and odour ratings by male raters

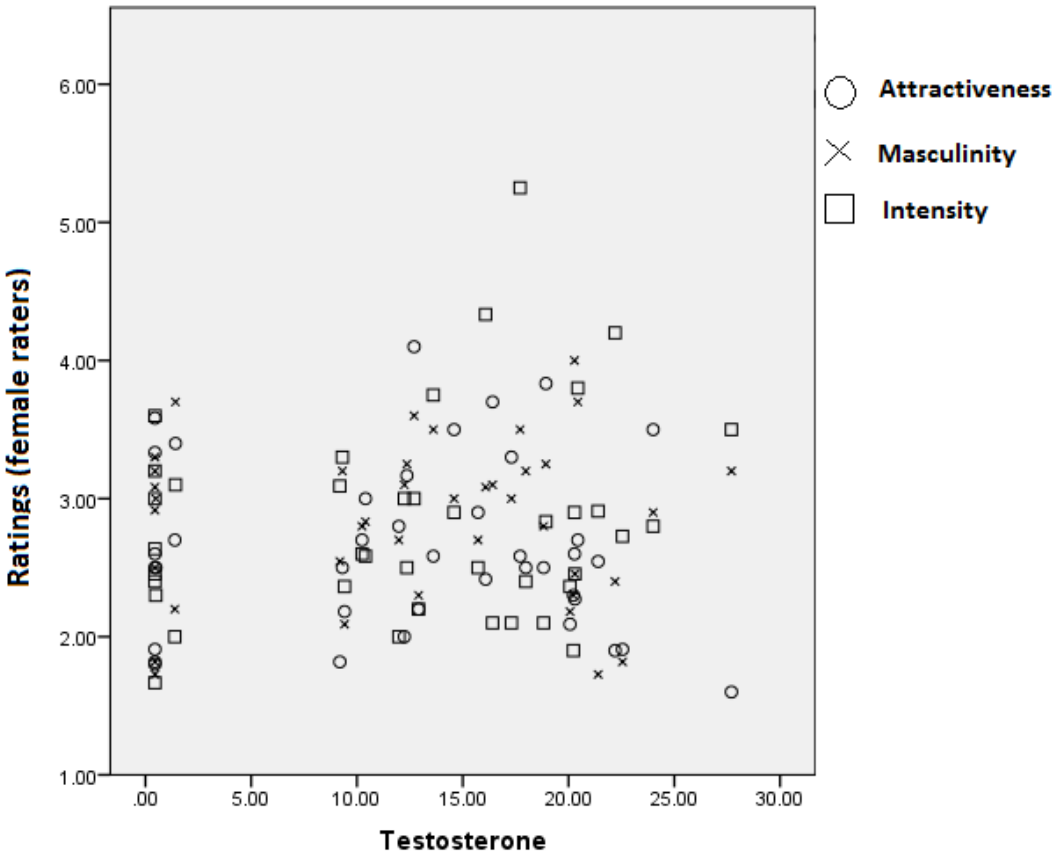


Figure 56 Scatter diagram of serum testosterone and odour ratings by female raters

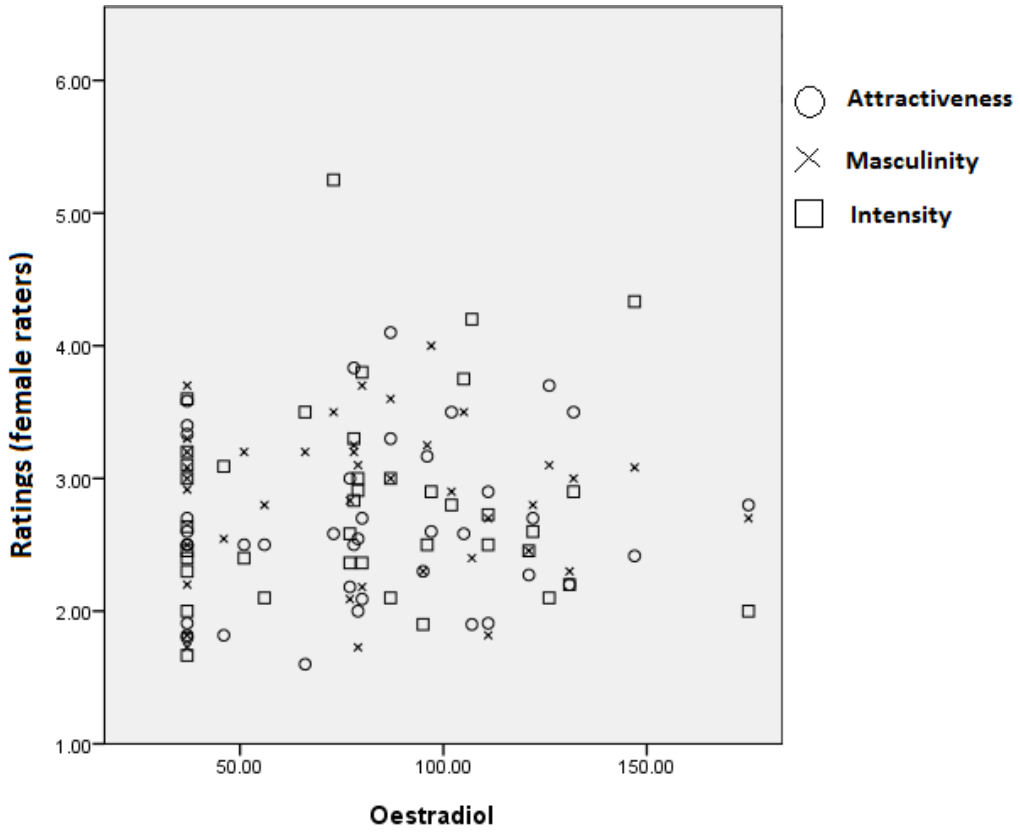


Figure 57 Scatter diagram of serum oestradiol and odour ratings by female raters

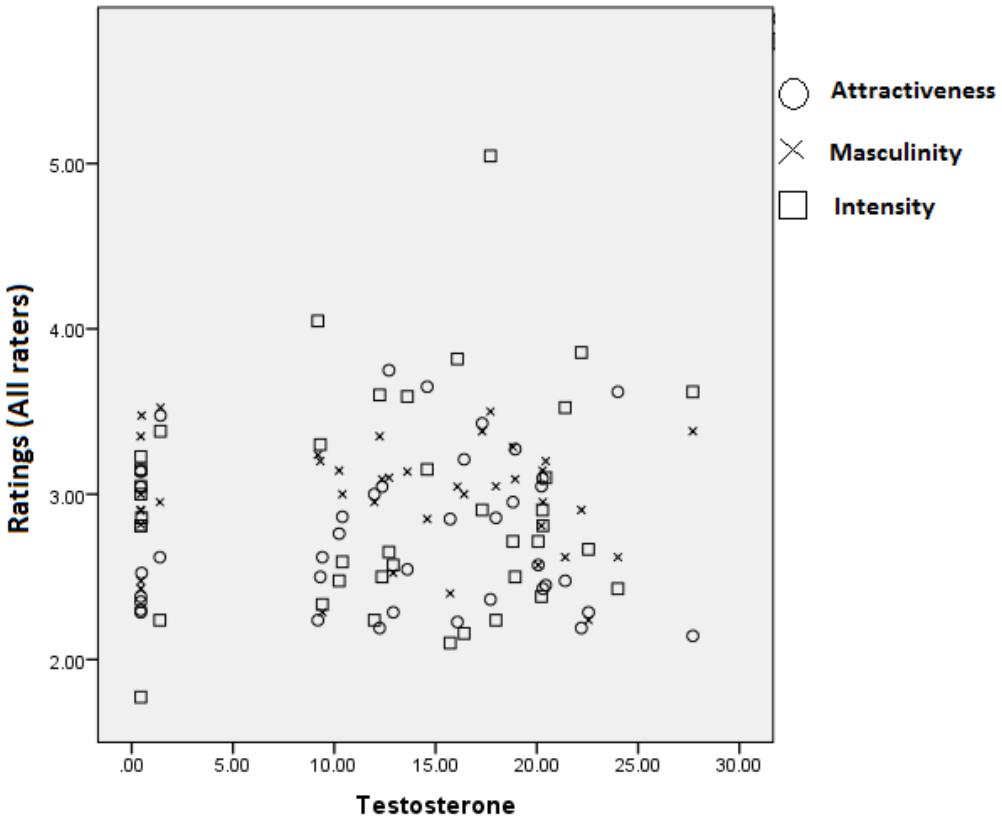


Figure 58 Scatter diagram of serum testosterone and odour ratings by all raters

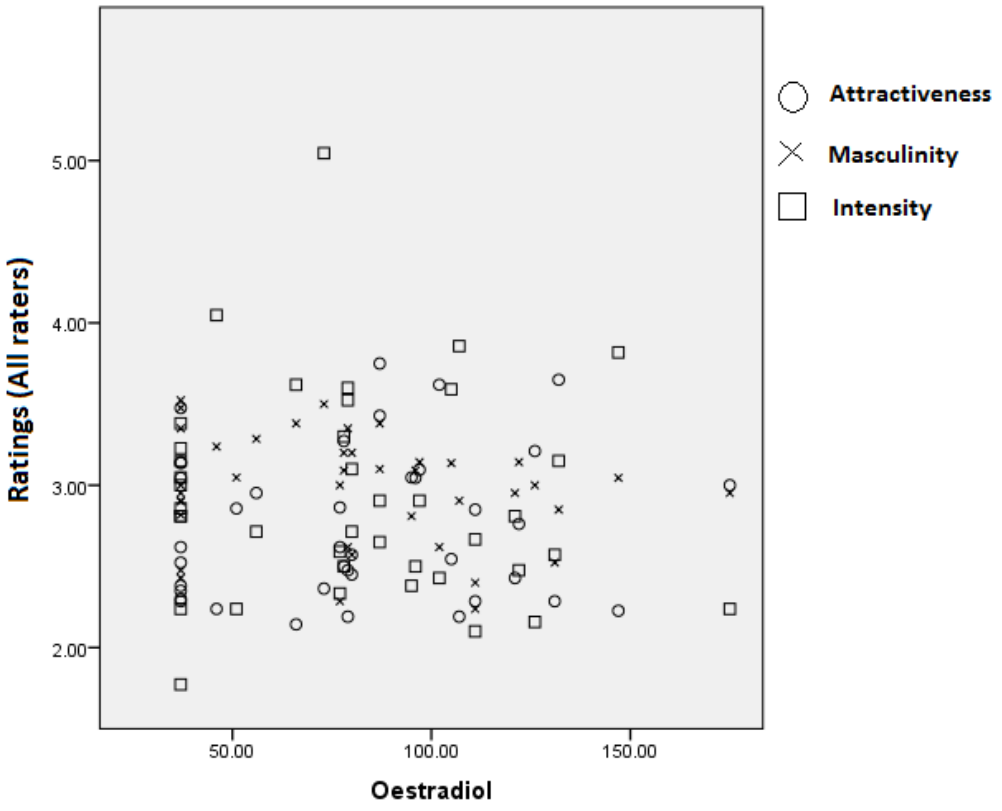


Figure 59 Scatter diagram of serum oestradiol and odour ratings by all raters

5. Discussion

5.1 Cognitive study

This current study explored for the first time the presence of neural pathological changes related to cognitive impairment in patients with PC undergoing ADT with LHRHa. The study employed PET brain imaging using radioligand [11C]PBR28 for the neuroinflammatory marker TSPO known to be overexpressed by the neuroimmune microglial cells activated by brain disease or damage [283, 284]. An increased global and regional uptake of the radioligand was seen in whole brain and frontal, parietal, temporal and occipital cortices of patients complaining of or noticing cognitive deterioration since starting on LHRHa treatment (Figure 41) (Table 12).

This work has yielded findings consistent with neuroinflammatory response in the setting of cognitive decline in PC patients receiving ADT. Studies on clinical conditions including stroke, multiple sclerosis, mild cognitive impairment (MCI) and AD have previously utilised TSPO PET imaging to visualise neuroinflammatory changes [284, 285, 344, 345]. The present study utilised mean SUVR between the scanning period of 60-90 minutes as the tissue uptake parameter of [11C]PBR28 to compare the groups (men reporting cognitive decline since starting LHRHa and men not reporting such change) as shown recently by Lyoo et al [333]. Their study of patients suffering from AD demonstrated no difference in cerebellar binding of [11C]PBR28 between healthy controls (n=21) and patients with MCI (n=11) or AD (n=25), thus justifying its use as a pseudo-reference region for clinical TSPO PET imaging to calculate SUVR. They further showed significantly higher [11C]PBR28 SUVR in patients with AD as compared to healthy controls in the regional cortices (temporal, parietal) ($p \leq 0.009$) [333].

The imaging component of the current study has a sample size smaller than other TSPO PET imaging studies which did not allow the use of statistical methods for assessing significance of the differences observed. However, the study had the advantage of including only those patients who were shown to have HAB TSPO polymorphism. It has been shown

previously that [11C]PBR28 binding is influenced by the TSPO genotype and up to a 50 fold difference in the uptake of [11C]PBR28 has been shown between HAB and LAB genotypes of TSPO [307]. The inclusion of HAB patients ensured maximal opportunity to pick up any difference in [11C]PBR28 TSPO signal in this pilot experiment and allowed consistency when interpreting the data.

The present study also assessed cognitive function in patients with PC reporting cognitive problems since starting ADT with LHRHa, using a detailed battery of paper-based and computerised neuropsychological tests. The neuropsychological tasks employed have also been used previously to pick early cognitive changes in clinical studies on neurodegenerative disorders, cerebrovascular disease and traumatic brain injury [308, 309]. Comparisons were made with a control group comprising of patients with PC matched for age, treatment and treatment duration. Patients with prior history of neuropsychiatric problems, neurodegenerative disorders or cerebrovascular incidents were excluded and so were the patients who were deemed to have cognitive issues before receiving ADT as determined by clinical history and MMSE. This study design was adopted based on clinical experience as well as the mixed findings from the existing literature which seem to suggest that not all patients on LHRHa exhibit cognitive side-effects to a similar extent. A meta-analysis conducted previously by Nelson et al. suggested that only 47% to 69% of patients on ADT develop cognitive decline [203].

The current work has shown differences between the two groups of patients on neuropsychological testing, demonstrating that some patients may possibly have a predisposition to early development of the deleterious cognitive effects of sex hormonal suppression as a result of LHRHa therapy. The paper-based tests revealed reduced performance in the study group (men reporting cognitive deterioration) on all cognitive domains assessed including working memory, attention span, verbal fluency, long term verbal memory, recognition, associative learning, verbal recall memory, visuospatial ability and executive functions (cognitive flexibility, reasoning ability, strategy-making and processing speed), as compared to the control group (mean ADT duration of ~7 months). However, the

difference was only statistically significant in mental flexibility and processing speed, associative learning and verbal recall fluency (Table 10). Although the design of the present study is uniquely different from all relevant studies conducted previously, but findings are consistent with published data suggesting reduction in sex hormones caused by ADT with LHRHa to be associated with early development of cognitive impairment and in multiple domains [203-206].

Yang et al. in their cross-sectional study on a Chinese population showed significantly impaired cognitive performance on tasks of attention span, processing speed and cognitive flexibility in patients who had received 6 months ADT with LHRHa for non-metastatic PC as compared to non-ADT patients and healthy controls ($P \leq 0.05$). In a longitudinal study assessing visual memory, verbal memory, executive function and attention, Gonzales et al. have also shown patients with PC receiving ADT to develop cognitive dysfunction within 6 months of the start of therapy as compared to PC patients not receiving ADT and healthy controls ($P < 0.05$) [205]. Previously, Beer et al. showed a significant decline in processing speed and long term verbal memory in PC patients on continuous ADT as compared to healthy controls ($P \leq 0.01$). However, the mean duration of ADT in their study was much longer (~6 years) than the current study and results were not stratified for the type of ADT which included LHRHa or orchidectomy or a combination of either with an anti-androgen.

The computerised battery of neuropsychometric tests assessing several cognitive areas was used for the first time in this population but did not show any difference between the two groups (Table 11). Some of the tests were modified digital versions of the paper-based questionnaires but did not contain measures of verbal fluency, verbal memory, verbal recall and associative learning which were the main cognitive domains shown to be statistically different on the paper-based tests. Further, the participants expressed visible difficulty in operating the computer and this lack of computer skills may also have contributed to the insignificant findings.

The observed increased neuroinflammation as evidenced by the greater binding of [11C]PBR28 in patients reporting cognitive problems as compared to those who did not notice

any cognitive change suggests the presence of predisposing factors that may potentially contribute to decline in cognitive ability. Moreover, as demonstrated by the neuropsychological testing (Table 10), the differential nature of the cognitive deficits caused by the treatment also points in the direction of a multi-factorial pathology. In this regard, genetic polymorphisms have recently been implicated as contributing influences on the development of cognitive behavioural changes in men on ADT [205]. Other factors such as low educational level, advanced age at the onset of treatment, concomitant treatments and co-morbid neurological conditions may add to cognitive deterioration particularly in susceptible individuals.

