

**IN VITRO MODULATION OF ANDROGEN AND ESTROGEN
RECEPTORS IN HUMAN PROSTATE CELLS BY
ESSENTIAL FATTY ACIDS**

BY

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SUBMITTED TO THE FACULTY OF HEALTH SCIENCES

SCHOOL OF MEDICINE

DEPARTMENT OF UROLOGY

UNIVERSITY OF PRETORIA

In partial fulfilment of the requirements for the degree ***Magister Scientiae with specialization in Reproductive Biology: Andrology***, Pretoria, South Africa, 2001



“Clearly there is need for a stronger evidence base, and more creative thinking on the part of the health-care profession, to help engage men of all ages in caring for their own health. Most importantly, it is essential that men themselves take an active part in the process of researching and developing their own care. The accomplishments of the women's health movements in recent years should provide ample stimulus and inspiration.”

The Lancet • June 9, 2001 • Vol 357 p. 1813 (editorial)



Hiermee erken ek met dank die finansiële hulp (beurs) wat ek van die "Struwig-Germeshuysen Kankemavorsingstrust" ontvang het vir die voltooiing van my studies. Menings wat in die publikasie uitgespreek word of gevolgtrekkings waartoe geraak is, is dié van die navorser alleen en strook nie noodwendig met dié van die "SGK-Trust" nie.



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ACKNOWLEDGEMENTS

On a personal note, I would like to extend my sincere gratitude to the following persons and organizations for the contributions they made in the fulfilment of this degree:

First of all, I would like to thank Dr. Casper van Aswegen for his patience in sharing his knowledge, insight and experience with me. I have learned that: "Any enterprise is built by wise planning, becomes strong through common sense, and profits wonderfully by keeping abreast of the facts." (Proverbs 24: 3,4 Living Bible). Thank you for your friendship and may you prosper in your future enterprises.

Dr. Marlene Fourie for her valued advice with my protocol and thesis and for many e-mails that made my day.

Prof. Riana Bornman for allowing me to study in her department.

Prof. Simon Reif, head of the Department of Urology for his support.

Prof. Dion du Plessis for his interest, encouragement and giving perspective and meaning to my project.

Miss Martie van der Walt and the staff from the Pre Medical Library for their friendliness and always excelling in obtaining the literature I needed. Smullerkie says thanks, NAMkie.

The Struwig-Germishuysen Trust for supporting me with a bursary for two years.

The Wolmarans Fund, Efamed SA, NAVKOM, CANSA, THRIP and the University of Pretoria for funding.

All my colleagues in the Department of Urology, including the secretaries, for their friendliness and Dr. Caren Hadders for helping me with my studies.

All my colleagues in the Department of Physiology, especially Dr. Petrus du Toit for teaching me cell culture work, Dr. Annie Joubert for her discussions and Prof. van Papendorf for the use of their facilities.

Mrs. Elize Gema and Prof. Leonora Dreyer from the Department of Anatomical Pathology who was so kind to assist me with immunocytochemistry.

Donovan Burger from Separations Scientific for helping me with valuable advice in the lab, Marinda van Kleef and Jeanni Fehrsen from Onderstepoort Research Institute for their interest and advice.

My parents, Piet and Elna Muller, my sister Marietjie and two brothers, Frikkie and Samuel, for always believing in me, for their support and for their love. Thanking you and loving you too!

My dear husband, Boet, for teaching this "slimjan" things about life that I shall carry with me always. Thanks Boet, I love you!

May you all be richly blessed with everlasting peace and joy!

To my Creator, Saviour and Encourager, I acknowledge Thee, I thank Thee and I praise Thee!

