Hormonal Regulation of the Class B Scavenger Receptors CD36 and SR-BI, in the Rat Liver

By

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DECLARATION

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Rebecca Fitzsimmons and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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Rebecca Fitzsimmons B.Sc.Hons.

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I wish to extend sincere thanks to both of my supervisors for having both the fortitude and the capacity to embrace a project that was not central to their primary areas of expertise. Thank you to Graham Mayrhofer, who has been for me an excellent educator and mentor. His remarkable ability to integrate individual systems and mechanisms into a whole body physiology is something I aspire to and this is the approach I atttempt to bring to each project I take on. The guidance and support provided was great, not to mention the many, many hours of hands on assistance with surgery. Few people would ever be so immersed in a long afternoon of castrations to forget to attend their own birthday dinner (still makes me laugh!). But on a serious note I owe a debt of gratitude especially for the editorial guidance and patience shown during the preparation of this thesis. I am truly grateful. Thanks also to Leslie Cleland for his many contributions to the project. I really appreciated his willingness to engage and his generosity with his time, despite an incredibly busy schedule. He provided a wealth of insight, encouragement and frequently a different perspective on issues, which was an invaluable resource.

Many people generously contributed reagents and expertise to this project. The testosterone radioimmunoassay kits were donated by Dr. Peter O'Loughlin (IMVS) and the Zoladex implants were provided by Dr Brian Setchell (University of Adelaide). Dr Jamie Taylor (Royal Adelaide Hospital) performed the magnetic resonance imaging. Drs Alan Rofe and Peter Coyle (IMVS) performed the hepatocyte perfusions and provided access to their laboratory and equipment. Prof Michael Waters provided the Dwarf rats from the colony at the University of Queensland. Financial support was provided by a Dawes PhD Scholarship from the Royal Adelaide Hospital Research Committee.

Thank you also to my colleagues and friends in the Arthritis Research Lab (Luigi, Sarah, Mahin and Zingqi) for their continual support - both moral and practical. From the School of Molecular Biosciences there are also many people to thank. To Gorjana who frequently assisted with the messy stuff and through all the blood and body parts became a good friend! To Nick for the endless discussions about CD36 over the bench, over coffee and over beer. It was really great working with you. To the Lec A's; Chris,

Alex and Jamie and also to Gary, all of whom generally made life in the department smoother and more fun. Thankyou.

On a personal note I would also like to acknowledge my current group leader Prof George Muscat who has provided tremendous encouragement, allowed me to take leave and has been very accommodating about the ongoing bleary-eyed arrival at work after many late nights, so that I could complete this thesis. Thanks also to my colleagues at the IMB and Steven Hodge at Deakin University who have assisted with the thesis preparation especially Nick, Mikey and Dennis. A friend who will proofread is a friend indeed!

I thank my parents who have been endlessly supportive and to my very own mini - scientist Elliot for allowing me to spend so much time “doing computer work”. Finally, heartfelt appreciation to my husband Brett who made many sacrifices and has juggled his own study commitments for some years around mine.

PRESENTATIONS AND PUBLICATION FROM THIS STUDY

Conference Presentations

ASMR SA Branch, Annual Scientific Meeting, Adelaide, June 2001, Oral Presentation "Sex Steroid Regulation of Hepatic CD36 Expression in Rats" **RL Fitzsimmons**, X Zhang, LG Cleland and G Mayrhofer.

Australian Health and Medical Congress, Melbourne, October 2002, Poster Presentation "Endocrine Regulation of Hepatic Class B Scavenger Receptor Expression in DA Rats" **RL Fitzsimmons**, X Zhang, LG Cleland and G Mayrhofer

ASBMB Annual Meeting, Boston, USA, June 2004, Poster Presentation "Hormonal Regulation of CD36 expression in the liver – implications for cholesterol metabolism". **RL Fitzsimmons**, LG Cleland and G Mayrhofer. Abstract published in FASEB, 18(8):C261, 2004

Publications

Zhang, W., **Fitzsimmons, R.L**., Cleland, L.G., Ey, P.L., Zannettino, A.C.W., Farmer, E-A., Sincock, P. and Mayrhofer, G. 2003. " CD36/Fatty Acid Translocase in Rats: Distribution, Isolation from Hepatocytes, and Comparison with the Scavenger Receptor SR-BI" Lab. Invest 83(3): 317-32.