These current findings highlight the need to ascertain factors that may predispose to impairments in cognitive functioning, thereby enabling identification of vulnerable individuals who may then be offered alternate PC treatment options mitigating the neurotoxic effects of diminished sex hormones due to LHRHa ADT. Parenteral oestrogen, a promising ADT modality [275, 279], may provide an alternative as oestrogen's neuroprotective role has been revealed in other clinical contexts [38, 40]. Improved cognitive performance following the use of transdermal oestradiol as a second-line agent for androgen insensitive PC has also been demonstrated in a previous small study [201]. Further research on the cognitive effects of ADT is also warranted to help establish changes in clinical practice involving discussion of the potential cognitive risks of LHRHa therapy and careful monitoring of cognitive function during treatment. Such work may potentially lead to an era of personalised medicine with a huge beneficial impact on the overall QoL of PC survivors and others without PC.

The study has several limitations. As a cross-sectional study, information about the cognitive changes over time from baseline is not provided. It lacks control groups of demographically matched healthy men as well as patients with PC not receiving ADT, inclusion of whom would have enabled effective comparisons. The high costs of the study procedures, particularly PET scans, and limited amount of funding available accounted for these limitations in study design. The statistical power of the study is also limited by the small sample size. However, this is a pilot exploratory study to generate preliminary data for

informing the feasibility and design of larger comprehensive future studies and therefore, no power calculation was involved for an estimate of sample size.

5.2 Bone study

BMD measurements are the current standard for assessing osteoporosis and the risk of associated fragility fractures [346]. Measured using the 2D DEXA technique, BMD only provides an areal measure of bone mass which is one of the many factors influencing bone strength. It does not take into account the contribution of other elements such as volumetric bone mass and micro-architecture to bone strength [296]. 3D high-resolution micro-CT has been used to calculate BVF *ex vivo* but is clinically inapplicable at present due to the risks of high radiation exposure [298]. This present study explored the potential of using low-resolution clinical-CT for estimating bone strength as most oncological patients receive CT scans clinically for staging tumours and planning interventions.

The study demonstrated that femoral head trabecular BVF can be calculated (> 90% correlation with BVF measured from high-resolution micro-CT images) from CT images downgraded in resolution comparable to clinical-CT scans of the pelvic region. BVF metrics from these low-resolution scans were also shown to have a 96% correlation with bone strength assessed *ex vivo*. These findings are concordant with results reported by Nazarian et al. who studied biopsy specimens of trabecular bone from spine and/or femur of patients with metastatic prostate, breast, lung, ovarian, or colon cancer (n=41) and non-cancer cadaveric samples (n=96). Specimens were imaged using micro-CT and mechanically tested by uniaxial compression. Measured BVF was shown to account for 84% of bone strength variations in all trabecular bone specimens irrespective of the skeletal site or pathology [300]. The results of the present study show that it is feasible to measure BVF at low voxel size (550um), which is the resolution offered by modern clinical CT scanners. The calculated low resolution measures of BVF are also highly predictive of bone strength assessed *ex vivo* (96% correlation) whereas BMD has previously been shown to have only a 50% correlation to bone strength [347].

A limitation of the study is the use of low resolution data downgraded from high-resolution micro-CT data as a simulation of low resolution clinical-CT data. The micro-CT data do not contain the same noise and artefacts that are present in clinical-CT data. Further work

using actual clinical CT data for determining BVF and its comparisons with BMD values from DEXA and measures calculated from finite element analysis (a method using computer modelling to predict effect of loading on mechanical behaviour of bone) is required to provide more conclusive results [348]. Future investigations showing that such clinical CT-based measurements predict fractures better than the current methods may help establish the potential future use of BVF as an easy access, non-invasive and cost-effective method for clinical bone health assessment.

BVF may readily serve as an effective metric for predicting bone strength in cancer patients who routinely undergo CT scans of the pelvis as part of their cancer management regime and changes in bone strength over time can be tracked using BVF measures derived from those CT scans. Timely and accurate identification of high risk patients such as those with diminishing BVF can allow clinicians to modify treatment and/or prescribe bone sparing agents [349]. Clinical trials of diet and/or exercise intervention for improving bone health can potentially utilise BVF instead of BMD [350, 351]. With advancements in CT technology leading to reduction in radiation dosage, this novel metric of bone strength could find widespread clinical use in the future for diagnosing osteoporosis in the general population, predicting fracture risk and monitoring treatment outcomes. BVF can be readily employed as a novel metric for predicting bone strength in PC patients receiving ADT with LHRHa.

As both osteoblastic and osteolytic processes are involved in bone remodelling and turnover, metabolites of bone formation and resorption have been used to assess changes in skeletal homeostasis following anti-resorptive therapy or treatment with bone forming agents. The data on changes in such biochemical parameters of bone metabolism following ADT with LHRHa in PC are limited. The present experiment looked at early changes in serum BALP levels of patients undergoing ADT for PC. No change was observed in serum BALP levels after 3 months of commencement of ADT (Table 13) although serum testosterone and oestradiol levels were significantly reduced as compared to baseline concentration and control group ($p \leq 0.001$) Table 14,15). No correlation was found between serum sex hormone and BALP levels (Figures 50,51). Unlike these findings, a previous study by Greenspan et al.

showed a rise in bone turnover markers in men on ADT for PC. Serum BALP, osteocalcin, procollagen type 1 amino-terminal propeptide (P1NP) and urinary NTX were elevated in men receiving ADT (n=30) compared with non-ADT users (n=72) and healthy controls (n=43) after 12 months ($p<0.05$). Urinary NTX was also elevated after 6 months in men on ADT compared with men not on ADT ($p<0.05$) [352]. Greenspan et al. measured bone markers at 6 and 12 months from baseline whereas in the present study, BALP was measured at 3 months from baseline. Further, the patients in the current study were ADT-naïve at baseline while patients included in the study by Greenspan et al. were already on ADT (mean duration of ADT at baseline was 2.9 $\pm$ 1.5 months).

In another study, Morote et al. showed increased serum BALP levels in patients with non-metastatic PC receiving CAB (n=35) over a 5 year follow-up period. After 5 years, there was a 65% increase in mean serum BALP concentration from the baseline pre-treatment values. The increase was highest (32% from baseline) after the first year of commencement of CAB [235]. Morote et al. measured BALP levels annually for five years but changes in BALP levels immediately after castration were not determined.

The present results suggest that biochemical changes in skeletal metabolism as reflected by serum BALP levels may not be evident as early as 3 months following ADT with LHRHa. However, this study is limited by the small sample size and comparison involving baseline and 3 month assessments. Another important limitation is the use of serum BALP measurements only which is one of the many markers of bone turnover. A larger prospective future study using serial assessments of multiple markers will help in determining the effects of acute reduction in sex hormones as a result of ADT on bone metabolism.

5.3 Odour study

The present pilot study investigated changes in odour of patients with PC following ADT with LHRHa considering the acute suppression of sex hormones seen with this treatment and the possible links between odour and sex steroid metabolism. The study was based on earlier work suggesting that body odour is mediated predominantly by axillary secretions and that metabolites of sex steroid biosynthetic pathway are important constituents of axillary secretions [74]. The results from this experiment did not show any perceived difference in odour after 3 months of LHRHa therapy suggesting odour signalling is not affected following LHRHa therapy (Tables 16-18). Serum testosterone and oestradiol levels were significantly reduced following treatment ($p=0.000$) (Table 19-21) (Figure 53).

An important consideration while interpreting these results is that in addition to the androstene steroids derived from metabolic pathways involving sex hormones, axillary secretions contain a wide array of odorant molecules including cholesterol, cholesteryl esters, unsaturated / hydroxylated branched fatty acids and sulfanylalkanols [73]. Thus, the suppression of sex hormone production as seen with ADT may not be a major factor influencing odour generation. Moreover, serum levels of sex hormones studied are in the nano- and pico-molar ranges (Table 20) and potential associated changes in secretory profile of axillary glands due to inhibition of sex steroid biosynthesis may not be sufficient to induce perceivable alterations in odour. A previous study by Gildersleeve et al. suggested that hormonal changes occurring during the menstrual cycle result in perceived differences in female odour. Their study showed men to discriminate scents of women in the high-fertility phase of menstrual cycle from the low-fertility phase ($p<0.001$). Men also rated high-fertility scents as more attractive than low-fertility scents ($p<0.001$) [66]. Unlike the present study, their study employed cotton gauze pads worn by women in both underarms for a period of 24 hours to collect odour samples. Differences in sampling method and length have been shown to influence perceived body odour quality [342].

Mitro et al. previously highlighted human ability to discriminate age based on emitted body odour and that effect was shown to be mediated mainly by odours of old age individuals [72]. An earlier study of body odour composition using gas-chromatography mass spectrometry (GCMS) detected the presence of 2-Nonenal, a strongly odoriferous aldehyde derived from oxidative degradation of fatty acids, only in older individuals (40 years or older) and its levels were shown to increase with advancing age [353]. In this current study, odour samples were provided by elderly men with PC (average age 69.6 years) and any potential changes in odour perception as a result of castration may have been masked by odour constituents such as 2-Nonenal which are known to be elevated in the older age group.

There are several limitations of this study. The sampling technique employed involved participant's wearing T-shirts in bed for two consecutive nights and following the instructions provided about dietary restrictions and hygiene. Many studies on odour signalling conducted previously employed this sampling method [71, 342, 343]. Although clear directions were given and patients were asked about any reservation or problem regarding compliance to those instructions, there were no objective means to determine non-adherence to instructions which could mask or contaminate the natural odour. Thus, any such deviation from given instructions is likely to have affected the outcome. Recruitment and subsequent odour sampling were done across all seasons which is another limitation of the study as anecdotal observations suggest seasonal variations in axillary sweat production. Personal grooming habits such as shaving of axillary hair has also been shown to have an effect on axillary odour [352]. The action of local cutaneous microflora on constituents of axillary sweat from apocrine, eccrine and sebaceous glands is known to alter odour characteristics [351]. The sampling method used did not control for these factors.

The donor odour samples were rated by young adult male and female volunteers for their attractiveness, masculinity and intensity. There is great diversity in human olfaction which is known to be mediated mainly by genotypic variations [354]. The reliability of ratings may have been compromised due to such variability in rater's individual olfactory ability although

relatively large number of raters were employed to minimise this effect. These inadequacies of the sampling methods and subjective assessments need to be revisited in future studies.

Future work to determine changes in odour profile in the clinical setting of ADT for PC should be done using analytical sampling procedures and instrumental techniques like GCMS [355-357] which can help identify and quantify any odoriferous component affected by the treatment.

6. Translational potential and future research

The cognitive data obtained from this pilot project will help design future research studies aimed at developing imaging biomarkers for earlier detection of ADT related cognitive decline and to improve understanding of the mechanisms of such worsening in cognitive function. Such work can potentially lead to the development of a commercial imaging agent for use in the early diagnosis of cognitive deterioration and enable identification of potential interventions for slowing / preventing this unwanted and serious treatment complication, thereby improving the QoL of not only PC survivors but their families as well. Mechanistic information on the development of cognitive dysfunction may provide important information to clinicians in assisting patients with PC to choose alternate treatments with potentially reduced toxicity (e.g. parenteral oestrogen). The standardised neuropsychometric testing employed in this study can also find routine use in the diagnostic and follow-up clinics, to gauge the cognitive health of patients under treatment for PC. Highlighting the pathological events in cognitive decline associated with contemporary ADT with LHRHa will have significant impact on future studies on dementia, far wider than ADT alone.

The CT-based methodology employed in this project for calculating BVF may possibly lead to improved clinical determination of the skeletal status of patients. Further work in-vivo on this non-invasive clinically accessible technique for assessment of bone quality may yield an efficient diagnostic and treatment modality which can be employed in a range of conditions affecting bone health such as ADT for PC. More widely, the BVF measurement technique could be useful for identifying early osteoarthritis and osteoporosis that are characterised by increasing and decreasing bone mass respectively. Measurement of bone turnover markers such as BALP is another approach for evaluating skeletal health which is commonly used in the management of postmenopausal women. The present work suggests a limitation of this approach as the sole method of monitoring early bone changes in men receiving ADT with LHRHa. This may be evaluated further in a larger study and also with alternative treatment options (parenteral oestrogen). Combination of skeletal imaging and bone markers can also

be investigated in future studies to ascertain their potential for clinical use in monitoring the rapid effects of androgen suppression on bone health.

The odour experiment did not show a change in perceived character of odour following LHRHa treatment. However, the study had some limitations which may be overcome in future work using objective analytical techniques like GCMS. Documenting changes in body odour of men receiving ADT for PC may pave way for investigating the psycho-socio-sexual impact of variations in smell signalling on dyadic relationships. Future metabolomic analysis of odour profile may help identify and characterise the chemo-signals involved in odour signalling. Such work may lead to development of pheromone-like odorants for patients' use to overcome any potential negative impact of ADT on patient-partner relationships.

The pilot results obtained and the observations noted from the current project will help in the development and execution of future comprehensive research studies whose findings are likely to benefit a large population of men with PC having iatrogenic sex hormone change as a result of ADT. On similar lines, further work can be designed to include other less well-studied but serious toxicities of LHRHa treatment like metabolic syndrome, renal and haematological problems. Moreover, the scope of this project can be extended to other clinical matters which involve physiological, pathological or therapeutic changes in sex hormones including menopausal syndrome, hormonal contraception, PCOS and breast cancer in females, late-onset hypogonadism and testosterone supplementation in males and gender dysphoria in both sexes.

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Appendix I - Publications

Original Research

- **Shah SIA**, Jin A, Wilson HCP, Abel PD, Price P, Hansen U, Abel RL. Novel computed tomography-based metric reliably estimates bone strength, offering potentially meaningful enhancement in clinical fracture risk prediction. European Journal of Medicine 2015;10:214-220.

Reviews

- Wilson HCP, **Shah SIA**, Abel PD, Price P, Honeyfield L, Edwards S, Abel RL. Contemporary hormone therapy with LHRH agonists for prostate cancer: avoiding osteoporosis and fracture. Central European Journal of Urology 2015;68:165-8.
- **Shah SIA**. Emerging potential of parenteral estrogen as androgen deprivation therapy for prostate cancer. South Asian Journal of Cancer 2015;4:95-7.
- Phillips I, **Shah SIA**, Duong T, Abel P, Langley RE. Androgen deprivation therapy and the re-emergence of parenteral estrogen in prostate cancer. Oncology & Hematology Review 2014;10:42–7.

Letters / Comments

- **Shah SIA**, Wilson HCP, Abel PD. First do no harm, second do some good, third give choice and fourth save cash – the 1, 2, 3 & 4 of transdermal estradiol as androgen deprivation therapy 'ticks all the boxes'. Internal Medicine Journal 2016;46:241-3.
- Wilson HCP, Abel PD, **Shah SIA**. Repeated vertebral augmentation for new vertebral compression fractures of postvertebral augmentation patients: a nationwide cohort study – how useful is the current clinical gold standard for fracture risk? Journal of Clinical Interventions in Aging 2015;10:1653-5.

- Wilson HCP, **Shah SIA**, Abel P, Price P, Abel R. Re: Overdiagnosis of bone fragility in the quest to prevent hip fracture- before we reach breaking point. British Medical Journal 2015 Published online, retrieved from http://www.bmj.com/content/350/bmj.h2088/rr-17
- Wilson HCP, **Shah SIA**, Price P, Abel PD. Re: State-of-the-art treatment in castration-resistant prostate cancer - forward to the past – again. European Medical Journal (Oncology) 2015 Published online, retrieved from http://emjreviews.com/therapeutic-area/oncology/re-state-of-the-art-treatment-in-castration-resistant-prostate-cancer-forward-to-the-past-again/
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- Abel PD, **Shah SIA**. Re: Finding Help for ADT-induced hot flashes: hormonal therapies. Johns Hopkins Health Alerts 2013. Published online, retrieved from http://www.johnshopkinshealthalerts.com/alerts/prostate_disorders/ADT-Induced-Hot-Flashes_6665-1.html

Conference abstracts

- **Shah SIA**, Jin A, Hansen U, Abel R, Cobb J. Quantitative assessment of bone quality using low-resolution clinical-CT. Conference Abstract #3135 EFORT Congress London 2014 (Oral presentation)
- **Shah SIA**, Saleem A, Mangar S, Price P, Hampshire H, Jones T, Puri BK, Coello C, Mukerji S, Abel PD. Cognitive impairments following androgen deprivation therapy for prostate cancer. Male Psychology Conference, University College London 2015 (Poster)
- Wilson HCP, **Shah SIA**, Abel PD. 'PATCH'-ing up toxicities of ADT for prostate cancer. Conference Abstract European Society for Medical Oncology (ESMO) Asia Congress, Singapore 2015 (Poster).
- Wilson HCP, **Shah SIA**, Price P, Saleem A, Craig C, Stewart M, Arnold M, Abel PD. Maximizing benefit of a clinical trial as new hypotheses are generated: 'PATCH'ing up toxicities of contemporary ADT for advanced prostate cancer. The British Association of Urologic Surgeons (BAUS) Section of Academic Urology Meeting, London 2015 (Poster).
- Saleem A, Mangar S, Abel P, Coello C, Mendoza N, Mukherjee S, **Shah SIA**, Jones T, Price P, Roncaroli F. Evaluation of the patho-physiological basis of elevated translocator protein (TSPO) expression in patients with solid tumours. Conference Abstract #B52 National Cancer Research Institute (NCRI) Cancer Conference, Liverpool 2015.
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Novel Computed Tomography-based Metric Reliably Estimates bone Strength, Offering Potentially Meaningful Enhancement in Clinical Fracture Risk Prediction

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Abstract

Osteoporosis with resultant fractures is a major global health problem with huge socio-economic implications for patients, families and healthcare services. Areal (2D bone mineral density (BMD) assessment is commonly used for predicting such fracture risk, but is unreliable, estimating only about 50% of bone strength. By contrast, computed tomography (CT) based techniques could provide improved metrics for estimating bone strength such as bone volume fraction (BVF; a 3D volumetric measure of mineralised bone), enabling cheap, safe and reliable strategies for clinical application, and to help divert resources to patients identified as most likely to benefit, meeting an unmet need.

