ENDOGENOUS SERUM TESTOSTERONE IN MAN: AGEING, THE METABOLIC SYNDROME, FUNCTIONAL DECLINE AND THE ROLE OF SUPPLEMENTATION

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In memory of my dear old dad Tim

And

For my mum Pat and sisters Marie-Ann and Carmel

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STATEMENT OF ORIGINALITY AND AUTHENTICITY

I declare that this thesis contains no material that has been accepted for the award of any other degree or diploma in any university or tertiary institution and to the best of my knowledge and belief, the thesis contains no material previously published or written by another person, except where due reference is made.

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying if accepted for the award of the degree.

Signed,

Matthew T. Haren _____ Date: _____

TABLE OF ABBREVIATIONS AND BIOCHEMICAL NAMES

ACCV	Australian Cancer Council of Victoria
ADAM	Androgen deficiency in the ageing male
AE	Adverse event
AIHW	Australian Institute of Health and Welfare
ANOVA	Analysis of variance
AR	Androgen receptor
ARCBS	Australian Red Cross Blood Service
ARE	Androgen response element
ARIP3	AR-interacting protein 3
ARR3-tk-luc	A construct containing a triple repeat of probasin ARE from rat ventral prostate
BDI	Beck depression inventory
BMD	Bone mineral density
BMI	Body mass index
BMR	Basal metabolic rate
BP	SF-36 domain: Bodily pain - intensity of bodily pain or discomfort

BPH	Benign prostatic hyperplasia
BT	Bioavailable testosterone
CaCo2	Human colon adenocarcinoma cells
cAMP	Cyclic adenosine mhey onophosphate
cBT	Calculated bioavailable testosterone
CCK	Cholecystokinin
cFT	Calculated free testosterone
CHO-K1	Chinese hamster ovary cells
CI	Confidence interval
COS-1	Kidney cells from an African green monkey
CV	Coefficient of variation
DBD	DNA binding domain
DBP	Diastolic blood pressure
DEXA	Dual energy x-ray absorptiometry
DHEA	Dehydropepiandrosterone
DHT	Dihydrotestosterone

DMEM	Dulbecco's modified Eagle's medium
DNA	Deoxyribonuceic acid
DSS	Department of Social Security
E2	Oestradiol
ED	Erectile dysfunction
ER	Oestrogen receptor
EWP	Electronic White Pages
FAI	Free androgen index
FAMAS	Florey Adelaide Male Ageing Study
FCS	Foetal calf serum
FLUT	Flutamide
FOME	Fuld object memory evaluation
fPSA	Free prostate specific antigen
FSH	Follicle stimulating hormone
FT	Free testosterone
FTT	Finger tapping test

GDS	Geriatric depression scale
GH	SF-36 domain: General health - general health perceptions
GHR	Generational Health Review
GIR	Global impotence rating
GnRH	Gonadotropin releasing hormone
hAR	Human androgen receptor
Hb	Haemoglobin
HbA1c	Glycated haemoglobin
HCG	Human chorionic gonadotropin
Hct	Haematocrit
HDL	High density lipoprotein
HeLa	Human epithelial cervix carcinoma cells
HPLC	High pressure liquid chromatography
ICC	Inter-class correlation coefficient
ICH	International Conference on Harmonisation
IGF-1	Insulin-like growth factor 1

IIEF	International index of erectile function
IMVS	Institute of Medical and Veterinary Science
IPSS	International prostate symptom scale
Kd	Dissociation constant
LBD	Ligand binding domain
LBM	Lean body mass
LDL	Low density lipoprotein
LH	Lutenising hormone
LMHS	Lyell McEwin Health Service
LUTS	Lower urinary tract symptoms
MH	SF-36 domain: Mental health - psychological distress and wellbeing
MMSE	Mini mental state examination
MNA	Mini nutritional assessment
mRNA	messenger ribonucleic acid
NaCl	Sodium chloride
OSA	Obstructive sleep apnoea

PBS	Phosphate buffered saline
PC3	Human prostate adenocarcinoma, metastic cells from bone
PDE-5	Phosphodiesterase 5
PF	SF-36 domain: Physical function - limitations in physical activities because of health problems
PLB	Passive lysis buffer
pRL-tk	A reporter vector containing herpes simplex virus thymidine kinase promoter upstream of *Renilla luciferase*
PROS	Population Research and Outcome Studies (unit, SA Department of Human Services)
QC	Quality control
Qmax	Maximal urinary flow rate
QoL	Quality of life
QPL	Questionnaire programming language
RAH	Royal Adelaide Hospital
RE	SF-36 domain: Role emotional - limitations in usual role activities because of emotional problems
RP	SF-36 domain: Role physical - limitations in usual role activities because of physical health problems