Fitzsimmons, R.L., Eyre, N.S., Radisic, G., Waters, M.J. and Mayrhofer, G."Gender and endocrine regulation of CD36 (fatty acid translocase) and SR-B1 expression in the liver" *Manuscript in preparation.*

CORRIGENDUM

The following corrections have been made to the original thesis manuscript that was submitted for assessment. Both assessors are sincerely thanked for their constructive and considered contributions.

Chapter 1

A brief discussion on the potential role of mitochondrial CD36, with respect to fatty acid transport and β-oxidation has been added into section 1.4.1.4.

Chapter 2

The text on p51, line 1 has been changed from "… in this study is…" to "…in this study are…"

The text on p56, line 10 has been changed from "…enumerated usign…" to "…enumerated using…"

Chapter 3

Figure 3.4(C) has been altered to indicate clearly that the Western blot in this figure has been cut.

Figure 3.6 (A) The position of the marker for Leydig cells has been moved to be more clearly visible in the figure.

Figure 3.8 (B) was incorrectly labelled female liver instead of male liver and vice versa. This has now been corrected. The label (C) has also been inserted into the figure legend.

The text on page 73, lines 6-7 the words "was observed" have been omitted as these were duplicated. In Paragraph 4 the description of analysis on Fig 3.8C has been changed from "….one-tailed t-test.." to "…two-tailed t-test.." This was a typographical error.

The text on page 75, line 4 has been changed from “utiliation” to “utilisation”.

Chapter 6

The text in page 117, hypothesis 1) has been changed from “disregulation” to “dysregulation”. In addition the title in 6.2 has been changed to remove capitals from prepositions and connector words.

In Tables 6.1 and 6.2, the sign +/- has been changed to ± in all instances and the number of significant figures has been reduced to 2, to improve clarity and the appearance of the tables. The title for Table 6.2 has been altered to include the age of the rats.

The text on page 122, paragraph 2, line 5 has been changed from “…the hypophysis-gonadal axis…” is changed to “…. Hypothalamic-pituitary-gonadal axis…”

The text on page 123, third last line has been changed from “…this biometric data was…” to “… these biometric data were…”

The text on page 127, paragraph 2, last sentence has been changed from “…this experiment demonstrates that upregulation of CD36 expression in these animals is not a direct consequence of the indirect effects of MSG in lowering concentrations of serum testosterone.” to “…this experiment demonstrates that GH, rather than testosterone, is responsible for male suppression of hepatic CD36 expression.”

The text on p127 (and elsewhere), dw/dw has been changed to *dw/dw* as it refers to the genotype of these mice.

On page 128, paragraph 2, a misplaced comma has been corrected from line 12 to line 10. The word “has” has been replaced with “have” in the text on line 14.

On page 129, paragraph 1, line 15, the word “ were” has been replaced with “was”.

On page 130, section 6.3.3.4, paragraph 2, the term “received” has been replaced with the phrase “subjected to” on lines 3, 6 and 7.

On page 132, paragraph 2 the sentence “However, in this case it could be concluded that it was the lack of testosterone, rather than the post-castration surge in LH production by the pituitary gland that was responsible either directly or indirectly for the increase in expression of CD36.” has been replaced with “However, because the effects of both experimental strategies to lower serum testosterone were similar, one of which lowered LH while the other did not, the conclusion reached was that lack of testosterone rather than post-castration surge in pituitary LH release was responsible for the increased hepatic expression of CD36.”.

On page 133, line 6 from the bottom, the word “after” has been deleted, as it was superfluous.

On page 135, paragraph 1, line 3, “this data” has been changed to “these data”. A double reference citation has also been deleted from line 21. On the eighth line from the bottom, the word “desaturase” has been inserted after “long chain -4 and -5”.

On page 137, paragraph 1, line 13, the term P4502C11 has been corrected to read CYP2C11.

Chapter 7

On page 148, paragraph 3, line 10 the word “an” has been deleted so that the phrase now reads “…towards understanding the complexity…”

PREFACE

For purpose of clarification, there are two points I wish to highlight in respect to this study.