Here we describe a novel method for measuring BVF at clinical-CT like low-resolution (550μm voxel size). Femoral heads (n=8) were micro-CT scanned *ex-vivo*. Micro-CT data were downgraded in resolution from 30μm to 550μm voxel size and BVF calculated at high and low resolution. Experimental mechanical testing was applied to measure *ex vivo* bone strength of samples. BVF measures collected at high-resolution showed high correlation (correlation coefficient r^2=0.95) with low-resolution data. Low-resolution BVF metrics showed high correlation (r^2=0.96) with calculated sample strength. These results demonstrate that measuring BVF at low resolution is feasible, which also predicts bone strength. Measures of BVF should be useful for

REVIEW PAPER

Contemporary hormone therapy with LHRH agonists for prostate cancer: avoiding osteoporosis and fracture

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Introduction Prostate cancer is a large clinical burden across Europe. It is, in fact, the most common cancer in males, accounting for more than 92,300 deaths annually throughout the continent. Prostate cancer is androgen-sensitive; thus an androgen deprivation therapy (ADT) is often used for treatment by reducing androgen to castrate levels. Several ADT agents have achieved benefits with effective palliation, but, unfortunately, severe adverse events are frequent. Contemporary ADT (Luteinising Hormone Releasing Hormone agonist- LHRHa injections) can result in side effects that include osteoporosis and fractures, compromising quality of life and survival.

Methods In this review we analysed the associated bone toxicity consequent upon contemporary ADT and based on the literature and our own experience we present future perspectives that seek to mitigate this associated toxicity both by development of novel therapies and by better identification and prediction of fracture risk.

Results Preliminary results indicate that parenteral oestrogen can mitigate associated osteoporotic risk and that CT scans could provide a more accurate indicator of overall bone quality and hence fracture risk.

Conclusions As healthcare costs increase globally, cheap and effective alternatives that achieve ADT, but mitigate or avoid such bone toxicities, will be needed. More so, innovative techniques to improve both the measurement and the extent of this toxicity, by assessing bone health and prediction of fracture risk, are also required.

Key Words: prostate cancer ‹› androgen deprivation therapy ‹› luteinising hormone releasing hormone agonist ‹› osteoporosis ‹› fracture imaging

INTRODUCTION

The discovery by Huggins in the 1940s that prostate cancer is androgen-sensitive led to the development of therapies, with differing mechanisms of action, to achieve castrate levels of androgen (ADT). Unfortunately, these agents often also had major unwanted side effects, such as osteoporosis and fractures, with ADT achieved with contemporary LHRH agonists (LHRHa) [1]. As both the clinical and financial burden surrounding prostate cancer grows [2], cheap and effective alternatives that achieve ADT but mitigate such bone toxicities are required.

Mechanisms of action and toxicity with contemporary hormonal treatment of prostate cancer

Prostate cancer cell growth is usually androgen-dependent [3], through stimulation of androgen receptors for growth and proliferation [4]. ADT in men, either medically (LHRHa) or by surgical orchiectomy, suppresses serum concentrations of both androgens and oestrogen to less than 5% and 20% of normal values respectively (oestradiol is synthesised in males by the aromatisation of testosterone) [5]. These very low sex hormone levels result in potentially major toxicities, including hot flushes, sarcopenia, erectile

Emerging potential of parenteral estrogen as androgen deprivation therapy for prostate cancer

Syed Imran Ali Shah

Abstract

Androgen deprivation therapy (ADT) is a key management strategy for prostate cancer (PC), achieved commonly by administration of luteinizing hormone-releasing hormone agonist (LHRHa). ADT markedly suppresses both male and female sex hormones which results in "castration syndrome", a constellation of adverse events such as muscle weakness, impairment of glucose and lipid metabolism, impotence, osteoporosis, and fractures. Recent evidence suggests that estrogen, in the parenteral form, may emerge as an alternative to LHRHa as it offers potential benefits of arresting PC growth as well as avoiding some of the estrogen deficiency related toxicities of LHRHa by maintaining endogenous levels of estrogen.

Key words: Androgen deprivation therapy, parenteral estrogen, prostate cancer

Introduction

Prostate cancer (PC) is the most common malignancy and the second most common cause of cancer death affecting men in the western world.[1] PC incidence has risen rapidly in Asia where people are known to have the lowest risk of this disease. Factors responsible for this rapid rise include aging population, westernized dietary habits, and increasing use of prostate-specific antigen (PSA) testing.[2] Prostate cells, normal or cancerous, are dependent upon androgens for survival and growth. Consequently, androgen deprivation therapy (ADT) (commonly called hormone therapy) is the mainstay of PC treatment. Surgical or medical interventions resulting in the reduction of testosterone or blockade of the androgen receptor are referred to as ADT. ADT was initially achieved by orchiectomy as the testes are the principal source of circulating androgens (producing nearly 95% of total); the remaining 5% are produced by the adrenal glands. Luteinizing hormone-releasing hormone agonist (LHRHa) is the most widely administered contemporary ADT modality usually offered following a diagnosis of advanced (incurable) disease either at presentation, following failure of radical therapy with curative intent and as adjuvant or neo-adjuvant to radical radiotherapy for localized disease.[3] This review focuses on the potential of parenteral estrogen as an alternative option to LHRHa for ADT.

Luteinizing Hormone-Releasing Hormone Agonist and Castration Syndrome

Introduced in the 1980s, LHRHa acts by down-regulating gonadotrophin receptors in the pituitary, thereby causing central hypogonadism [Figure 1]. However, initial exposure to LHRHa leads to a "testosterone flare", which can exacerbate symptoms in a few patients like worsening bone pain from skeletal metastasis. The flare phenomenon is blocked by giving anti-androgens, a week before administering LHRHa.[4] Contemporary LHRHa as ADT delivers up to a 95% reduction in endogenous testosterone levels, which in turn results in suppression of endogenous estrogen (by about 80%) as it is derived from testosterone.[5]

The iatrogenic hypogonadism resulting from LHRHa therapy causes unwanted side effects including sarcopenia, anemia and erectile dysfunction (from testosterone deficiency) and osteoporosis (with high risk of fractures), hot flushes and probably, cognitive impairment (menopausal symptoms from estrogen deficiency) [Figure 2].[6] These LHRHa toxicities are labeled as "castration syndrome" which has a huge impact not only upon the quality-of-life (QOL) but also on the overall cost of treating PC and on the health economy.[7]

Oral Estrogen

Long before the advent of LHRHa, diethylstilbestrol (DES), a synthetic oral estrogen, was the first pharmacological agent used as an effective and inexpensive ADT for PC. DES acts by lowering androgen production via a negative feedback loop affecting the hypothalamic-pituitary-testicular axis [Figure 1]. The Veterans Administration Cooperative Urological Research Group (VACURG) conducted a series of randomized clinical trials between 1960 and 1975, comparing surgical orchiectomy, DES, and combination of both for the treatment of newly diagnosed PC.[8] Despite showing greater efficacy than orchiectomy, DES was discontinued from routine clinical use as results from the VACURG trials showed that DES caused cardiovascular (CVS) toxicity in up to 35% of patients with 15% experiencing a thromboembolic event. CVS mortality was shown to be lower after therapy with low dose DES (1 mg) as compared to high dose DES (5 mg) without any change in oncological effect. More recently, fosfestrol, another synthetic estrogen, was shown to be effective in controlling castration-resistant PC in terms of declining PSA levels but its toxicity profile needs elaboration.[9]

It is now known that the thromoboembolic and CVS complications of oral estrogen are a consequence of direct exposure of the liver to high concentrations of estrogen through the portal circulation which leads to hepatic overexpression of proteins, including those involved in coagulation.[10]

Parenteral Estrogen

Parenteral estrogen appears to be a more suitable alternative to both LHRHa and oral estrogen in the treatment of PC. Research over the last two decades suggests that parenteral administration of estrogen as ADT (intramuscular or transdermal) avoids first-pass through the liver, thereby avoiding hepatic induction of pro-coagulant proteins and circumventing the CVS toxicity. Several recent studies have demonstrated that castrate levels of testosterone for PC growth arrest can be achieved by this strategy, with little effect on hemostatic profile [Table 1].

A series of studies was conducted by the Scandinavian PC Group (SPCG) using polyestradiol phosphate (PEP) administered

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Androgen Deprivation Therapy and the Re-emergence of Parenteral Estrogen in Prostate Cancer

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Abstract

Androgen deprivation therapy (ADT) resulting in testosterone suppression is central to the management of prostate cancer (PC). As PC incidence increases, ADT is more frequently prescribed, and for longer periods of time as survival improves. Initial approaches to ADT included orchiectomy or oral estrogen (diethylstilbestrol [DES]). DES reduces PC-specific mortality, but causes substantial cardiovascular (CV) toxicity. Currently, luteinizing hormone-releasing hormone agonists (LHRHa) are mainly used; they produce low levels of both testosterone and estrogen (as estrogen in men results from the aromatization of testosterone), and many toxicities including osteoporosis, fractures, hot flashes, erectile dysfunction, muscle weakness, increased risk for diabetes, changes in body composition, and CV toxicity. An alternative approach is parenteral estrogen, it suppresses testosterone, appears to mitigate the CV complications of oral estrogen by avoiding first-pass hepatic metabolism, and avoids complications caused by estrogen deprivation. Recent research on the toxicity of ADT and the rationale for revisiting parenteral estrogen is discussed.

Keywords

Prostate cancer, estrogen, testosterone, LHRH agonist, PATCH trial, androgen deprivation therapy (ADT)

Disclosure: Paul Abel, MB ChB, FRCS, and Ruth E Langley, MBBS, MRCP, are co-chief investigators of the PATCH trial, which is an academic study funded by Cancer Research UK sponsored by the MRC Clinical Trials Unit at UCL. Iain Phillips, MBBS, MRCP, Syed I A Shah, MBBS, and Trinh Duong, MSc, have no conflicts of interest to declare.
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This review describes strategies for producing castrate levels of testosterone in men with androgen-sensitive prostate cancer (PC) and the associated toxicities, with particular focus on the re-emergence and potential benefits of parenteral estrogen. In the developed world, PC is the commonest malignancy and second commonest cause of cancer death affecting men. Its incidence is increasing with an aging population and frequent prostate-specific antigen (PSA) testing.[1] Almost 240,000 new cases of PC are diagnosed each year in the US and nearly 30,000 American men die from PC annually.[2]

Androgen Deprivation Therapy

The androgen dependence of PC has been recognized since the 1940s and remains a major component of the strategies used to manage PC today.

Surgical Orchiectomy and Oral Estrogen

Androgen deprivation therapy (ADT) was initially achieved by surgical orchiectomy, as the testes produce nearly 95 % of circulating androgens; the remaining 5 % is produced by the adrenal glands.[3] As surgical castration is invasive and can cause significant psychological trauma it became less common following the introduction of medical (also called chemical) ADT. Diethylstilbestrol (DES), a synthetic oral estrogen, was the first pharmacological agent used as ADT for PC. The primary mechanism of action of DES involves a negative feedback loop affecting the hypothalamic–pituitary–testicular axis.[4,5] The pulsatile secretion of hypothalamic luteinizing hormone-releasing hormone (LHRH) stimulates the release of follicle stimulating hormone (FSH) and LH from the anterior pituitary, which then stimulates testicular Leydig cells to produce testosterone. DES remained an effective and low-cost option for ADT from the 1950s up to the 1980s, but its use was discontinued following findings of adverse cardiovascular system (CVS) outcomes from the Veterans Administrative Cooperative Urological Research Group (VACURG) trials. Initiated in the early 1960s, this series of randomized clinical trials compared DES with orchiectomy, placebo, DES plus orchiectomy, and placebo plus orchiectomy. Although DES improved PC outcomes, the DES groups were shown to have increased CVS toxicity (36 % increase in non-cancer-related deaths mostly CVS) with the highest risk in the first year of starting therapy.[6]

Luteinizing Hormone-releasing Hormone Agonists and Anti-androgens

Luteinizing hormone-releasing hormone agonists (LHRHa), also called gonadotrophin-releasing hormone analogs (GnRHa), were introduced

Tripodi has stated that the widely held and promoted notion that laboratory monitoring is not required for patients treated with non-vitamin K antagonist oral anticoagulants has been overemphasised, potentially leading clinicians to believe that laboratory testing is not needed for these patients.[4] Our data support Tripodi's viewpoint. Other potential reasons for our findings include greater familiarity with warfarin than dabigatran and uncertainty in the interpretation of coagulation test results with dabigatran. Management guidelines that utilise these tests, for both thromboembolic and haemorrhagic events, have been proposed.[5,6] Studies assessing clinical outcomes from using these guidelines will be valuable in informing practice in this area of uncertainty.

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General correspondence

First, do no harm, second, do some good, third, give choice and fourth, save cash: the 1, 2, 3 and 4 of transdermal oestradiol as androgen deprivation therapy ticks all the boxes

It was interesting to read the review by Lomax *et al.* offering current perspectives on management and prevention of the complications of androgen deprivation therapy (ADT) for prostate cancer (PC) to improve patient quality of life (QoL).[1] Contemporary ADT with luteinising hormone-releasing hormone agonist (LHRHa) delivers a drop in testosterone levels of up to 95% followed by lowered oestradiol of about 80% (because testosterone is the substrate for synthesis of oestradiol by aromatase).[2] This iatrogenic hypogonadism is at the heart of the multiple metabolic toxicities of LHRHa including sarcopenia, adiposity and diabetes (linked to testosterone deficiency), loss of bone mass and dyslipidaemia (linked to oestrogen deficiency) and heightened cardiovascular (CVS) risk.[3]

Before the introduction of LHRHa, oestrogen given orally as diethylstilboestrol was used as ADT, but this use was rapidly diminished in the 1980s owing to concerns over thromboembolic and CVS toxicities.[4] It is now known that delivery by an oral route escalates this risk by hepatic exposure to high concentrations of oestrogen via the portal circulation, causing upregulation of synthesis of coagulation factors and a hypercoagulable state.[5] Oestrogen given parenterally (e.g. intramuscular or transdermal) avoids hepatic-portal flow thereby circumventing the CVS complications due to its oral administration.[6,7] In this context, it was somewhat disappointing that this review overlooked the slow but gradual renaissance of parenteral oestrogen, offering the potential to deliver both the following: (1) ADT for PC and (2) avoiding unwanted side-effects of oestradiol

Disclosure: S. I. A. Shah is a Commonwealth Scholar, funded by the UK government, and has served as an advisor and speaker for BHR Pharma, LLC.

Clinical Interventions in Aging

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LETTER

Repeated vertebral augmentation for new vertebral compression fractures of postvertebral augmentation patients: a nationwide cohort study – how useful is the current clinical gold standard for fracture risk?

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Dear editor

Further to the recent publication on the "Repeated vertebral augmentation for new vertebral compression fractures of postvertebral augmentation patients: a nationwide cohort study",[1] current data highlight the limitations of dual-energy X-ray absorptiometry scans. In this context, at best, dual-energy X-ray absorptiometry scans (which measure bone mineral density) can account for no greater than 50% of overall bone strength (defined as the ability to resist fracture). This is because the resulting images are two-dimensional and therefore unable to capture skeletal micro-architecture, which also contributes to bone strength.[2]

A better clinical measure of overall bone strength that more accurately reflects the ability of that bone to resist fracture and hence fracture risk reflect an unmet need and is urgently required. Recent evidence suggests that micro-computed tomography scans, which enable three-dimensional imaging, might provide a solution but use so far has necessarily been limited to ex vivo assessment owing to radiation hazards as well as technical and accessibility issues.[3,4] However micro-computed tomography images have identified bone volume fraction (the volumetric distribution of bone mass) as a strong determinant of bone strength ($r^2>0.8$).[5,6]

Further, perhaps other potential tools, alone or in combination with imaging may also play a role. For example, serum biomarkers of bone metabolism[7,8] along with other imaging modalities such as magnetic resonance imaging could capture the complex factors that make up bone strength.[9] Preexisting algorithms like the FRAX (a fracture risk assessment tool calculator)[10] might help reduce the overprediction issue currently faced.