ABBREVIATIONS

A ₂₆₀	-	Absorbance at 260 nm
A ₂₈₀	-	Absorbance at 280 nm
ALA	-	α-Linolenic acid
AR	-	Androgen receptor
ARE	-	Androgen response element
bp	-	base pair
BP	-	Binding protein
BPH	-	Benign prostatic hyperplasia
°C	-	degrees Celsius
Ca ²⁺	-	Calcium catione
CAG	-	Cytosine Adenine Guanine
cDNA	-	complementary deoxyribonucleic acid
Ci	-	Curie
COX	-	Cyclooxygenase
CSPD	-	Disodium 3-(4-methoxyspiro{1,2-dioxetane-3,2'-(5' chloro)-tricyclo[3.3.1.1 ^{3,7}]decan}-4-yl) phenyl phosphate
CsTFA	-	Cesium trifluoroacetate
DAB	-	3,3'-diaminobenzidine tetrahydrochloride
DHA	-	Docosahexaenoic acid
DTT	-	Dithiotreitol
ddATP	-	Dideoxyadenine triphosphate
ddNTP	-	Dideoxynucleotide triphosphate
ddUTP	-	Dideoxyuridine triphosphate
DEPC	-	Diethyl pyrocarbonate
DES	-	Diethylstilbestrone
DESdP	-	Diethylstilbestrone diphosphate
DHT	-	Dihydrotestosterone



DIG	-	Digoxigenin
DNA	-	Deoxyribonucleic acid
dpm	-	disintegrations per minute
E	-	Estrogen
E ₂	-	Estradiol
EMEM	-	Eagle's minimum essential medium
EDTA	-	Ethylenediamine tetraacetic acid disodium salt
EFA	-	Essential fatty acid
EFAs	-	Essential fatty acids
EGF	-	Epidermal growth factor-urogastrone
EPA	-	Eicosapentaenoic acid
ER	-	Estrogen receptor
ER α	-	Estrogen receptor alpha
ER β	-	Estrogen receptor beta
EpRE	-	electrophile/antioxidant response element
<i>et al.</i>	-	and others
EVS	-	Essensiële vetsure
FAs	-	Fatty acids
FAS	-	Fatty acid synthase
FCS	-	Fetal calf serum
FGF-2	-	Fibroblast growth factor 2
FSH	-	Follicle-stimulating hormone
G3PDH	-	Glyceraldehyde-3-phosphodehydrogenase
GAPDH	-	Glyceraldehyde-3-phosphodehydrogenase
GH	-	Growth hormone
GLA	-	γ -Linolenic acid
[³ H]-E ₂	-	Radiolabelled estradiol
[³ H]-T	-	Radiolabelled testosterone
HRE	-	Hormone response element
HRP	-	Horse radish peroxidase
hsp	-	heat shock protein



IGEPAL	-	(Octylphenoxy)polyethoxyethanol
IGF-I	-	Insulin-like growth factor I
IgG	-	Immunoglobulin Gamma
kDa	-	kilo Dalton
KGF	-	Keratinocyte growth factor
LH	-	Luteinizing hormone
LH-RH	-	Luteinizing hormone-releasing hormone
LA	-	Linoleic acid
M	-	Molarity
MDA	-	Malondialdehyde
ml	-	millilitre
mmol	-	millimoles
MOPS	-	3-[N-Morpholino]propanesulphonic acid
mRNA	-	Messenger ribonucleic acid
MUFAs	-	Monounsaturated fatty acids
NLS	-	Nuclear localization signal
nM	-	nano Molar
NO	-	Nitric oxide
NGS	-	Normal goat serum diluted 1 in 5 with TBS
NSAID	-	Nonsteroidal anti-inflammatory drugs
OA	-	Oleic acid
PAGE	-	Polyacrylamide gel electrophoresis
par	-	pararagraph
PBS	-	Phosphate buffered saline
PEP	-	Phosphoenolpyrovate
PIN	-	Prostatic intraepithelial neoplasia
PKC	-	Protein kinase C
pmol	-	picomoles
PMS	-	Premenstrual syndrome
PMSF	-	Phenyl-methyl-sulphonyl fluoride
PR	-	Phenol red



PSA	-	Prostate specific antigen
PUFAs	-	Poly unsaturated fatty acids
RIPA	-	Radioimmuno precipitation assay
RNA	-	Ribonucleic acid
RNase	-	Ribonuclease
RSHBG	-	SHBG receptor
SA	-	South Africa
SDS	-	Sodium dodecyl sulphate
SFAs	-	Saturated fatty acids
SHBG	-	Steroid hormone binding globulin
SH	-	Steroid hormone
SHR	-	Steroid hormone receptor
SH-SHR	-	Steroid hormone – steroid hormone receptor complex
SS	-	Saline solution
SSC	-	Saline sodium citrate
T	-	Testosterone
TAF	-	Transcription activation factor
TBS	-	Tris buffered saline
TBS-T	-	Tris buffered saline Tween 20
TE	-	Tris-EDTA
TEBG	-	Testosterone estradiol binding globulin
TeBG	-	Testosterone binding globulin
TGF- α	-	Transforming growth factor alpha
Tris	-	Tris(hydroxymethyl)-amino-methane
Tween 20	-	Polyoxyethylenesorbitan
UK	-	United Kingdom
uPA	-	Urokinase type plasminogen activator
USA	-	United States of America
μ l	-	microlitre
VEGF	-	Vascular endothelial growth factor

