Preclinical evaluation of the role of TIMP-1 and isoflavones on the development of obesity and the metabolic syndrome

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FACULTY OF HEALTH AND MEDICAL SCIENCES
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PhD thesis

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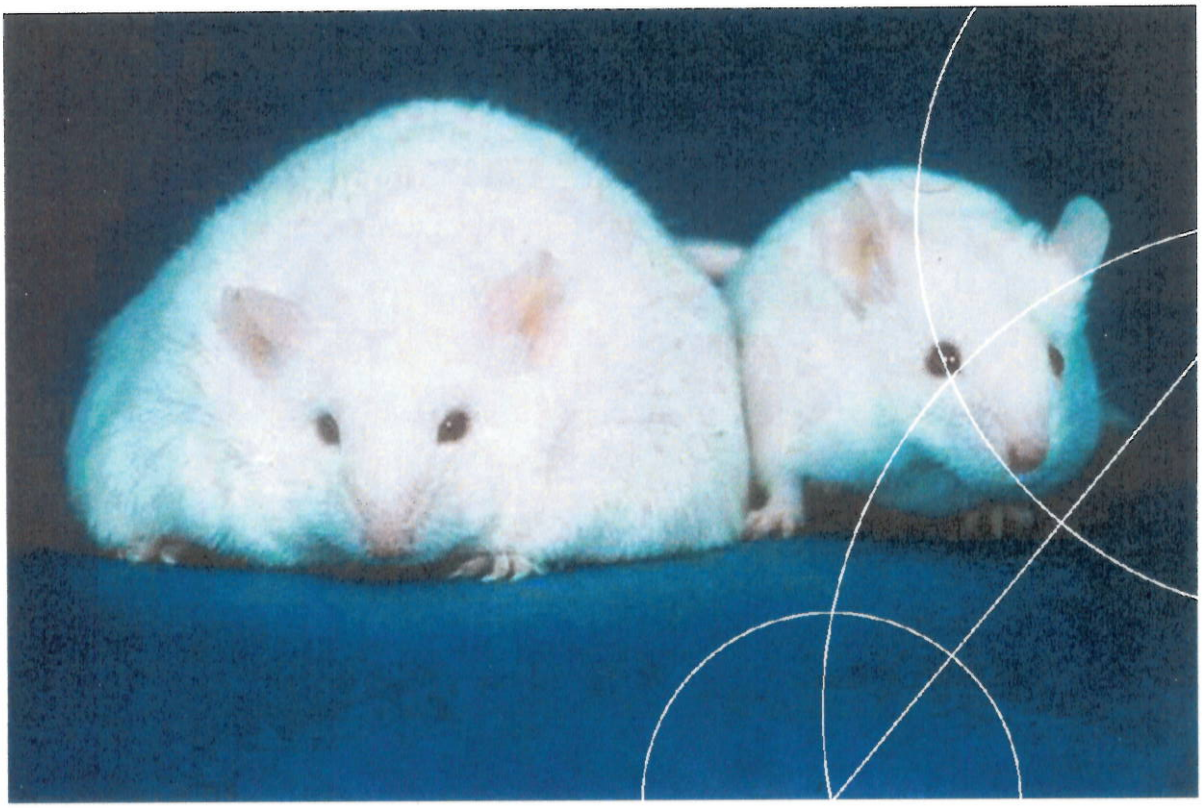

Department of Veterinary Disease Biology • August 2012

Preclinical evaluation of the role of TIMP-1 and isoflavones on the development of obesity and the metabolic syndrome

PhD thesis by Charlotte Andersen

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Preface

The PhD project was financed by a scholarship from the Faculty of Life Sciences, University of Copenhagen (now part of the Faculty of Health and Medical Sciences). The PhD was based on two separate projects in which the research group was involved. The primary focus of the research group is the role of TIMP-1 in the development of cancers. In connection to this there was a desire to investigate the role of TIMP-1 in the development of obesity using TIMP-1 knock-out mice generated in the group. The other project was on the role of isoflavones in development of obesity and was part of the strategic research project at LIFE called BEST (Byg En Sund Tilværelse). There is no link between the isoflavones and TIMP-1 and thus no common hypothesis. Yet, the two projects in PhD study are connected by the overall topic and the methods used.

The work was carried out at the Department of Veterinary Disease Biology, Faculty of Life Sciences and at the Department of Biology, Faculty of Science, both at University of Copenhagen. Furthermore, I had the opportunity to go to the lab of Professor Clifton Baile at University of Georgia, USA for five month.

Charlotte Andersen
Frederiksberg, August 2012

Content

List of included papers

The thesis includes the following publication and manuscripts:

Paper I: **Phytochemicals and adipogenesis** (review)
Charlotte Andersen, Srujana Rayalam, Mary Anne Della-Fera and Clifton A. Baile
Biofactors. 2010, 36(6):415-22.