With regard to the aforementioned evidence, there is a pressing need to consider first how we use bone densitometry in the diagnosis of osteoporosis in prostate cancer patients, before the National Health Service itself becomes fractured.

Disclosure

The authors report no conflicts of interest in this communication.

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Re: Overdiagnosis of bone fragility in the quest to prevent hip fracture- before we reach breaking point

05 June 2015

Hannah CP Wilson
Dr Imran Shah, Prof Paul Abel, Prof Pat Price, Dr Richard Abel
Imperial College London

Further to the recent publication on over-prediction of hip fracture risk and ensuing challenges (1), current data highlight the limitations of Dual-Energy-X-ray-Absorptiometry scans (DEXA). In this context, at best, DEXA scans (which measure bone mineral density (BMD)) can account for no greater than 50% of overall bone strength (defined as the ability to resist fracture). This is because the resulting images are two-dimensional (2D) and therefore unable to capture skeletal micro-architecture, which also contributes to bone strength (2).

Clearly, better clinical measures of overall bone strength that more accurately reflect the ability of that bone to resist fracture represent an unmet need and are urgently required. Recent evidence suggests that micro-Computed Tomography (CT) scans, which enable 3D imaging, might provide a solution but use so far has necessarily been limited to ex vivo assessment owing to radiation hazards as well as technical and accessibility issues (3, 4). However micro-CT images have identified bone volume fraction (BVF, the volumetric distribution of bone mass) as a strong determinant of bone strength ($r2 > 0.8$) (5,6).

Other potential tools, alone or in combination with imaging may also play a role. For example serum biomarkers of bone metabolism (7,8) along with other imaging modalities such as magnetic resonance imaging could capture the complex factors that make up bone strength (9). Input of such data into pre-existing algorithms like the FRAX (a Fracture Risk Assessment tool calculator) (10) might help reduce the over-prediction issue currently faced.

Clinical trials of medications for non-bone disease, that nonetheless may also have the unwanted effect of inducing rapid changes to bone strength, could offer opportunities for accelerating the assessment of currently developing fracture prediction tools. An example is a sub-study of PATCH, (Prostate Adenocarcinoma TransCutaneous Hormones (MRC PR09)), an ongoing National Phase III randomized clinical trial (ClinicalTrials.gov number NCT00303784) comparing efficacy and toxicity of LHRHa (lutenising hormone releasing hormone analogues) and oestradiol transdermal patches in suppressing testosterone to castrate levels (androgen deprivation therapy (ADT)). LHRHa delivers this through suppression of (initially) testosterone (by about 95%), and then oestradiol (by about 80%; oestradiol is synthesized in men from testeosterone through aromatase). Lack of these sex hormones rapidly alters the balance of activity between osteoblasts and osteoclasts resulting in a loss of up to 10% BMD in the first year of ADT and 2-4% annually thereafter (11) and so development of osteoporosis. Conversely, whilst transdermal oestradiol patches (EP) also lead to suppression of testosterone, the 'lost' endogenous oestradiol is replaced by the exogenous oestradiol patches, resulting overall in a gain of BMD (12,13).

RE: STATE-OF-THE-ART TREATMENT IN CASTRATION-RESISTANT PROSTATE CANCER - FORWARD TO THE PAST - AGAIN

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Castration-resistant prostate cancer (CRPC) management is enjoying a renaissance following the marginal but significant beneficial impact of recent novel advances in medication on progression-free and overall survival, timely reviewed by Castro et al.[1] The Nobel prize-winning work of Huggins in the 1940s revealed the prostate to be hormone-sensitive and to shrink when androgen-deprived, and this was the inspiration for the era that followed and what has taken us through to this point in time.[2] Androgen deprivation therapy (ADT), as first-line therapy in the management of advanced prostate cancer (PrC), was initially prescribed in the form of oral oestrogen (diethylstilbestrol [DES]; i.e. medical orchiectomy at a very high dose of 5 mg three times per day) or surgical orchiectomy. However, the use of DES was abandoned when it was shown to be associated with serious and often fatal thromboembolic and cardiovascular (CVS) events in more than one-third of men.[2] This outcome was later shown to be due to the effect of oral oestrogen absorption via the hepatic–enteric circulation, which bathes the liver in a high concentration of oestrogen and induces pro-coagulation factors. The VACURG studies later showed this effect to be markedly reduced when oral DES was used at lower doses (1-3 mg once daily), which led to oestrogen regaining some of its reputation as ADT in certain instances. Consequently, it is now often forgotten that (low-dose) DES has also played a role in second-line treatment for CRPC and has been reported to lead to improved outcomes in some men. For example, Wilkins et al.[3] described how almost 30% of 231 men with CRPC treated with DES at a dose between 1-3 mg once daily exhibited a prostate-specific antigen response for a median duration of 4.6 months. So it is disappointing, if unsurprising, that once again and despite many supportive data,[2,4-7] the long-established and still-evolving contemporary literature demonstrating a role for oestrogen as effective and possibly safer therapy in men with CRPC received no mention in this review.

Oestrogen acts on PrC cell growth by several mechanisms. One example is suppression of the hypothalamic—pituitary—gonadal axis through feedback inhibition, which is the same mechanism through which contemporary ADT (luteinising hormone receptor hormone analogue [LHRHa], the compound which replaced DES as first-line medical orchiectomy) acts.[8] However, with the passage of time, LHRHa is itself increasingly recognised to be associated with major toxicity because it leads not only to a reduction in testosterone levels of up to 95%, but also to suppression of endogenous oestrogen by about 80%.[9,10] This suppression is due to oestrogen in men being derived from testosterone through the action of the enzyme aromatase. In contrast, if sufficient oestrogen is applied parenterally, then not only are castration-associated levels of testosterone reached, but exogenous oestrogen replaces the lost endogenous oestrogen so that overall oestrogen levels remain high, with the liver being avoided and the CVS effects minimised.[5,11] This allows for the potential mitigation of toxicities due to oestrogen deficiency itself, such as osteoporosis, improving bone mineral density,[12] cognitive impairment, and disturbances of lipid metabolism,[11] and thus makes it possible to reconsider oestrogen as an attractive option in hormonal therapy.

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LETTER TO THE EDITOR

Parenteral oestrogen: Effective and safer than both oral oestrogen and contemporary androgen deprivation therapy for prostate cancer?

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Dear Editor,

In this era of emerging novel, complex prostate cancer treatments, it was refreshing to review a historical perspective of the merits of oestrogen, the first pharmacological agent used as androgen deprivation therapy (ADT), i.e. suppression of testosterone by about 95% to castrate levels [1]. Prescribed for oral use as diethylstilboestrol, it had to be discontinued because of the resulting high cardiovascular toxicity and deaths, despite evidence of greater efficacy than orchiectomy as a prostate cancer treatment [2]. Although oestrogen suppresses testosterone primarily through the hypothalamic–pituitary–gonadal axis, additional mechanisms similar to those of some of the newest therapies under investigation may account for this greater efficacy, e.g. inhibition of 17,20-lyase and consequent extragonadal steroid synthesis, an abiraterone-like action [1].

ADT was subsequently achieved by replacing oral oestrogen with luteinizing hormone-releasing hormone agonist (LHRHa), which also suppresses testosterone to castrate levels. The rate of testosterone suppression is even more marked with an LHRH antagonist [3]. However, this reduction in testosterone also results in suppression of the endogenously produced oestrogen by about 80%. Oestrogen in men is derived from aromatization of testosterone [4,5]. The resulting iatrogenic hypogonadism (of both male and female sex hormones) causes unwanted side-effects including sarcopenia, anaemia and erectile dysfunction (from testosterone depletion), and osteoporosis (with a high risk of fractures), hot flushes and, probably, cognitive impairment (from oestrogen depletion) [6].

Can the potential benefits of oestrogen be realized without the cardiovascular and hypogonadal risks of the oral route?

Parenteral oestrogen administration (intramuscular as polyoestradiol phosphate or transdermal as oestradiol) avoids first pass through the liver and the consequent hepatic overexpression of proteins, including those affecting coagulation, thereby diminishing cardiovascular toxicity substantially [7,8]. Exogenous oestrogen also replaces suppressed endogenous oestrogen, potentially diminishing the oestrogen depletion-related toxicity of LHRHa. Thus, parenteral oestrogen as single-agent therapy offers potential benefits over either oral diethylstilboestrol or LHRHa through (i) treating the cancer, by suppressing testosterone to castrate levels, (ii) reducing the risk of cardiovascular hazards of oral oestrogen, and (iii) possibly avoiding some of the adverse effects of LHRHa by maintaining endogenous levels of oestrogen.

The Prostate Adenocarcinoma TransCutaneous Hormones (PATCH) study, funded by Cancer Research UK, is a randomized phase II trial comparing LHRHa with transdermal oestrogen patches in men with locally advanced or metastatic prostate cancer. Stage 1 of this study (n = 254) specifically addressed cardiovascular toxicity as the primary outcome, and data showed similar rates of cardiovascular

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Re: Androgen Deprivation Therapy: Impact on Quality of Life and Cardiovascular Health

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The recent comprehensive review by Trost et al. highlighting the impact of androgen deprivation therapy (ADT) with luteinising hormone-releasing hormone agonists (LHRHa) on quality of life (QoL) of prostate cancer survivors is timely in view of increasing usage and accumulating evidence of its harms [1]. LHRHa were developed because oral estrogen, a major option for treating prostate cancer through ADT in the second half of the 20th century, was discontinued due to its excessive cardiovascular (CVS) mortality [2]. The authors report a wide range of adverse events with LHRHa and include advice for monitoring and management options [1]. That contemporary ADT using LHRHa suppresses endogenous testosterone levels (by about 95%) is well known but that estrogen is derived from testosterone via aromatization and endogenous estrogen levels are consequently also reduced (by about 80%) is far less appreciated [3,4]. Thus in addition to achieving the treatment objective of arresting prostate tumor growth, the castrated male suffers unwanted side effects from this iatrogenic hypogonadism including sarcopenia, anemia, and erectile dysfunction (testosterone lack) and osteoporosis (with high risk of fractures), hot flushes and probably, cognitive impairment (estrogen lack) [5]. We concur that definitive evidence for the impact of castration on CVS mobidity/mortality remains wanting, but several observational studies support an increased risk of CVS complications [1,6]. Ironically, alternatives to LHRHa are now sought following the multiple comorbidities consequent on its use.

Parenteral administration of estrogen (intramuscular or transdermal) for ADT appears to mitigate the CVS toxicity associated with oral estrogen by circumventing first-pass liver metabolism [7–9]. By contrast, oral estrogen leads to direct exposure of the liver to high levels of estrogen through the portal circulation resulting in a hypercoaguable state through upregulation of liver biosynthesis including procoagulant proteins, and increasing the risk of serious CVS events [10]. Parenteral exogenous estrogen administration has the added benefit of replacing the reduced endogenous estrogen, thereby potentially avoiding the menopausal (estrogen lack) symptoms seen with LHRHa while simultaneously delivering the beneficial arterial effects associated with estrogen therapy [11]. Thus exogenous parenteral estrogen as monotherapy (especially important in older age groups) has the potential of both treating prostate cancer and reducing side effects when compared with LHRHa.

More recent evidence supporting parenteral estrogen therapy comes from the PATCH (Prostate Adenocarcinoma Trans Cutaneous Hormones) study; a phase II randomized clinical trial comparing transdermal estrogen patches with LHRHa in locally advanced and metastatic prostate cancer. In the first stage of the trial (n = 254, median follow-up time 19 months, primary outcome measure CVS events), the rate of CVS events was similar in the two trial arms [12]. Testosterone suppression rates were also similar. At 6 and 12 months, mean fasting cholesterol increased in the LHRHa arm but decreased in the estrogen arm whereas high-density lipoprotein cholesterol increased in both. Mean fasting glucose increased in the LHRHa group at 6 months and again further at 12 months but showed a decrease in the estrogen group at 6 months, which was maintained unchanged at 12 months. The second stage of the phase II study with a planned recruitment target of 730 men assessing progression-free survival as the primary outcome measure is nearing completion and will inform the design of a phase III clinical trial with overall survival as primary end point.

Further research should lead to the re-emergence of estrogen, in parenteral form, as an effective and cheaper alternative to LHRHa for the treatment of prostate cancer with reduced adverse events, enhancing patient choice when considering ADT. Until the availability of definitive results, effective monitoring is required for instituting timely prophylactic and/or therapeutic interventions for CVS and other serious toxicities of contemporary ADT with LHRHa.

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Letters

Fracture after androgen deprivation therapy among men with a high baseline risk of skeletal complications

Contemporary medical androgen deprivation therapy (ADT) to suppress testosterone for men with prostate cancer is achieved using LHRH agonists (LHRHa). This results, however, not only in reduced testosterone levels (by ~95%) but also reduced endogenous oestrogen levels (by ~80%); testosterone is the substrate for oestrogen through aromatization [1]. The use of LHRHa has increased such that it is now being employed progressively earlier during the natural history of the disease and for longer periods, often exceeding 10 years [2]; however, as a result of the loss of both male and female sex hormones, men on such long-term LHRHa also experience 'castration syndrome', a constellation of adverse events encompassing both a menopause and an analogous 'andropause', and can thereby develop serious toxicity including skeletal-related events (SKEs) such as osteoporosis and bone fracture. Shao *et al.* [3] add to the accumulating data concerning SKEs in this scenario. Their study identified men from a large population-based cohort with a diagnosis of localized prostate cancer ($n = 75\,994$) who had increased pre-existing risk of SKEs at the time of diagnosis. Should such men subsequently be treated with LHRHa, as their risk of SKEs would be expected to be correspondingly higher? Is there a way to achieve medical castration whilst at the same time avoiding SKEs?

Before LHRHa therapy, oral oestrogen was one of the main therapeutic options for treating prostate cancer. Use as first-line therapy was discontinued in the 1980s, as studies showed that men treated with oral oestrogen had cardiovascular toxicity [4], thought to be a direct result of hepatic first-pass of oestrogen-inducing pro-coagulant proteins. More recent research suggests that parenteral administration of oestrogen appears to circumvent this cardiovascular toxicity by avoiding hepatic first-pass metabolism [5–7]. Additionally, there is a benefit (through exogenous oestrogen replacement) of maintaining endogenous levels of oestrogen, potentially avoiding menopausal symptoms. Oestrogen is inexpensive compared with contemporary ADT (LHRHa) and, as a single therapy, not only treats the cancer (suppressing testosterone to castrate levels) but also replaces lost endogenous oestrogen (thereby avoiding some of the adverse events of LHRHa). Oestrogen is known to inhibit osteoclastogenesis and increase osteoclast apoptosis, thereby suppressing bone resorption. These effects have been studied extensively in the context of the female menopause [8].

In a previous small study of men ($n = 20$) treated with transdermal oestradiol patches for newly diagnosed locally advanced or metastatic prostate cancer, of 12 baseline osteoporotic/osteopenic regions (in five patients), four showed improvement based on the WHO grading after a year of therapy and bone mineral density increased at all measured sites over time [9]. In another, much larger, study ($n = 910$) with long follow-up (~9 years), no patient on parenteral oestrogen (i.m. polyestradiol phosphate) developed serious SKEs compared with 18 on combined ADT (anti-androgen with either LHRHa or bilateral orchidectomy) [6].

The Medical Research Council phase II randomized clinical trial, PATCH (Prostate Adenocarcinoma TransCutaneous Hormones), is comparing LHRHa with transdermal oestrogen patches in men with locally advanced or metastatic prostate cancer. In the first stage of this study ($n = 254$), similar rates of significant cardiovascular events (the primary outcome) were reported in both arms [10]. The second stage is ongoing and will assess efficacy based on progression-free survival in 730 men. A sub-study of the trial is comparing bone mineral density changes in the two trial arms, recruitment for which is now complete. Results from this sub-study, expected to be available in 2014, should further clarify the bone-protective role of oestrogen in this setting.

Based on such research, parenteral oestrogen may emerge as an effective single therapy for both treating prostate cancer and avoiding some of the adverse events associated with LHRHa administration, and may be particularly relevant for men with pre-existing risk of SKEs.

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BJC

LETTER TO THE EDITOR

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Comment on 'Endocrine therapy in prostate cancer: time for re-appraisal of risks, benefits and cost-effectiveness?'

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Sir,

Bourke *et al* (2013) raise important and topical issues concerning the expanding literature and consequent increasingly informed debate surrounding the risks, benefits and cost-effectiveness of androgen deprivation therapy (ADT) in advanced prostate cancer (Bourke *et al*, 2013). It was disappointing, therefore, that their review did not incorporate a more detailed perspective on the potential for a revival of oestrogen, particularly in the face of accumulating knowledge about its pharmacology, toxicity and costs. As they state, following the discovery of the excess cardiovascular toxicity with oral oestrogens, its use as first-line treatment was 'all but forgotten for the next 30 years', to be replaced by luteinising hormone releasing hormone agonist (LHRHa) therapy.

