DR ALEXANDRÉ DELPORT

PROJECT: Assessing the feasibility of combating obesity in women through targeted protein degradation

Background and Rationale

The global obesity pandemic has not left South Africa unaffected, with levels of overweight and obese individuals of all ages on the rise across the country[1]. Obesity's association with a higher risk of developing comorbidities (e.g., insulin resistance, type 2 diabetes mellitus, heart disease, stroke, cancers and COVID-19)[2] means this rise in incidence is cause for concern. Moreover, the prevalence of obesity in South Africa is higher in women than in men[3; 4] – making this disease uniquely important to South African women in science.

Metabolically, obesity is associated with adipose or fat tissue dysfunction which is directly linked with mitochondrial dysfunction. Recently, using targeted protein degradation we have developed a new technology which can restore adipose tissue function.

Preliminary results from our *in vivo* pilot study suggest that, phenotypically, mice treated with our new technology have better glucose tolerance and show weight loss during the treatment period. Moreover, adipose tissue exhibits improved mitochondrial function after treatment even though the mice remained on a high-fat diet. Our technology has significant potential to be developed further into a novel obesity and diabetes treatment, by directly combating adipose dysfunction.

Objectives and methodology

Preliminary results suggest that our new technology, based on targeted protein degradation, can improve the obese state in mice. However, most obesity studies (like ours — so far) are focused on male mice, with little data analysing the effect in female mice. Since obesity affects women more than men in South Africa, data to determine efficacy in females is imperative. Therefore, we aim to assess whether our new technology can yield similar improvements in

glucose tolerance, reduced weight gain and improved adipose tissue function *in vivo* using female mice.

The following objectives are being pursued:

Objective 1. Phenotypic analyses of the effect on weight in female mice.

Objective 2. Confirmation of the mechanism of action, and target and tissue specificity.

Objective 3. Analysis of the benefit to metabolic health as well as to adipose tissue function.

Experimental detail:

C57BL/6 female mice (male mice of this strain were used previously allowing direct comparison) were fed a high-fat diet (HFD, D12492, Research Diets, USA) from weaning until weight gain becomes significantly greater than control mice on normal diet (ND, 5058, LabDiet, USA) (± 4-6 weeks). Mice will then be grouped as a test group: 1 (HFD + treatment) and control groups: 1 (HFD + vehicle) and 2 (ND + vehicle). Treatment and vehicle controls will be administered once daily by intraperitoneal injection for 4 weeks. Food consumption will be monitored, and accurate body weight measured (grams, every day) (**phenotypic analyses – Objective 1**). Blood samples will be taken to compare non-fasting blood glucose (mg/dL) and glucose tolerance (mg/dL) via IPGTT protocols (**metabolic analyses – Objective 3**). Tissue collection (comprising whole blood, brain, kidney, liver, visceral and subcutaneous adipose tissues) on termination of the study will allow for the assessment of acute toxicity, confirmation of mechanism of action and determination of adipose tissue function via biochemical assays (**quantitative analyses – Objective 2 & 3**). Sample size was calculated through power analysis and all biological experiments will be repeated in, at least, triplicate ($n \geq 3$). All animal studies have been approved by the Animal Research Ethics Committee (AREC) of the University of KwaZulu Natal (AREC/00003469/2023) and by the national responsible authority (DALLRD).

Timeline

Large quantity degrader synthesis: 01/11/2022 - 15/05/2023 [Completed]

Feeding phase: 01/05/2023 – 18/06/2023 (~ 4-6 weeks feeding) [Completed]

Treatment and phenotypic and metabolic analyses: 19/06/2023 – 17/07/2023 [Completed]

Termination and quantitative tissue analyses: 17/07/2023 – 30/11/2023 [In Progress]