Paper II: **Tissue inhibitor of metalloproteinases-1 mediates high fat diet induced glucose intolerance and hepatic steatosis**
Charlotte Andersen*, Jakob B. Hansen*, Even Fjære, Hanne S. Tastesen, Lene S. Myrmel, Nils Brünner, Lise Madsen, Karsten Kristiansen, Thomas Mandrup-Poulsen and Maria Unni Rømer
In final preparation for submission to Diabetes
* Shared first authorship

Paper III: **2(2-bromophenol)-formononetin and 2-heptyl-formononetin are partial PPARγ agonists and reduce lipid accumulation in 3T3-L1 adipocytes**
Charlotte Andersen*, Dorota Kotowska*, Christian G. Tortzen, Karsten Kristiansen, John Nielsen and Rasmus Koefoed Petersen
Manuscript in preparation
* Shared first authorship

Paper IV: **2-Heptyl-Formononetin Increases Cholesterol and Induces Hepatic Steatosis in Mice**
Charlotte Andersen, Janne Gram Schioldager, Christian G. Tortzen, Andreas Vegge, Majbritt Ravn Hufeldt, Mette T. Skaanild, Finn Kvist Vogensen, Karsten Kristiansen, Axel Kornerup Hansen and John Nielsen
In final preparation for submission to PlusOne

Summary

Obesity is a complex disorder often leading to a range of chronic diseases like insulin resistance, dyslipidaemia and hepatic steatosis, all components of the metabolic syndrome. The aim of the PhD project was to obtain more insight in the biology of obesity and related disorders and in particular to investigate the effects of tissue inhibitor of metalloproteinases (TIMP)-1 and isoflavones using *in vitro* and *in vivo* models.

Expansion of the adipose tissue requires extensive modulation of the extracellular matrix. This is among other factors controlled by metalloproteinases and TIMP. TIMP-1 is a biomarker of obesity but the functional role is unclear. The hypothesis was that TIMP-1 promotes the development of diet-induced obesity, glucose intolerance and hepatic steatosis. The hypothesis was tested in male TIMP-1 knock-out mice fed obesogenic diets (Paper II). The study showed that TIMP-1 had limited effects on adiposity, but TIMP-1 knock-out mice were protected against the development of hepatic steatosis and glucose intolerance perhaps due to better hepatic insulin sensitivity.

Several isoflavones reduce the risk of developing the metabolic syndrome and may be used as inspiration in drug development. The hypothesis was that the isoflavone formononetin and analogues of formononetin reduce adiposity, dyslipidaemia and hepatic steatosis. The effects on adiposity were investigated using 3T3-L1 preadipocytes (Paper III). The study showed that formononetin had limited effects on lipid metabolism, whereas 2-heptyl formononetin, a synthetic analogue, was a partial PPARγ agonist and decreased lipid accumulation in maturing and mature adipocytes possibly by decreasing adipogenesis and lipogenesis and increasing lipolysis and β-oxidation. The hypothesis was further investigated in mice fed a cholesterol-enriched diet (Paper IV). Supplementation with formononetin and 2-heptyl formononetin to the diet induced dyslipidaemia and hepatic steatosis possibly by decreasing hepatic lipid metabolism and lipoprotein assembly. The mice did not gain sufficient weight to assess the effects on adiposity.

In conclusion, the studies showed new effects of TIMP-1 on hepatic steatosis which could have a pharmacological perspective but should be further investigated. Despite an interesting profile *in vitro*, the tested isoflavones had detrimental effects *in vivo* showing that although isoflavones have many promising aspects for drug development there are also many pitfalls and investigations should include various diets.

Resumé (Dansk)

Udvikling af fedme leder ofte til en række metaboliske forstyrrelser som insulin resistens, dyslipidæmi og hepatosteatose som alle er del af det metaboliske syndrom. Formålet med ph.d. projektet var at opnå større biologisk indsigt i fedme og relaterede sygdomme og specielt at undersøge effekten af tissue inhibitor of metalloproteinases (TIMP)-1 og isoflavoner ved anvendelse af *in vitro* og *in vivo* modeller.

Udvidelsen af fedtvæv kræver omfattende modulering af den extracellulære matrix. Det er blandt andet reguleret af metalloproteinaser og TIMPs. TIMP-1 er en biomarkør for fedme men den funktionelle rolle er uklar. Hypotesen var at TIMP-1 fremmer udviklingen diæt-induceret fedme, glukose intolerance and hepatosteatose. Hypotesen blev testet i TIMP-1 knock-out hanmus fodret med fedmefremmende diæter (Artikel II). Studiet viste at TIMP-1 havde begrænset effekt på fedme, men TIMP-1 knock-out mus var beskyttet mod udviklingen af hepatosteatose og glukose intolerance måske på grund af bedre insulin sensitivitet i leveren.

Flere isoflavoner mindsker risikoen for udvikling af det metaboliske syndrom og kan bruges som inspiration til udvikling af lægemidler. Hypotesen var at isoflavonen formononetin og analoger af formononetin reducerer fedme, dyslipidæmi og hepatosteatose. Effekten på fedme blev undersøgt i 3T3-L1 fedtceller (Artikel III). Studiet viste at formononetin havde begrænset effekt på fedtmetabolisme, mens 2-heptyl formononetin, en syntetisk analog, var en partiel PPARγ agonist og nedsatte fedtmængden i fedtceller muligvis ved at nedsætte adipogenese og lipogenese og øge lipolyse og β-oxidation. Hypotesen blev yderligere testet i mus fodret en kolesterolberiget diæt (Artikel IV). Berigelse af foderet med formononetin og 2-heptyl formononetin medførte dyslipidæmi og hepatosteatose muligvis ved at nedsætte fedtmetabolismen og samlingen af lipoproteiner i leveren. Musene tog ikke nok på til at effekten på fedme kunne undersøges.

Studierne viste nye effekter af TIMP-1 på hepatosteatose som kunne have farmakologisk perspektiv men bør undersøges nærmere. På trods af en interessant profil *in vitro* havde de undersøgte isoflavoner uønsket effekt *in vivo*, hvilket viser at selv om isoflavoner har mange lovende effekter i forhold til udviklingen af lægemidler, så er der også mange faldgruber og undersøgelser bør inkludere forskellige diæter.