Castration with LHRHa as ADT delivers up to a 95% reduction in endogenous testosterone (T) levels, but results in toxicity including, for example, sarcopenia and erectile dysfunction (sometimes referred to as the male menopause or andropause). As noted by Bourke *et al* (2013), there is now also some evidence indicating an increased risk of cardiovascular disease. Further, as endogenous oestrogen is derived from T, castrate T levels result in suppression of oestrogen (by about 80%) causing toxicity, including osteoporosis and bone fractures, cognitive impairment and hot flushes (like in the female menopause; The Leuprolide Study Group, 1984; Garnick, 1986).

Exogenous oestrogen for ADT offers two major theoretical therapeutic benefits. First, the route of administration of oestrogen is of paramount importance for the development of cardiovascular toxicity. Oral oestrogen undergoes first pass through the liver, which gets bathed in high concentrations switching on pro-coagulant proteins. This does not appear to occur, at least not to the same extent, when oestrogen is given parenterally (Ockrim *et al*, 2005; Hedlund *et al*, 2008; Langley *et al*, 2008). Second, exogenous oestrogen replaces endogenous oestrogen, which would be lost through contemporary LHRHa administration (Ockrim *et al*, 2004). By contrast with the alternatives, exogenous oestrogen is also cheap and can, as a single agent, not only treat the cancer through T suppression but also avoid the use of additional, usually expensive, drugs to counter the often unpleasant toxicities associated with the menopausal side effects of LHRHa.

The Cancer Research UK funded PATCH study (Prostate Adenocarcinoma TransCutaneous Hormone) compares LHRHa with transdermal oestrogen patches in a phase II randomised clinical trial of men with locally advanced or metastatic prostate cancer. Stage 1 of this study ($n = 254$) specifically addressed cardiovascular toxicity as the primary outcome and the data showed similar rates of cardiovascular events in both arms (Langley *et al*, 2013). The phase II trial continues to recruit with a new primary outcome of progression-free survival in order to gain data on efficacy and help inform the decision to proceed to a phase III study with overall survival as the primary outcome. Data from the study, which include changes in lipid profiles and other metabolic factors over time, will also contribute to the evidence-base regarding an association between cardiovascular risk and LHRHa.

Further research may yet establish the use of parenteral oestrogen as a safe, effective and cheap single therapy for the treatment of prostate cancer, which could avoid some of the toxicities of present-day castration.

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BMJ
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Androgen deprivation treatment in prostate cancer
Re: Androgen deprivation treatment in prostate cancer

13 March 2013

The recent therapeutics review on androgen deprivation therapy (ADT, i.e. castration) in prostate cancer [1] is timely as several promising new treatments have been reported over the past year or so, although their effectiveness and timing in treatment pathways remain to be fully elucidated. Additionally, awareness of the many side-effects of castration with contemporary therapy (Luteinising Hormone Releasing Hormone Agonists (LHRHa) is increasing as indications for ADT expand, it is prescribed ever earlier in the natural history of the disease and men survive for increasingly long periods. These side-effects are due not only to endogenous testosterone suppression (resulting in loss of libido and sarcopenic obesity) but also suppression of endogenous oestrogen (as oestrogen production requires testosterone as a substrate) which can lead to osteoporosis and higher fracture risk, adverse lipid changes, hot flushes and potentially cognitive impairment [2].

Missing from this recent paper however, was a discussion about the gradual re-emergence of oestrogen to the ADT armamentarium. Oral oestrogen was an effective and low-cost option for ADT in the 1950's and up to the 1980's but was then abandoned as randomized trials showed that it was associated with increased cardiovascular toxicity and thromboembolism [3] and LHRHa were introduced. It is now apparent that the route of administration of oestrogen is pivotal to development of this toxicity; taken orally, entero-hepatic first pass exposes the liver to high levels of oestrogen resulting in up-regulation of pro-coagulant proteins and a hypercoaguable state which in turn increases the risk of thromboembolism and cardiovascular events [4]. This appears to be mitigated by giving oestrogen parenterally e.g. intramuscular [5] or transdermally [6]. Moreover, exogenous oestrogen not only suppresses testosterone but also replaces the endogenous oestrogen lost with LHRHa, and thereby has the potential to achieve the same therapeutic effect but with reduced side-effects, without the need for additional agents.

More evidence in favour of parenteral oestrogen therapy has recently been published with cardiovascular data from the first stage of the PATCH (Prostate Adenocacinoma Trans Cutaneous Hormones) study; a phase II randomized clinical trial comparing transdermal oestrogen patches with LHRHa in locally advanced and metastatic prostate cancer. In a cohort of 254 men (randomized 2:1 to patches or LHRHa) with a median follow-up of 19 months, the rate of cardiovascular events was similar in the two trial arms [7]. Castration rates were also similar. PATCH has since progressed to the second stage of the phase II study, assessing progression-free survival as the primary outcome measure with a non-inferiority design. Current recruitment exceeds 650 of the planned 730 men required for statistical analysis and to inform the decision to proceed to a confirmatory phase III study.

In summary, parenteral oestrogen seems to avoid the thromboembolic and cardiovascular toxicities associated with oral administration, and has the potential to both suppress testosterone to castrate levels and reduce or eliminate some of the serious toxicities of contemporary castration which result in significant morbidity and mortality (including osteoporosis with the increased risk of fracture, cognitive impairment and hot flushes), all in one single therapy. Furthermore, parenteral oestrogen is inexpensive. Thus, if efficacy is confirmed, this could be an important alternative to LHRHa in the management of prostate cancer.

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#3135

Quantitative Assessment Of Bone Quality Using Low-Resolution Clinical-CT

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keywords:Bone, Quality, Computed Tomography

Introduction: Bone is a metabolically dynamic tissue, undergoing structural remodelling in response to several factors like hormones, mechanical loading etc. Ageing is associated with imbalanced turnover and net loss of bone mass and structure. The ability of bone to resist fractures is dependent upon material composition and structure. Bone mineral density (BMD) measurement by dual energy x-ray absorptiometry (DEXA) is currently employed for clinical assessment of fracture risk. Bone densitometry, however, does not provide a complete picture of bone strength and quality, which includes various micro-structural parameters such as cortical distribution, trabecular microarchitecture, bone volume fraction etc. Sophisticated imaging techniques like micro computed tomography (µCT) are employed for this purpose but their use is limited to in vitro assessments owing to technical issues and radiation hazards. Development of non-invasive methods and clinically accessible techniques is required for clinical application. Many hospitals already posses and utilise medical grade CT scanners. Typically voxel size is about 0.5mm and resolution 2.5 mm.

Objectives: To develop a method for measuring bone mass (volume and density) distribution at 0.5mm voxel size using clinical-CT scanners. Then validate the clinical-CT method against high-resolution micro-CT data. Data will be interpreted with regard to usefulness for clinical assessment.

Methods: Femoral heads collected following hip replacement surgery for osteoarthritis and osteoporosis were micro-CT scanned at a voxel size of 30 µm using a Nikon HMX-ST CT system. The micro-CT data was serially downgraded in quality at 50, 100, 200, 400 and 550 µm voxel sizes. Bone image analysis was done on a high power workstation (HP Z800). Spheres of trabecular bone were virtually sectioned from 3D scans in the femoral head then a frequency distribution plot of the voxel grey values was plotted. A threshold grey value was calculated by finding the trough that separates bone and non-bone peaks. Trabecular bone volume and density were measured using the BoneJ plugin for ImageJ software. The accuracy of lower-resolution metrics was validated against the high-resolution scans.

Results: Measures of bone volume fraction/density collected at 30 µm were correlated with data collected at larger voxel size. At the largest voxel size r2=0.91 with 550 µm data.

Conclusions: The results show that it is feasible to measure bone mass at low voxel size. This is an easy access, non-invasive and non-destructive methodology, which can be applied clinically, and it can give much more information to the clinicians about bone health as compared to the currently employed bone densitometry technique. For example perhaps in-vivo medical grade CT data of femoral heads can be analysed using 3D finite element modelling to obtain objective assessments of bone strength. The CT scans collected for this study will be used to calculate the mechanical properties of the bone samples, which will be validated using experimental mechanical testing. Potentially relatively low resolution measures of bone mass distribution can be used to analyse variation in bone quality. The measures could be used to diagnose, monitor or value treatment outcomes for osteoporosis.

Cognitive impairment following androgen deprivation therapy for prostate cancer

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Background

Prostate cancer (PC) is androgen-dependent and androgen deprivation therapy (ADT) is a standard treatment for advanced disease. ADT has been linked to cognitive impairment (CI) but its pathologic basis is unknown and neuroinflammatory processes may possibly be involved. Positron emission tomography (PET) imaging using radioligand for neuroinflammatory marker translocator protein (TSPO) may serve as a sensitive pre-symptomatic imaging biomarker for ADT induced CI.

Objectives

- To demonstrate the presence of neuroinflammation in ADT induced CI using TSPO PET imaging
- To determine association between neuropsychological test scores and structural/functional magnetic resonance imaging (MRI) in ADT induced CI

Methods

PC patients aged 50-80 years on ADT for at least 3 months and up to a year are being recruited. Men who report CI since starting ADT will be compared with matched controls without noticeable cognitive change. All participants are administered a detailed battery of neuropsychological tests and only those with high-affinity binding TSPO polymorphism (determined by blood test) proceed for imaging.

Results

	Control (n=6) Mean ± SEM	CI (n=4) Mean ± SEM	p-value
Age (years)	64.8 ± 2.2	71.7 ± 4.3	0.156
ADT (months)	7.3 ± 1.1	7.2 ± 1.4	0.960
MMSE	27.8 ± 0.8	26.2 ± 1.3	0.324
WTAR	114 ± 3.8	105 ± 5.4	0.202
PT - Immediate	21.3 ± 3.7	13.0 ± 1.4	0.003*
PT - Delayed	6.8 ± 0.47	1.5 ± 0.64	0.000*
Letter Fluency	48.8 ± 2.4	31.0 ± 3.3	0.002*
CWT Word Reading	22.8 ± 0.7	26.7 ± 1.4	0.029*
CWT Inhibition	67.8 ± 2.5	114.0 ± 24.7	0.048*

Table. Descriptive and neuropsychological characteristics of study participants. **Difference is significant at p < 0.05*

MMSE- Mini-Mental State Examination; WTAR- Wechsler's Test of Adult Reading, PT- People Test, CWT- Colour Word Test

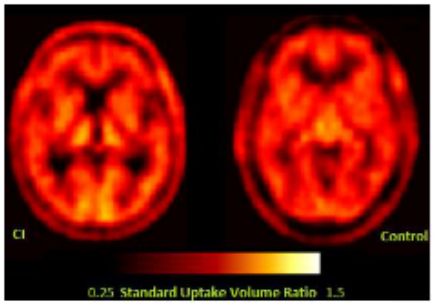

Figure. TSPO radioligand uptake as seen on PET imaging

Conclusions

ADT affects multiple cognitive domains including verbal fluency and memory, attention and processing speed. PET TSPO radioligand uptake suggests a possible role of neuroinflammation in development of CI. Final evaluation will provide important pilot data to help us better understand CI.

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Evaluation of the patho-physiological basis of elevated Translocator protein (TSPO) expression in patients with solid tumours

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Background and Rationale:

Tumour microenvironment consisting of both the tumour cells and surrounding non-malignant cells (e.g. endothelial cells, fibroblasts, immune and inflammatory cells, etc.) plays an important role in disease progression and response to therapy. Some of the non-tumour cells in the microenvironment are actively recruited by the tumour cells to collaborate in their neoplastic activity. Multiple lines of evidence indicate that inflammatory cells can actively promote tumour growth, by fostering angiogenesis, cancer cell proliferation, and invasiveness.

The 18KDa translocator protein (TSPO) formerly known as the peripheral benzodiazepine receptor (PBR) is widely expressed in the body but is particularly enriched up to 20- to 50- fold in steroid synthesising tissues. Other functions of the TSPO include protein import, porphyrin transport and haeme biosynthesis, cellular proliferation and differentiation, immunomodulation, apoptosis, regulation of mitochondrial metabolism and involvement in cellular oxidative process. The high expression of TSPO in macrophages and monocytes [1] has been used to evaluate neuro-inflammation using positron emission tomography (PET) with TSPO ligands. However, TSPO is also overexpressed in breast, colorectal, prostate, ovarian, glioma and hepatic cancer cells [2] and has shown to be involved in the modulation of cell proliferation and tumorigenesis. Recent ionizing radiation research has recognized the increasing role of mitochondria in radiation therapy. It has been proposed that despite the induction of canonical apoptotic genes by ionising radiation, dysfunctional mitochondria may lead to radiation resistance [3]. It has also been shown that TSPO inhibits mitochondrial autophagy and abolishes mitochondrial relocation of the autophagic marker LC3 (microtubule-associated protein 1 light chain 3), thus leading to an accumulation of dysfunctional mitochondria [4]. The accumulation of TSPO after radiotherapy may therefore reflect mitochondrial activity and response to ionising radiation.

[^{11}C]PBR28 is a second generation TSPO ligand that has been developed for PET studies and has superior specificity and properties compared to the earlier generation of TSPO ligands. Clinical PET studies have been performed in patients to evaluate neuro-inflammation have shown that the pituitary gland demonstrated the highest uptake for TSPO radioligands in the brain.

We aim to evaluate the relevance of TSPO expression in patients in patients with solid tumours in this pilot feasibility study with an adaptive design. In addition, we aim to compare uptake of [^{11}C]PBR28 with uptake of [^{18}F]fluorodeoxyglucose (FDG), which is also known to increase in inflammatory conditions. Finally, we aim to assess the relationship between tissue TSPO and macrophage immunohistochemistry with imaging uptake of [^{11}C]PBR28, [^{18}F]FDG to understand the patho-physiology of [^{11}C]PBR28 uptake.

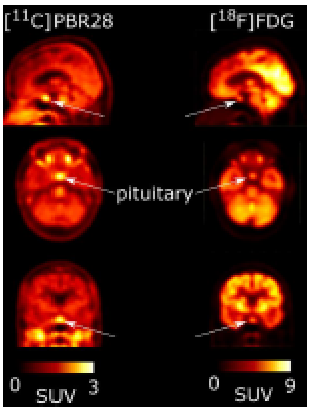


Figure 1. Uptake of [^{11}C]PBR28 (10 - 60min post injection; right) and [^{18}F]FDG (60 – 80 min post injection; right) in a subject (M,69y) with pituitary macroadenomas showing good contrast in uptake in the tumour compared to normal brain with [^{11}C]PBR28. Sagittal, axial and coronal planes are illustrated in the top, middle and bottom panels. SUV: standardised uptake value. uptake in pituitary

* Contact details: azeem.saleem@imanova.co.uk

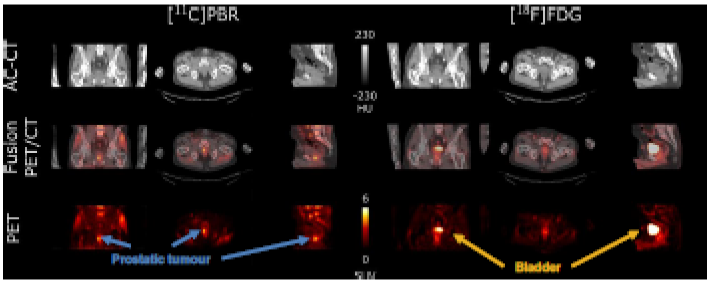


Figure 2. Uptake of [^{11}C]PBR28 (10 - 60min post injection; right) and [^{18}F]FDG (60 – 80 min post injection; right) in a subject (M,69y) with prostate cancer showing [^{11}C]PBR28 uptake in the prostatic tumour. There is minimal bladder elimination of radioactivity during this period with [^{11}C]PBR28 compared to [^{18}F]FDG. Coronal, axial and sagittal planes are illustrated in the left, middle and right panels for each of the images. SUV: standardised uptake value.

Methods:

Ten patients with solid tumours will be recruited, with patients with pituitary macroadenomas and prostatic tumours being initially included. All patients will either have archival or tissue pathological samples available from future procedures. Subjects with tumours > 1 cm, who are able to have PET imaging will be included. Pregnant and lactating women will be excluded from the study. Each subject will have a dynamic PET scan for 60 minutes after administration of [^{11}C]PBR28 and a static FDG PET scan for 20 minutes, 60 minutes after administration of [^{18}F]FDG. Regions of interest (ROIs) will be defined outlining the tumour and other normal tissues and will be applied to the dynamic [^{11}C]PBR28 data to generate mean voxel radioactivity for the full duration of the scan. Tissue uptake will be normalised to injected dose and body weight to generate semi-quantitative Standardised Uptake Value (SUV) images. Average SUV in each region was calculated and will be reported as SUV_{mean}.