REAGENTS AND CHEMICALS

All Reagents were of analytical grade and were supplied by the following companies:

Amersham Pharmacia Biotech (Buckinghamshire, UK)

The ExcelGel 7.5 and 12.5 homogenous PAGE gels
Rainbow™ coloured protein molecular weight markers
Kodak Scientific imaging film, X-OMAT™AR AEC-Amersham (Kelvin, SA).

Beckman (Irvine, USA)

Ready Protein⁺

BioWhitaker (Walkerville, MD, USA)

Trypsin

Bio-Rad Laboratories (Richmond, CA, USA)

Bio-Rad Protein Assay kit

Clontech Laboratories (Palo Alto, CA, USA)

G3PDH and β -actin probes

Corning Glass Works (New York, USA)

Corning culture flasks

Dako (Cambridgeshire, UK)

LSAB[®]2 (K0675)
DAB chromogen (K3466)

Delta Bioproducts (Kempton Park, SA)

Fetal calf serum

DuPont NEN (Boston, MA, USA)

[2,4,6,7-³H(N)]Estradiol-17β (92 Ci/mmol)

[1,2,6,7-³H(N)]Testosterone (95 Ci/mmol)

Flow Laboratories (Irvine, UK)

Ham's nutrient F-10 mixture

Microsep (Bramley, SA)

Immobilon P membranes

Millipore Sterivex-GS filters

Millipore filters AP 15 and 20

NEN™ Life Science Products (Boston, MA, USA)

3'-End Labelling Fluorescein kit with Antifluorescein-HRP

Oncogene Products (Cambridge, MA, USA)

ER and GAPDH probes

Novo Castra Laboratories (Newcastle upon Tyne, UK)

Mouse antihuman antibody for AR (NCL-AR-318)

Mouse antihuman antibody for ER α (NCL-ER-6F11/2)

Pierce (Rockford, Illinois, USA)

Super Signal West Picochemiluminescence Substrate

Roche Diagnostics (Boehringer Mannheim Roche Diagnostics SA)

TriPure isolation reagent, positively charged nylon membranes, DIG nucleic acid labelling and detection kits, washing, blocking, maleic acid and detection buffers.

Serotec (Oxford, UK)

Secondary antibody F(ab')₂ goat antimouse IgG, conjugated with Horseradish peroxidase

Sigma-Aldrich (Atlasville, SA).

Linoleic acid (LA), α -linolenic acid (ALA), γ -linolenic acid (GLA), arachidonic acid (AA), eicosopentaenoic acid (EPA) and oleic acid (OA), Testosterone and DES, PMSF, aprotinin, leupeptin, MOPS buffer, Tween 20. Primary rabbit antihuman antibodies for ER α and ER β , as well as the secondary monoclonal antirabbit immunoglobins, which were conjugated with Horseradish peroxidase, DEPC.

Sterilab (Johannesburg, SA)

DU-145 cell lines

Tec Med Imaging (Halfway House, SA).