SAE	Serious adverse event
SBP	Systolic blood pressure
SD	Standard deviation
SDI-2	Sexual desire inventory (version 2)
SE	Standard error
SF	SF-36 domain: Social functioning - limitations in social activities due to physical or emotional problems
SF-36	36-item Short form health survey
SHBG	Sex hormone binding globulin
SHBG-R	SHBG receptor
T	Testosterone
TC	Testosterone cypionate
TE	Testosterone enenthate
TMT	Trail making test
tPSA	Total prostate specific antigen
TQEH	The Queen Elizabeth Hospital
TU	Testosterone undecanoate

TURP	Trans-urethral resection of prostate
VAS	Visual analogue scale
VSP	Visuospatial (block design test)
VT	Vitality; energy and fatigue
WHO	World Health Organisation

PAPERS ARISING FROM THIS THESIS

Haren MT, Nordin BEC, Pearce CEM, O'Loughlin P, Chapman I, Morley JE, Wittert GA. The calculation of bioavailable testosterone. Andrology in the 21st Century [short communication]. *Proceedings of the VII International Congress of Andrology.* Robiare B, Chemis H, Morales C (eds.). 2001. Medimond: Englewood, NJ pp.209-213.

Haren MT, Morley JE, Chapman IM, O'Loughlin PD, Wittert GA. Defining 'relative' androgen deficiency in aging men: how should testosterone be measured and what are the relationships between androgen levels and physical, sexual and emotional health? *Climacteric 5(1):15-25, 2002*.

Wittert GA, Chapman IM, Haren MT, Mackintosh S, Coates P, Morley JE. Oral testosterone supplementation increases muscle and decreases fat mass in healthy elderly males with low-normal gonadal status. *J Gerontol A Biol Sci Med Sci. 58(7):618-25, 2003.*

Haren MT, Wittert GA, Chapman IM, Coates P, Morley JE. Effect of 12-month oral testosterone undecanoate on visuospatial cognition in healthy elderly men with low-normal gonadal status. *Maturitas* 50(2):124-33.

Haren MT, Wittert GA, Chapman IM, Coates P, Morley JE. Effect of 12-month oral testosterone on testosterone deficiency symptoms in symptomatic elderly males with low-normal gonadal status. *Age & Ageing* 2004 (Dec 13) Epub ahead of print.

SUMMARY

This thesis investigates the age-related decline in the various available measures and estimates of serum testosterone levels in men. Testosterone circulates predominantly bound with high affinity to sex-hormone binging globulin (SHBG) in plasma (~60%) and with lower affinity to albumin (<40%); approximately 1-2% circulates unbound in plasma. It is the albumin-bound and free fractions (termed "bioavailable testosterone") that are most likely to have biological effects on target tissues. This thesis reports the establishment, validation and derivation of normal ranges for an ammonium sulphate precipitation method for the measurement of bioavailable testosterone in serum. This method is in use by a number of laboratories at present including the laboratory of Professor John Morley at St Louis University with whom we collaborated.

Testosterone has been shown, both cross-sectionally and longitudinally, to decline progressively beginning around the age of thirty. Total testosterone declines at approximately 0.4% per year while bioavailable and free testosterone decline at approximately 1.2% per year. The mechanisms that may be responsible for this include age-related changes to the hypothalamic-pituitary-testicular axis, increased SHBG levels, environmental factors, medication and chronic illness. This decline may contribute to a multitude of physiological, psychosexual and cognitive changes associated with ageing in men. This thesis cross-sectionally examines the possible determinants of the various fractions of serum testosterone and the associations with various physical, psychosexual and lifestyle variables and with chronic disease and medication use. These cross-sectional data were generated from the Florey Adelaide Male Ageing study, which randomly recruited 568 men from the north and west suburbs of Adelaide, between August 2001 and August 2002.

Moreover, this thesis includes a randomised controlled trial of testosterone replacement therapy in men aged 60 years and over with low-normal testosterone levels at baseline, recruited by newspaper

advertisement. The goals of testosterone replacement therapy might be to prevent osteoporosis, age related frailty and falls, and to maintain optimal physical, sexual, emotional and cognitive health during the ageing process. This intervention study focused on the effect of treatment on body composition and muscle strength, symptoms of testosterone deficiency, visuospatial cognition, mood, wellbeing and quality of life.

Finally, preliminary work was initiated to develop an in vitro bioassay for the measurement of serum testosterone bio-action. This was done using a transient transfection protocol in cultured cells, where androgen receptor and androgen response elements were introduced into the cells, subsequently treated with testosterone containing media and the amplitude of response quantified using a dual-luciferase-reporter assay.

In summary, this thesis discusses the issues with the measurement of testosterone in plasma and the factors that determine the concentration of the various fractions of testosterone in plasma. A cross-sectional study, using random recruitment procedures was used to investigate associations between testosterone levels and health-related-factors and finally a randomised-controlled-trial of testosterone replacement in ageing men with low-normal testosterone levels is reported. Throughout the thesis, the following themes are common; body composition, physical function and strength, sexual function, lower urinary tract symptoms and the prostate, visuospatial cognition, mood, quality-of-life and wellbeing.