Immunohistochemical (IHC) analysis of the archival tissue samples (obtained prior to scanning) or fresh tumour tissue obtained after the imaging will be performed. IHC analysis will be performed following standard protocols for the assessment of TSPO, macrophage markers and other relevant markers of interest using a digital image analysis system and positive staining for each of the antigens will be scored and recorded. Genotyping for the TSPO polymorphism Ala147Thr will be performed from blood and tumour samples from patients recruited to the study on completion of all the scans.

Results:

So far, 4 patients - 2 with pituitary macroadenomas (Figure 1) due to undergo surgery and 2 patients with prostate tumours (Figure 2) due to receive RT have been included to the study. All 4 subjects have had both PET scans.

Interim analysis of the images have revealed similar uptake of [^{11}C]PBR28 (average SUV_{mean} = 1.33) and [^{18}F]FDG (average SUV_{mean} = 1.70) in the prostatic tumour (Figure 3). However, there was minimal bladder elimination of PBR28 enabling better discrimination of prostatic tumour with PBR28. Uptake was observed in one subject with an inflammatory pelvic lymph node as determined by diagnostic MRI with [^{11}C]PBR28 but not with FDG. In patients with pituitary macroadenomas, higher [^{11}C]PBR28 uptake was observed in the pituitary macroadenomas. In contrast, with FDG, there was generally high uptake throughout the brain.

Time-radioactivity curves plotted for the full duration of the [^{11}C]PBR28 PET scan (Figure 4) showed good discrimination between prostatic and bladder radioactivity uptake during the first 20 minutes, suggesting that a shorter duration PBR28 scan may suffice in future imaging studies with [^{11}C]PBR28.

Based on the interim results, which have shown good signal to noise characteristics for [^{11}C]PBR28 in the prostate, we have modified the study to exclude FDG PET scans in patients with prostate tumours. We hypothesise that TSPO would be indicative of intra-tumoural steroidogenesis and therefore a potential imaging biomarker of intra-tumoural steroidogenesis modifying agents. Additionally, changes in [^{11}C]PBR28 uptake may indicate response to RT due to its role in the mitochondrial function. We therefore plan to image prostate cancer patients before and during or after anti-androgen therapy in future patients recruited to this pilot study.

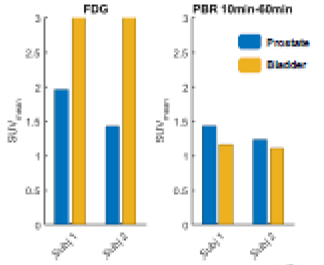


Figure 3. Mean standardised uptake value (SUVmean) for [^{18}F]FDG (left) and [^{11}C]PBR28 (10 – 60min post injection) (right) for both the prostate cancer patients imaged.

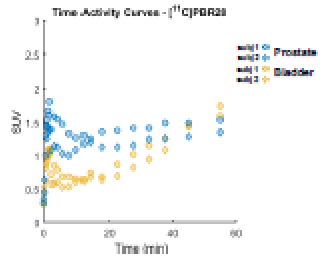


Figure 4. Time- radioactivity curves for [^{11}C]PBR28 for the two subjects with prostatic tumours. Regions are prostatic tumour (blue) and bladder (yellow).

Discussion:

Study accrual is continuing. Preliminary data from this study has shown that [^{11}C]PBR28 imaging is feasible and that [^{11}C]PBR28 may be a good molecular imaging radiotracer for imaging prostate tumours due to the relative absence of confounding noise from bladder elimination of radioactivity. Pathological analysis will be undertaken in the subjects accrued so far to evaluate the relevance and prognostic significance of [^{11}C]PBR28 uptake in pituitary macroadenomas and the relationship between imaging uptake and pathological markers of TSPO and inflammation.

References:

1. Canat, X., et al., Life Sci, 1993. 52(1) p. 107-18.
2. Batarseh, A. and V. Papadopoulos, Mol Cell Endocrinol, 2010. 327(1-2) p. 1-12.
3. Rameshandrayan [illegible] and Price [illegible]. Cancer Res. 2013 Feb 15;73(4):1[illegible]-23. doi: 10.1158/0008-5472.CAN-14-3175. Epub 2013 Jan 29.
4. Gatliff J, et al., Autophagy. 2014;10(12):2279-96. doi: 10.4161/15548627.2014.991665.

'PATCH'-ing up toxicities of contemporary ADT for advanced prostate cancer: maximizing benefit of a clinical trial as new hypotheses are generated

Hannah CP Wilson, S Imran A Shah, Paul D Abel
Department of Surgery and Cancer, Imperial College London

Introduction Embraced when discovered by Huggins in 1940, 'hormone therapy' (androgen deprivation therapy (ADT)) for advanced prostate cancer, using oral estradiol (E2) was abandoned following data revealing good cancer specific but poor overall survival. Replaced by Luteinizing Hormone Releasing Hormone analogues (LHRHa), testosterone (T), but also E2, were suppressed to castrate levels. Multiple toxicities resulted which, remain today both clinically and financially burdensome. The Phase III PATCH (Prostate Adenocarcinoma Trans-Cutaneous Hormones) trial, re-explores benefits and toxicities of E2 (transdermally) vs LHRHa. Transdermal E2 offers single medication effective both as ADT yet simultaneously mitigating toxicity. PATCH has enabled new hypotheses concerning sex hormone deficiency to be addressed **Methodology** Summaries of sub-studies include: *Cognitive:* previously well men in the 3rd to 12th month of LHRHa ADT achieving castrate T were divided into 2 groups, impaired or not, (they, family/friends were asked about changes consistent with cognitive impairment). They underwent complex neuro-psychometric testing, positron emission tomography scanning and structural/functional magnetic resonance imaging. *Odour:* Ten men starting LHRHa ADT provided axillary sweat on t-shirts worn over 2 successive nights pre and 3 months post-treatment. Non-cancer controls provided samples for comparison. Sweat samples were frozen at -80˚C until rated simultaneously for masculinity, attractiveness and intensity by young adult females **Results** *Cognitive:* Preliminary results suggest memory and executive function deficits plus diffuse cortical neuroinflamation. *Odour:* Data are currently being analyzed **Conclusion** PATCH reflects potential for addressing hypotheses additional to main primary and secondary end-points of a large clinical trial through design and performance of novel sub-studies.

Conference Abstract IX Congress of the International Society of Men's Health and Aging (ISSAM), Prague 2015.

Imperial College London

'PATCH'-ing up toxicities of contemporary ADT for advanced prostate cancer

H Wilson, S Shah, P Abel

imanova
Centre for Imaging Sciences

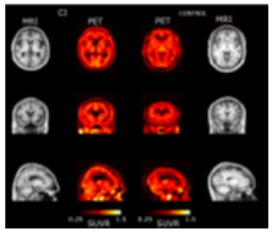
INTRODUCTION

- Embraced when discovered by Huggins in 1940, 'hormone therapy' (androgen deprivation therapy (ADT)) for advanced prostate cancer (PC), using oral estradiol (E2) was abandoned following data revealing good cancer specific but poor overall survival
- Replaced by Luteinizing Hormone Releasing Hormone analogues (LHRHa), serum testosterone (95%), but also E2 (80%) were suppressed to castrate levels, resulting in multiple-toxicities
- The Phase III PATCH (Prostate-Adenocarcinoma-Trans-Cutaneous-Hormones) trial, re-explores benefits and toxicities of E2 (transdermally) vs LHRHa
- PATCH has enabled new hypotheses concerning sex-hormone-deficiency to be addressed in several pilot sub-studies utilizing men on LHRHa

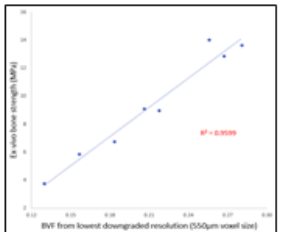
RESULTS

Recruitment in these translational studies was achieved:

- *Cognitive:*



- *Bone density:*



METHODS

Effect of LHRHa on men with PC:

- *Cognition:* Previously well men in-between start of month 4 and end of month 12 were divided into 2 groups; a) experiencing treatment related cognitive decline and b) normal cognition. Patients underwent complex neuro-psychometric questionnaire testing, positron-emission-tomography scanning and structure/function magnetic resonance imaging
- *Bone density:* Dual Energy X-ray Absorptiometry (DEXA) and Computed Tomography (CT) scans were identified and compared to determine bone strength and fracture risk
- *Body Odour:* Newly diagnosed PC men provided axillary sweat on t-shirts worn over 2 successive nights pre- and post- 3 months treatment. Non-cancer controls provided samples for comparison. Sweat samples were frozen at -80'C until rated simultaneously for masculinity, attractiveness and intensity by young adult females

CONCLUSION

- Studies like PATCH allow potential to address hypotheses additional to primary and secondary end-points of a large clinical trial
- Through design and performance of novel multidisciplinary sub-studies performed in parallel more outputs are generated
- We hope to take such sub-studies forward and address if such toxicities can be mitigated by using oestrogen patches

Imperial College London

Maximizing benefit of a clinical trial as new hypotheses are generated: 'PATCH'-ing up toxicities of contemporary ADT for advanced prostate cancer

H Wilson, P Price, M Stewart, M Arnold, C Craig, A Saleem, S Shah, T Jones, P Abel

imanova
Centre for Imaging Sciences

INTRODUCTION

- Embraced when discovered by Huggins in 1940, 'hormone therapy' (androgen deprivation therapy (ADT)) for advanced prostate cancer (PC), using oral estradiol (E2) was abandoned following data revealing good cancer specific but poor overall survival
- Replaced by Luteinizing Hormone Releasing Hormone analogues (LHRHa), testosterone (95%), but also E2 (80%) were suppressed to castrate levels, resulting in multiple-toxicities
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- PATCH has enabled new hypotheses concerning sex-hormone-deficiency to be addressed in several pilot sub-studies utilizing men on LHRHa

METHODS

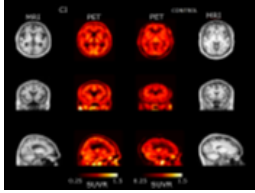
Effect of LHRHa on men with PC:

- *Cognition:* Previously well men in-between start of month 4 and end of month 12 were divided into 2 groups; a) experiencing treatment related cognitive decline and b) normal cognition. Patients underwent complex neuro-psychometric questionnaire testing, positron-emission-tomography scanning and structure/function magnetic resonance imaging
- *Body Odour:* Newly diagnosed PC men provided axillary sweat on t-shirts worn over 2 successive nights pre- and 3 months post-treatment. Non-cancer controls provided samples for comparison. Sweat samples were frozen at -80'C until rated simultaneously for masculinity, attractiveness and intensity by young adult females
- *Bone density:* Dual Energy X-ray Absorptiometry (DEXA) and Computed Tomography (CT) scans were identified and compared to determine bone strength and fracture risk

RESULTS

Recruitment and participation in these translational studies was achieved:

- *Cognitive:*



- Body Odour

Table. Group characteristics of odour ratings for attractiveness, masculinity and intensity by female raters

Parameter	Control group n=10 Mean ± S.E.M		Study group n=10 Mean ± S.E.M		p-value
	Baseline	3 months	Baseline	3 months	
Attractiveness	2.68 ± 0.22	2.72 ± 0.24	2.55 ± 0.11	2.61 ± 0.20	0.933
Masculinity	2.94 ± 0.13	2.82 ± 0.22	2.85 ± 0.17	2.74 ± 0.20	0.900
Intensity	3.03 ± 0.30	2.73 ± 0.18	2.87 ± 0.24	2.63 ± 0.18	0.650

CONCLUSION

- Studies like PATCH allow potential to address hypotheses additional to primary and secondary end-points of a large clinical trial
- Through design and performance of novel multidisciplinary sub-studies performed in parallel more outputs are generated
- We hope to take such sub-studies forward and address if such toxicities can be mitigated by using oestrogen patches

Appendix II - Regulatory approvals

NHS
Health Research Authority

NRES Committee London - Queen Square
HRA Head Office
Skipton House
80 London Road
London
SE1 6LH

Telephone: 020 797 22580

***Reissued Letter- 22nd October 2013**

04 October 2013

Prof Paul Abel
Professor of Urology
Imperial College London
Room BN1/5
Dept of Surgery, Hammersmith Campus
Imperial College, Faculty of Medicine, DuCane Road,
W12 0NN

Dear Prof Abel

Study title:	**A PET imaging study to detect the presence of activated microglia in the brains of prostate cancer patients who develop mild cognitive impairment following androgen deprivation therapy.**
REC reference:	**13/LO/0731**
Protocol number:	**13HH0558**
IRAS project ID:	**123476**

Thank you for your letter of 05 September 2013, responding to the Committee's request for further information on the above research.

The further information was considered by a sub-committee of the REC at a meeting held on 19th September 2013. A list of the sub-committee members is attached.

We plan to publish your research summary wording for the above study on the NRES website, together with your contact details, unless you expressly withhold permission to do so. Publication will be no earlier than three months from the date of this favourable opinion letter. Should you wish to provide a substitute contact point, require further information, or wish to withhold permission to publish, please contact the Co-ordinator Hayley Fraser NRESCommittee.London-QueenSquare@nhs.net

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation subject to the conditions specified below.

Imperial College London

Imperial College Healthcare NHS Trust

Joint Research Compliance Office
Academic Health Science Centre
Imperial College London and
Imperial College Healthcare NHS Trust
Room 5L10C, 5th Floor, Lab Block
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Fulham Palace Road
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Fax: +44 (0)20 3311 0203
Gary.roper@imperial.ac.uk/
www.ic.ac.uk/clinicalresearchgovernanceoffice

Gary Roper
Head of Regulatory Compliance

27/11/2013

Prof Paul Abel
Professor of Urology
Imperial College London
Room BN1/5
Dept of Surgery, Hammersmith Campus
Imperial College, Faculty of Medicine, DuCane Road,
W12 0NN

Dear Professor Abel,

RE: JRCO Study Approval

Project Title: A PET imaging study to detect the presence of activated microglia in the brains of prostate cancer patients who develop mild cognitive impairment following androgen deprivation therapy.

Short Title: PET imaging in MCI following ADT for PCa

Joint Research Compliance Office Reference number: 13HH0558 **CSP Ref:** 123476

Ethics reference number: 13/LO/0731 **Principal Investigator:** Dr Shah

I confirm that this project has now been approved by the Joint Research Compliance Office. The project may now start at Imperial College Healthcare NHS Trust sites. Please note that the start date of the project is the date of this letter and the duration is the same as that provided in your application form.

The list of documents reviewed and approved by the Joint Research Compliance Office under requirements of the Research Governance Framework are as follows:

Document	Version	Date
REC Approval		04/10/13
R&D Form	123476/489089/14/683	
SSI Form	123476/491191/6/234/189403/279259	
Protocol	2	16/07/13
GP/Consultant Information Sheets		11/04/13
Participant Consent Form	2	16/07/13
Participant Information Sheet: Arterial Line Incision	1	11/04/13
Participant Information Sheet: Patients	2	16/07/13
Participant Information Sheet: Control Participants	2	16/0713
Participant Information Sheet: Cases	3	
Participant Information Sheet: Controls		02/09/13

NHS
Health Research Authority

NRES Committee London - Queen Square
HRA Head Office
Skipton House
80 London Road
London
SE1 6LH

Tel: 020 797 22580

03 February 2014

Prof Paul Abel
Professor of Urology
Imperial College London
Room BN1/5
Dept of Surgery, Hammersmith Campus
Imperial College, Faculty of Medicine, DuCane Road,
W12 0NN

Dear Prof Abel

Study title:	**A PET imaging study to detect the presence of activated microglia in the brains of prostate cancer patients who develop mild cognitive impairment following androgen deprivation therapy.**
REC reference:	**13/LO/0731**
Protocol number:	**13HH0558**
Amendment number:	**1**
Amendment date:	**03 January 2014**
IRAS project ID:	**123476**

The above amendment was reviewed by the Sub-Committee in correspondence.

Ethical opinion

After review of the amendment no ethical issues raised.

The members of the Committee taking part in the review gave a favourable ethical opinion of the amendment on the basis described in the notice of amendment form and supporting documentation.

Approved documents

The documents reviewed and approved at the meeting were:

Document	Version	Date
Protocol	3	20 November 2013
Participant Information Sheet: Controls	4	20 November 2013
Participant Information Sheet: Cases	4	20 November 2013
Covering Letter		03 January 2014
Notice of Substantial Amendment (non-CTIMPs)	1	03 January 2014

Membership of the Committee

The members of the Committee who took part in the review are listed on the attached sheet.

Imperial College London

Imperial College Healthcare NHS
NHS Trust

Joint Research Compliance Office
Academic Health Science Centre
Imperial College London and
Imperial College Healthcare NHS Trust
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24 March 2014

Paul Abel
Professor of Urology
Imperial College London
Room BN1/5, Dept of Surgery
Hammersmith Hospital
Du Cane Road
London. W12 OHS

Dear Pau; Abel,

RE: Non-Substantial Amendment

Project Title: A PET imaging study to detect the presence of activated microglia in the brains of prostate cancer patients who develop mild cognitive impairment following androgen deprivation therapy.