Film developer and fixer solutions

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ABSTRACT

Cancer of the prostate is one of the leading causes of cancer related deaths in black and white men. An important role in the development of prostate cancer is played by androgens and androgen ablation is therefore currently used in cancer treatment. In the past, estrogens were widely used in treatment of prostate cancer, but there are indications that estrogens could also be involved in carcinogenesis. In contrast with saturated fats, some essential fatty acids (EFAs) are associated with a decrease in prostate cancer incidence. Lately, much research has been done on the modulation of the binding of steroid hormones to their receptors by essential fatty acids (EFAs), which could interfere with the steroid hormone's message. Therefore, the aim of this study was to determine in whole DU-145 human prostate cells the effect of EFAs and their metabolites on the binding and affinity of the estrogen receptor (ER) and androgen receptor (AR) to estradiol (E_2) and testosterone (T), respectively. Binding studies were carried out with radioactive T and E_2 with and without an excess of cold steroid. Data was analyzed according to the Scatchard method. Western and northern blotting were applied. The results showed that the EFAs under investigation inhibited the AR's capacity (B_{max}), in contrast to the ER's capacity, which was stimulated. However, the dissociation constants (K_d) of the AR and ER complexes in the presence of the fatty acids (FAs) were as follows. Except for eicosapentaenoic acid (EPA) which decreased the AR dissociation constant and EPA and alpha-linolenic acid (ALA) which increased the ER dissociation constant, the remaining FAs had no significant effect on the K_d values of both the AR and ER complexes. The decrease in the AR K_d and B_{max} values implied that EPA caused less T to bind to AR, but more tightly. In contrast, the increase in the ER B_{max} and K_d values implied that both EPA and ALA caused more E_2 to bind to ER, but more loosely. The overall inhibition of T binding to AR and stimulation of E_2 binding to ER by n-3 EFA may be beneficial during androgen withdrawal with or without estrogen therapy. Molecular studies showed that EFAs had no effect on the expression of the steroid receptors and further studies are necessary to



investigate the effect of EFAs on steroid receptor expression. According to these preliminary results it is concluded that men should benefit from a diet rich in certain essential polyunsaturated fatty acids although its function remains to be clarified.

KEY WORDS: Estrogen receptor α , Estrogen receptor β , Androgen receptors, Prostatic cancer, DU-145 cells, Essential fatty acids, Scatchard analysis, Northern blotting, Immunocytochemistry, Western blotting.

OPSOMMING

Prostaatkanker is een van die belangrikste oorsake van kankerverwante sterftes onder swart en wit mans. Androgene speel 'n belangrike rol in die ontwikkeling van prostaatkanker en derhalwe word androgeen onttrekking in die behandeling van kanker behandeling gebruik. In die verlede was estrogene algemeen gebruik in die behandeling van prostaatkanker, maar daar is aanduidings dat estrogene ook betrokke kan wees in karsinogenese. Onlangs is heelwat navorsing gedoen op die modulering van die binding van steroïed reseptore aan hulle reseptore deur essensiële vetsure (EVS), wat met die steroïed hormoon se boodskap kan inmeng. Daarom was die doel van hierdie studie om die effek van EVS en hulle metaboliëte op die bindingskapasiteit en die affiniteit van die estrogeen reseptor (ER) en die androgeen reseptor (AR) aan estradiol (E_2) en testosteroon (T) in heel DU-145 menslike prostaat selle onderskeidelik te bepaal. Scatchard binding studies, asook noordelike en westelike klad is uitgevoer. Die resultate het getoon dat die EVS wat ondersoek was die AR se kapasiteit (B_{max}) geïnhibeer het, in teenstelling met die ER se kapasiteit wat gestimuleer was. Die dissosiasie konstantes (K_d) van die AR en ER komplekse in die teenwoordigheid van EVS was as volg. Behalwe vir eicosapentaenoaat (EPA) wat die AR dissosiasie konstante verminder het en EPA en alpha-linolenaat (ALA) wat die ER dissosiasie konstante vermeerder het, het die oorblywende vetsure geen betekenisvolle effek op die K_d waardes van beide die AR en ER komplekse gehad nie. The afname in die AR K_d en B_{max} waardes beteken dat EPA veroorsaak het dat minder T aan die AR gebind het, maar stywer. In teenstelling daarmee, het EPA en ALA veroorsaak dat meer E_2 aan ER gebind het, maar lossier. Die algehele inhibering van T binding aan ER deur EPA en stimulering van E_2 binding aan ER mag voordelig wees tydens androgeen onttrekking met of sonder estrogeen behandeling. Hoewel geen effek op die uitdrukking van AR en ER verkry was nie, word verdere studies benodig om te bepaal of EVS die uitdrukking van steroïed reseptore sou affekteer. Volgens hierdie voorlopige resultate kan afgelei word dat mans bevoordeel sou



word deur 'n diëet ryk in sekere essensiële poli-onversadigde vetsure, hoewel die funksies nog opgeklaar moet word.

SLEUTELWOORDE: Estrogeen reseptor α , Estrogeen reseptor β , Androgeen reseptore, Prostaat kanker, DU-145 selle, Essensiële vetsure, Scatchard analise, Noordelike klad, Immunositochemie, Westelike klad.