Firstly, in the interests of transparency, I would like to clearly outline my contributions to the publication “CD36/Fatty Acid Translocase in Rats: Distribution, Isolation from Hepatocytes, and Comparison with the Scavenger Receptor SR-BI” (Laboratory Investigation 2003 83(3):317-332). With assistance from collaborators where noted, the experiments presented in Figures 1-6,9,10 and 13 were performed by Dr Xingqi Zhang whilst I performed the experiments presented in Figures 7, 8, 11 and 12. I also assisted Assoc Prof. G. Mayrhofer with the preparation of the manuscript, which was submitted after Dr. Zhang had left the laboratory.

Secondly, it appears pertinent to highlight the timeframe of the work presented in this thesis. The experimental work in this study was performed between 2001 and 2004. Consequently the rationale for the study and hypotheses that were investigated reflect the background knowledge and resources that were available during this time. In the time period from 2007 to the present there have been several studies of particular relevance and the implications of these findings to the work presented in this thesis are addressed in the final discussion.

ABSTRACT

CD36 or fatty acid translocase (FAT) is an 88 kDa cell surface protein. It is characterised functionally as a Scavenger Receptor, and it is the founding member of the Class B subtype, which also includes the HDL receptor, Scavenger Receptor Class B-I (SR-BI). A common feature of all scavenger receptors is a broad binding specificity and many ligands have been identified for CD36. Of particular interest to this project are the metabolic ligands, such as native and modified lipoprotein particles and long chain fatty acids. In the rat, others have reported that CD36 is expressed in heart muscle, intestine, spleen, skeletal muscle, adipose tissue and testis. Previous work in our laboratory, using a new monoclonal antibody (mAb UA009), extended the distribution of CD36 to include the adrenal cortex, the ovary and the parenchymal cells of the liver. Furthermore it was noted that hepatocytes from female DA rats expressed considerably more CD36 than their male counterparts. The scope and objective of this project was to investigate the effects of gender on expression of CD36 in the liver, and to compare it with expression of SR-B1.

Expression of CD36 was examined *in vivo,* in normal and experimentally manipulated female and male rats and mice, using immuno-histochemistry plus video-image analysis, quantitative Western blot and real-time PCR techniques. It was shown that female predominant expression of CD36 i) is specific to the liver, ii) occurs in many strains of laboratory rats, and iii) is also present in three mouse strains. Investigation of CD36 expression during post-natal development revealed that in neonates, the protein is not expressed by parenchymal cells of the liver. Although CD36 is expressed by hepatocytes from approx. 4 weeks of age, the adult pattern (female predominant) was not observed until the onset of puberty. No changes in the levels of SR-BI expression were observed during development. The role of hormones in regulating expression of CD36 was investigated by observing the effects of gonadectomy and supplementation with sex steroid hormones. Hepatic expression of CD36 was reduced by oophorectomy and increased by castration. Sexually dimorphic expression was restored by administration of estrogen or testosterone to gonadectomised females and males, respectively. Inhibition of gonadotropin release did not alter the effect of castration but the gender difference, and the response to gonadectomy, was reduced in growth hormone-deficient rats. In contrast, SR-BI was barely detectable in liver by immuno-

histochemistry in either sex, although modest differences between females and males and changes in response to gonadectomy were observed in levels of *Sr-bI/II* transcripts.

The results demonstrated the importance of the endocrine milieu in the regulation of CD36 expression by the liver. Although the initial acquisition of CD36 expression during postnatal development is sex steroid hormone independent, the sexually dimorphic adult pattern of expression coincides with the onset of puberty. The influence of sex steroids on hepatic CD36 expression is mediated via their interplay with the hypothalamic-pituitary axis, rather than by a direct action on the liver. Thus, the expression of CD36 in hepatocytes is regulated by mechanisms that are distinct from those that regulate fellow class B scavenger receptor family member, SR-BI. In conclusion, CD36 is a known to be a receptor for long chain fatty acids and for both native and modified lipoproteins. If hepatic expression of CD36 is also regulated by steroid sex hormones in humans, these findings could have important implications for understanding differences in lipid handling by the liver in females and males, and the possible consequences of therapeutic manipulation of these hormones.