Short Title: PET imaging in MCI following ADT for PCa

Joint Research Compliance Office Reference number: 13HH0558 **CSP Ref:** 123476

Ethics reference number: 13/LO/0731 **Principal Investigator:** Paul Abel

Thank you for forwarding the details for the amendment to the original study documentation for the above study. The Trust is happy to approve the amendments with respect to risk assessment, research governance and cost implications.

The documents reviewed and approved were:

Document	Version	Date
Notice of Substantial Amendment (non-CTIMP)	1.0	03 January 2014
NRES Committee London – Queens Square – Favourable Opinion letter for Substantial Amendment 1	Substantial Amendment 1	03 February 2014
Protocol	3.0	20 November 2013
Participant Information Sheet:: Controls	4.0	20 November 2013
Participant Information Sheet:: Cases	4.0	20 November 2013

Before you commence your research, please note that you must be aware of your obligations to comply with the minimum requirements for compliance with the Research Governance indicators 17 (Data Protection); 25 (Health and Safety) and 22 (Financial Probity). Details of the requirements to be met can be found in the on the Trust Research Governance Framework available on www.dh.gov.uk.

I wish you well in your research and look forward to seeing regular annual progress reports, funding details as well as information about any publications resulting from the study.

Yours sincerely

Ms Becky Ward
Research Governance Manager

NHS
Health Research Authority
National Research Ethics Service

NRES Committee London - Queen Square
HRA NRES Centre Manchester
Barlow House
3rd Floor
4 Minshull Street
Manchester
M1 3DZ

22 October 2014

Prof Paul Abel
Professor of Urology
Imperial College London
Room BN1/5
Dept of Surgery, Hammersmith Campus
Imperial College, Faculty of Medicine, DuCane Road,
W12 0NN

Dear Prof Abel

Study title:	**A PET imaging study to detect the presence of activated microglia in the brains of prostate cancer patients who develop mild cognitive impairment following androgen deprivation therapy.**
REC reference:	**13/LO/0731**
Protocol number:	**13HH0558**
Amendment number:	**2**
Amendment date:	**18 September 2014**
IRAS project ID:	**123476**

The above amendment was reviewed at the meeting of the Sub-Committee held on 16 October 2014.

Ethical opinion

The members of the Committee taking part in the review gave a favourable ethical opinion of the amendment on the basis described in the notice of amendment form and supporting documentation.

Approved documents

The documents reviewed and approved at the meeting were:

Document	*Version*	*Date*
Notice of Substantial Amendment (non-CTIMP)		18 September 2014
Other [GP letter]	2	18 September 2014
Participant consent form [Consent form PET]	3	18 September 2014

Imperial College London

Imperial College Healthcare NHS

NHS Trust

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05 December 2014

Becky Ward
Research Governance Manager

Professor Paul Abel
Post Professor of Urology
Imperial College London
Room BN1/5, Dept of Surgery
Hammersmith Campus
Imperial College, Faculty of Medicine
DuCane Road,
W12 0NN

Dear Professor Paul Abel

RE: Approval of Substantial Amendment 2 Dated 18 September 2014

Project Title: A PET imaging study to detect the presence of activated microglia in the brains of prostate cancer patients who develop mild cognitive impairment following androgen deprivation therapy.

Short Title: PET imaging in MCI following ADT for PCa

Joint Research Compliance Office Reference number: 13HH0558 **CSP Ref**: 123476

Ethics reference number: 13/LO/0731 **Principal Investigator:** Professor Paul Abel

Thank you for forwarding the details for the amendment to the original study documentation for the above study. The Trust is happy to approve the amendments with respect to risk assessment, research governance and cost implications.

The documents reviewed and approved were:

Document	Version	Date
Notice of Substantial Amendment (Non CTIMP)	Amendment 2	30 September 2014
NRES Committee London- Queens Square- Favourable Opinion Letter For Substantial Amendment 2	Amendment 2	22 October 2014
Other [GP letter]	2.0	18 September 2014
Participant consent form [Consent form PET]	3.0	18 September 2014
Participant information sheet (PIS) [PIS cases]	5.0	18 September 2014
Participant information sheet (PIS) [PIS controls]	5.0	18 September 2014
Research protocol or project proposal	4.0	18 September 2014
CPG/Divisional Approval		05 December 2014

Before you commence your research, please note that you must be aware of your obligations to comply with the minimum requirements for compliance with the Research Governance indicators 17 (Data Protection); 25 (Health and Safety) and 22 (Financial Probity). Details of the requirements to be met can be found in the on the Trust Research Governance Framework available on www.dh.gov.uk.

I wish you well in your research and look forward to seeing regular annual progress reports, funding details as well as information about any publications resulting from the study.

Arthur J. Gallagher International

30th July 2012

TO WHOM IT MAY CONCERN

We, the undersigned Insurance Brokers hereby certify that we have place the following Insurance:

VERIFICATION OF INSURANCE

Unique Market Reference:	B1262FI0103012
Type:	No Fault Compensation for Clinical Trials and/or Human Volunteer Studies
Insured:	Imperial College London
Period:	From: 1st August 2012 To: 1st August 2013 Both days inclusive at Local Standard Time.
Interest:	This Policy will indemnify/cover the Insured in respect of their Legal Liabilities arising out of the Insured's activities and as more fully disclosed within the Policy Wording.
Limit of Indemnity:	GBP 10,000,000 Any One Claim and GBP 12,500,000 in the Aggregate, including costs and expenses
Excess:	GBP 2,500 Each and Every Claim, including costs and expenses
Underwriter:	100.0000% Newline Syndicate 1218

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Should the above mentioned Contract of Insurance be cancelled, assigned or changed during the above policy period in such manner as to affect this document, no obligation to inform the holder of this document is accepted by the undersigned or by the Insurers. The information provided is correct at the date of signature.

Authorised Signatory
Gallagher London.

Imperial College London

Imperial College Healthcare NHS
NHS Trust

Joint Research Compliance Office
Academic Health Science Centre
Imperial College London and
Imperial College Healthcare NHS Trust
Room 5L10A, 5th Floor, Lab Block
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www.ic.ac.uk/clinicalresearchgovernanceoffice

Becky Ward
Research Governance Manager

12/04/2013

Professor Paul Abel
Professor of Urology
Imperial College London
Room BN2\11
Department of Surgery
Hammersmith Campus
DuCane Road
London, W12 0NN

Dear Professor Abel

RE: A PET imaging study to detect the presence of activated microglia in the brains of prostate cancer patients who develop mild cognitive impairment following androgen deprivation therapy

Joint Research Compliance Office Reference number: 13HH0558

This is to confirm that the above named research project utilises human participants, their organs, tissue and/or data as defined under the sponsorship requirements of the Research Governance Framework for Health and Social Care 2005, incorporating the Medicines for Human Use (Clinical Trials) Regulations 2004.

On behalf of Imperial College of Science, Technology and Medicine, we undertake to act as the identified Research Sponsor for this project.

This letter confirms:

- The research proposal has been discussed, assessed and registered with the Joint Research Compliance Office, Imperial College Academic Health Science Centre, Imperial College London and provisional sponsor approval granted.
- The Chief Investigator has undergone a process of scientific critique commensurate with the scale of the project.
- Indemnity and insurance arrangements have been put in place to cover the project.
- Resources and support are available to the research team to aid delivery of the research as proposed.
- Management, monitoring and reporting responsibilities for the research have been approved.
- Imperial College will undertake and enforce those sponsor duties set out in the NHS Research Governance Framework for Health and Social Care.

Imperial College Sponsorship is conditional on the project receiving applicable ethical and regulatory approval for all research related aspects of its conduct. It is also conditional on successful contract and agreement negotiations and sign off via the Joint Research Office, where relevant, and before the study commences.

A copy of the ethics approval letter **must** be sent to the Research Governance Manager prior to the study commencing. Sponsorship is dependant on obtaining R&D Office approval for all NHS sites where the research is being conducted.

Yours sincerely

Ms Becky Ward
Research Governance Manager

Imperial College London

Project Title	A PET imaging study to detect the presence of activated microglia in the brains of prostate cancer patients who develop mild cognitive impairment following androgen deprivation therapy
Chief Investigator	Professor Paul D Abel
Reference number	0057/IC0031
Date of Submission	19th April 2013

This is to confirm that the protocol of the above project was submitted to the Imperial College Peer Review Office for consideration, and that:

☐ It was judged not to need peer review.

Details:

☒ The peer review already obtained was judged satisfactory for the type of project.

Details: Funded by the joint BRC/Imanova programme and received review as part of the funding approval process.

☐ Two independent peer reviews were obtained by the office, and that these were judged satisfactory for the type of project. The reviews should be supplied and considered along-side this certificate.

Details:

☐ This level 4 review was carried out under the pre-funding exemption, and undertaken by internal reviewers.

This certificate confirms that the correct peer review process was undergone, that the review (if applicable) was carried out at an appropriate level of independence, and that (for a review obtained by the Peer Review Office) the questions asked of the reviewer were addressed.

This certificate is not a commentary on the scientific or methodological merit of the study.

This certificate is digitally signed and can be verified. Please contact the PRO for more details.

Naomi Gardner, Administrator,
On behalf of Chairman of Peer Review Committee

Imperial College Healthcare Tissue Bank
Department of Surgery and Cancer,
Hammersmith Hospital,
Du Cane Road,
London W12 0HS
Tel: +44 7711 701382
Email: gerry.thomas@imperial.ac.uk

ICHTB HTA licence: 12275
REC Wales approval: 12/WA/0196

Professor Paul Abel
Charing Cross Hospital
W6 8RF

5th January 2015

Dear Paul,

Re: Tissue Bank application number Project R14110; do changes in body odour signalling follow androgen deprivation therapy for prostate cancer.

I am pleased to confirm that the Tissue Management Committee of the ICHTB has approved your application for access.

In order to satisfy HTA tracking requirements, would you please ensure the samples you use are reported to the tissue bank via the online database and sub-collection, along with any publications you produce, that should contain the acknowledgment wording as per the signed MTA.

Yours sincerely,

Professor Gerry Thomas
DI, Hammersmith HTA Research Licence.

Imperial College London

Appendix III - Study materials

Mini-Mental State Examination (MMSE)

Patient's Name: _____ Date: _____

Instructions: Score one point for each correct response within each question or activity.

Maximum Score	Patient's Score	Questions
5		"What is the year? Season? Date? Day? Month?"
5		"Where are we now? State? County? Town/city? Hospital? Floor?"
3		The examiner names three unrelated objects clearly and slowly, then the instructor asks the patient to name all three of them. The patient's response is used for scoring. The examiner repeats them until patient learns all of them, if possible.
5		"I would like you to count backward from 100 by sevens." (93, 86, 79, 72, 65, …) Alternative: "Spell WORLD backwards." (D-L-R-O-W)
3		"Earlier I told you the names of three things. Can you tell me what those were?"
2		Show the patient two simple objects, such as a wristwatch and a pencil, and ask the patient to name them.
1		"Repeat the phrase: 'No ifs, ands, or buts.'"
3		"Take the paper in your right hand, fold it in half, and put it on the floor." (The examiner gives the patient a piece of blank paper.)
1		"Please read this and do what it says." (Written instruction is "Close your eyes.")
1		"Make up and write a sentence about anything." (This sentence must contain a noun and a verb.)
1		"Please copy this picture." (The examiner gives the patient a blank piece of paper and asks him/her to draw the symbol below. All 10 angles must be present and two must intersect.) 
30		TOTAL

wtar WORD CARD

WECHSLER® TEST OF ADULT READING™

1. again
2. address
3. cough
4. preview
5. although
6. most
7. excitement
8. know
9. plumb
10. decorate
11. fierce
12. knead
13. aisle
14. vengeance
15. prestigious
16. wreathe
17. gnat
18. amphitheatre
19. lieu
20. grotesque
21. iridescent
22. ballet
23. equestrian
24. porpoise
25. aesthetic
26. conscientious
27. homily
28. malady
29. subtle
30. fecund
31. palatable
32. menagerie
33. obfuscate
34. liaison
35. exigency
36. xenophobia
37. ogre
38. scurrilous
39. ethereal
40. paradigm
41. perspicuity
42. plethora
43. lugubrious
44. treatise
45. dilettante
46. vertiginous
47. ubiquitous
48. hyperbole
49. insouciant
50. hegemony

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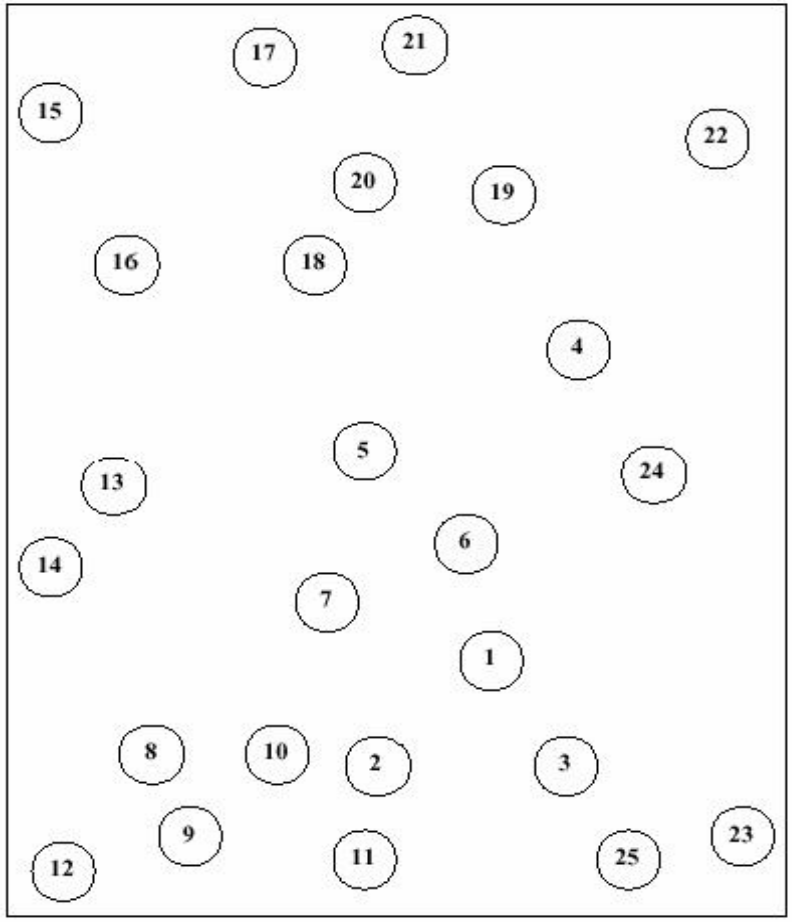
TRAIL MAKING TEST

A



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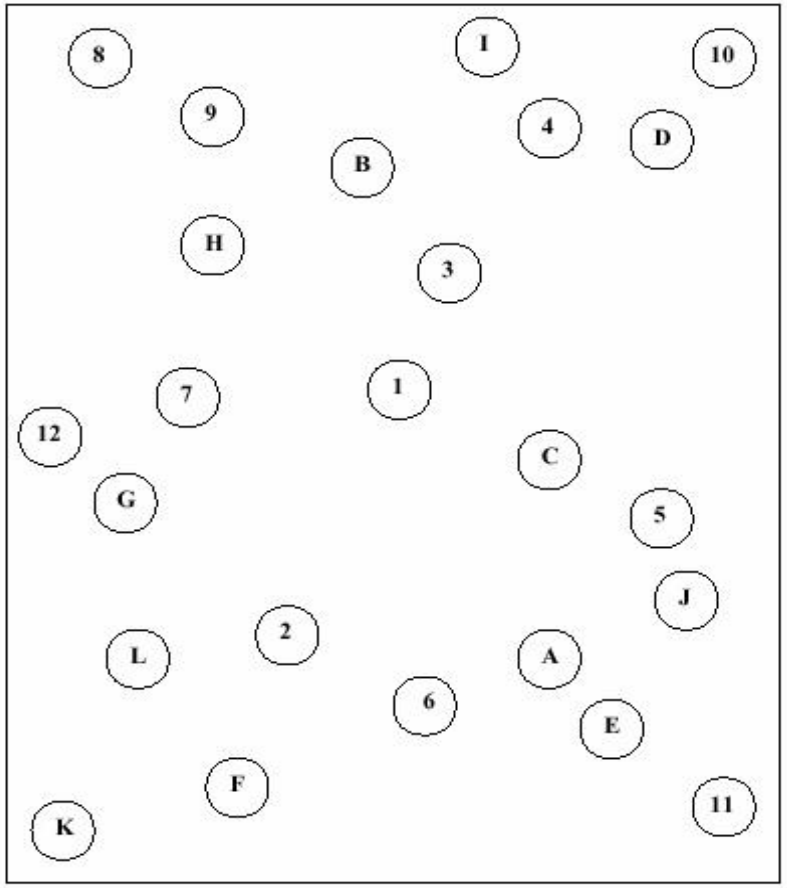
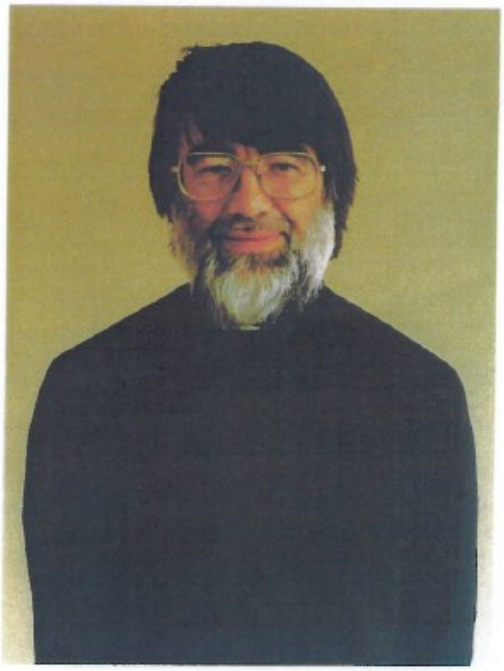


PEOPLE TEST

JIM GREEN – DOCTOR



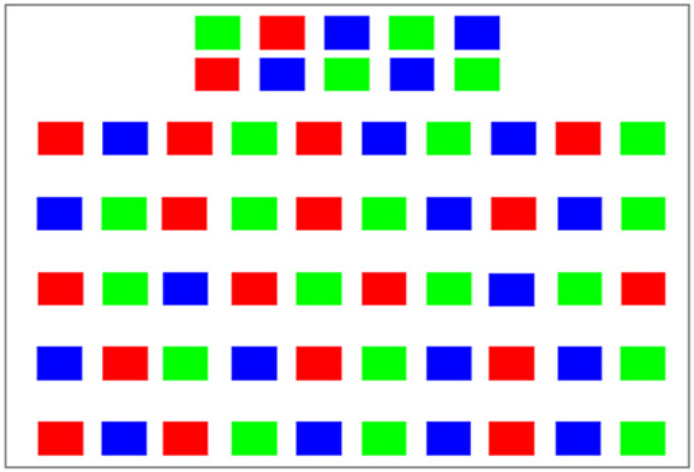
CUTHBERT CATTERMOLE – MINISTER

TOM WEBSTER – POSTMAN

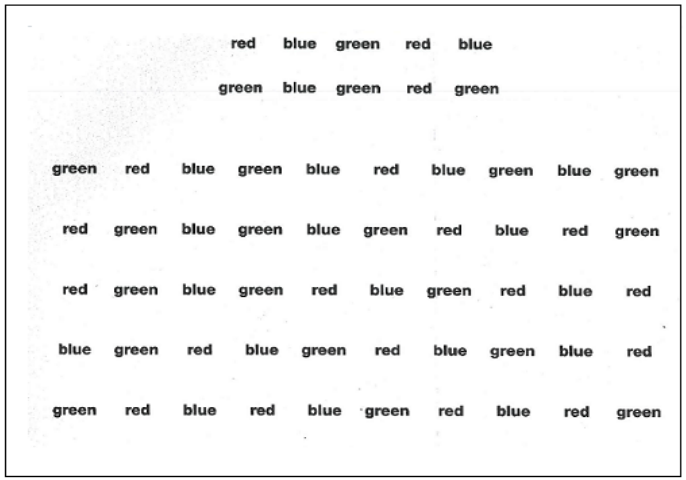
PHILIP ARMSTRONG – PAPERBOY

COLOUR-WORD INTERFERENCE TEST (CWIT)

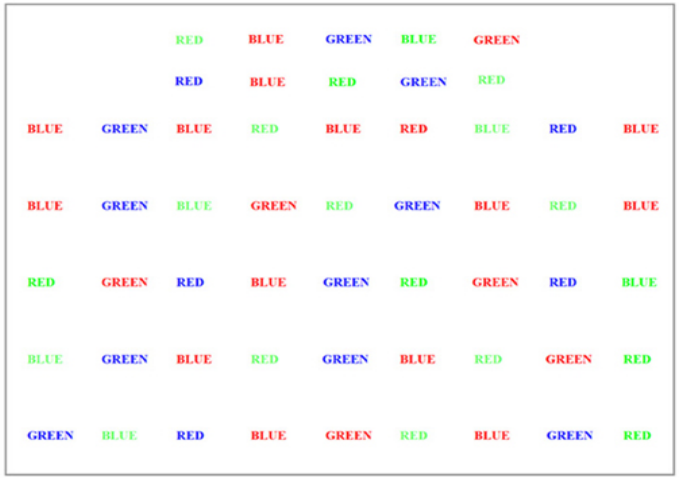
Colour naming



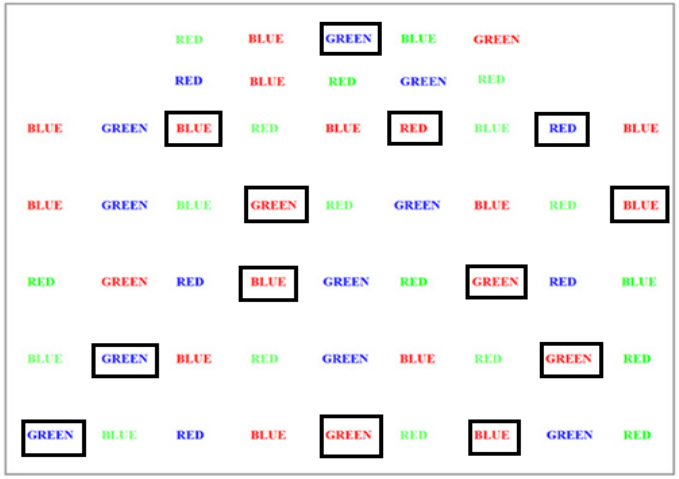
Word reading

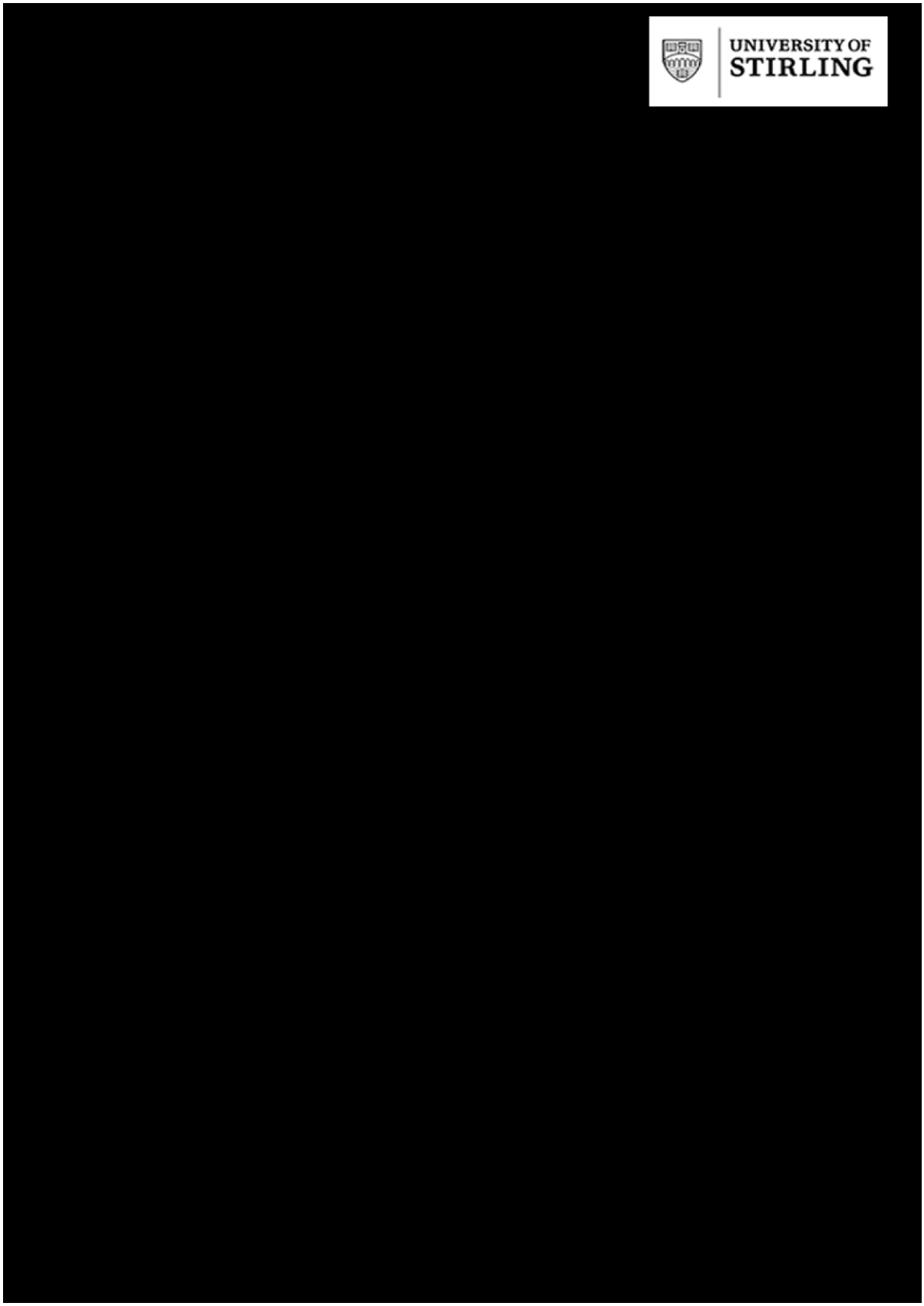


Inhibition



Inhibition Switching




UNIVERSITY OF
STIRLING

Appendix IV – Study Data

Bone Study

Bone Volume Fraction Experiment

No.	Femoral Head Bone Volume Fraction measured at different resolutions					
	30 µ	50 µ	100 µ	200	400 µ	550 µ
1	0.17	0.17	0.15	0.17	0.15	0.16
2	0.26	0.26	0.27	0.26	0.25	0.24
3	0.22	0.23	0.22	0.22	0.22	0.21
4	0.21	0.20	0.21	0.20	0.20	0.20
5	0.25	0.24	0.24	0.24	0.25	0.24
6	0.21	0.22	0.22	0.22	0.20	0.22
7	0.26	0.25	0.25	0.25	0.25	0.26
8	0.19	0.19	0.20	0.19	0.19	0.18
9	0.21	0.21	0.21	0.21	0.21	0.21
10	0.21	0.19	0.21	0.21	0.21	0.20
11	0.24	0.26	0.24	0.24	0.24	0.24
12	0.27	0.27	0.27	0.27	0.28	0.27
13	0.20	0.19	0.20	0.20	0.19	0.19
14	0.28	0.25	0.27	0.27	0.28	0.28
15	0.25	0.25	0.25	0.25	0.24	0.23
16	0.27	0.25	0.23	0.27	0.26	0.26
17	0.17	0.15	0.15	0.15	0.11	0.13
18	0.18	0.17	0.15	0.12	0.12	0.13
19	0.22	0.21	0.22	0.22	0.22	0.21
20	0.25	0.25	0.24	0.24	0.24	0.23

Bone alkaline phosphatase experiment

Sr. No.	Group	Testosterone nmol/L	Oestradiol pmol/L	BALP U/L	Age Years
1	Control Baseline	20.07	80	24.9	58
2	Control Baseline	9.19	46	34.2	60
3	Control Baseline	16.41	126	28.9	72
4	Control Baseline	14.59	132	27.1	73
5	Control Baseline	13.61	105	23.1	65
6	Control Baseline	17.71	73	65	50
7	Control Baseline	27.7	66	22.4	64
8	Control Baseline	11.98	175	20.4	53
9	Control Baseline	10.24	122	42.1	64
10	Study Baseline	20.3	121	21.3	73
11	Study Baseline	21.4	79	28.9	67
12	Study Baseline	9.31	78	16.7	74
13	Study Baseline	20.44	80	29	69
14	Study Baseline	10.4	77	17.4	75
15	Study Baseline	16.08	147	34.4	72
16	Study Baseline	17.3	87	30.9	75
17	Study Baseline	17.98	51	33.5	59
18	Study Baseline	20.23	95	23.3	68
19	Control 3month	22.55	111	24	58
20	Control 3month	15.72	111	32	60
21	Control 3month	9.41	77	35.9	72
22	Control 3month	12.7	87	28.6	73
23	Control 3month	12.36	96	24.4	65
24	Control 3month	18.93	78	56.6	50
25	Control 3month	18.82	56	24.5	64
26	Control 3month	20.28	97	65.2	53
27	Control 3month	2.91	131	34.1	64
28	Study 3month	0.45	37	45.6	73
29	Study 3month	0.45	37	21	67
30	Study 3month	0.45	37	15.7	74
31	Study 3month	0.45	37	20.3	69
32	Study 3month	0.45	37	16.2	75
33	Study 3month	0.45	37	38.6	72
34	Study 3month	0.45	37	27	75
35	Study 3month	1.39	37	17.2	59
36	Study 3month	1.42	37	34	68

Cognitive Study

Paper-based Neuropsychology testing

No.	Age	Group	MMSE	WTAR	SM	MR	DS	PI	PD	LMI	LMD	LMR	TMA	TMB	CWCN	CWWR	CWI	CWIS	VF
1	77	CI	28	114	36	19	12	13	1	36	18	25	73	136	49	26	110	114	41
2	77	CI	24	90	16	8	9	11	0	22	7	17	49	146	67	31	180	180	28
3	59	CI	29	104	26	7	16	14	3	37	14	22	26	85	38	26	60	56	28
4	74	CI	24	112	30	18	14	14	2	31	13	25	37	120	37	24	106	90	27
5	60	Control	27	111	35	21	13	26	8	36	21	25	48	130	44	25	70	80	51
6	70	Control	24	97	36	18	14	23	7	36	16	21	34	113	41	23	57	65	44
7	57	Control	29	115	38	25	13	21	8	38	17	24	33	95	30	23	66	78	58
8	71	Control	28	122	44	23	18	17	5	23	12	23	51	118	33	22	76	88	50
9	66	Control	29	116	27	9	17	24	6	31	18	23	35	90	35	24	70	76	41
10	65	Control	30	123	34	20	19	17	7	40	21	25	34	94	29	20	68	65	49

Key:

MMSE; Mini-Mental State Examination
WTAR; Wechsler's Test of Adult Reading
SM; Similarities
MR; Matrix Reasoning
DS; Digit Span
PI; People Test Immediate Recall
PD; People Test Delayed Recall
LMI; Logical Memory Immediate Recall
LMD; Logical Memory Delayed Recall
LMR; Logical Memory Recognition
TMA; Trail-making A
TMB; Trail-making B
CWCN; Colour Word Interference Test Colour Naming
CWWR; Colour Word Interference Test Word Reading
CWI; Colour Word Interference Test inhibition
CWIS; Colour Word Interference Test Inhibition Switching
VF; Verbal Fluency

Computerised Cognitive testing

No.	Age	Group	Digit Span	Spatial Span	Paired Associates	Self Ordered Search	Rotations	Feature match	Polygon	Monkey ladder	Double Trouble	Verbal reasoning	Odd one out	Tree Task
1	77	CI	5	2	2	5	53	56	24	5	9	3	12	6
2	77	CI	5	3	3	4	33	43	0	4	6	7	11	3
3	59	CI	6	5	4	8	69	72	34	7	13	14	12	6
4	74	CI	6	5	3	7	44	72	20	6	3	10	14	6
5	60	Control	6	5	4	6	18	36	4	7	16	8	8	3
6	70	Control	5	4	3	6	15	61	13	6	6	3	13	10
7	57	Control	5	2	3	5	44	56	13	8	8	9	13	3
8	71	Control	6	3	3	7	46	70	43	6	16	14	13	6
9	66	Control	4	4	2	6	49	60	22	5	3	3	12	14
10	65	Control	7	5	4	6	56	132	16	6	5	6	13	10

Odour Study

No.	Group	Age Years	Ethnicity	Testosterone Baseline	Testosterone 3 months	Oestradiol Baseline	Oestradiol 3 months
1	Control	58	S American	20.07	22.55	80	111
2	Control	60	White	9.19	9.41	46	77
3	Control	72	Chinese	16.41	15.72	126	111
4	Control	73	White	14.59	12.7	132	87
5	Control	65	White	13.61	12.36	105	96
6	Control	50	Asian	17.71	18.93	73	78
7	Control	64	White	27.7	18.82	66	56
8	Control	53	White	11.98	20.28	175	97
9	Control	74	White	24	22.2	102	107
10	Control	64	White	10.24	12.91	122	131
11	Study	73	White	20.3	0.45	121	37
12	Study	67	White	21.4	0.45	79	37
13	Study	74	Black	9.31	0.45	78	37
14	Study	69	Asian	20.44	0.45	80	37
15	Study	75	Chinese	10.4	0.45	77	37
16	Study	72	Black	16.08	0.45	147	37
17	Study	75	White	17.3	0.45	87	37
18	Study	59	Asian	17.98	1.39	51	37
19	Study	68	Asian	20.23	1.42	95	37
20	Study	64	Black	12.24	0.49	79	37
Units: Testosterone nmol/L, Oestradiol pmol/L							