Administration of protein hydrolysates from *Palmaria palmata* improved glycaemic parameters and lipid profile in streptozotocin diabetes induced mice.

C. McLaughlin¹, P.A. Harnedy², P.J. Allsopp¹, E.M. McSorley¹, R.J. FitzGerald², & F.P.M. O'Harte¹

¹School of Biomedical Sciences, Ulster University, Cromore Road, Coleraine, N. Ireland and ²Department of Life Sciences, University of Limerick, Castletroy, Limerick, Ireland.

INTRODUCTION

- Interest in low value/high protein products has peaked for the ability to yield an abundance of potentially useful functional food components
- Palmaria palmata*, is a species of red seaweed native to Irish shores which has a favourable composition with 10–35% protein content dependent on season
- Using proteolytic food grade enzymes, *Palmaria palmata* protein has been extracted and enzymatically digested to yield multiple small molecular weight peptides (SMWPs)
- The potential benefits of marine biomass derived SMWPs for type 2 diabetes therapy has received limited attention to date
- This study examined the efficacy of protein hydrolysates for improving glucose homeostasis in a diabetic induced animal model of multiple low dose streptozotocin (STZ).

AIMS & OBJECTIVES

To firstly establish the diabetic state in male HsdOla:T0 mice (n=24) using low dose STZ (3 x 40 mg/kg/BW) over 9 days. A twice daily oral dose of *Palmaria palmata* protein hydrolysates (50 mg/kg/BW) or metformin (200 mg/kg/BW) were given for 18 days and blood markers of diabetes were assessed including lipids and body composition analysis performed.

METHODOLOGY

Multiple low-dose STZ (40 mg/kg bw)

Administer STZ by ip injection once every 3 days for 9 days

Further 9 days given for acclimatization

Oral administration of twice daily treatments (**Metformin 200mg/kg** or ***Palmaria palmata* hydrolysate 50 mg/kg**) started after 6 days of saline gavage conditioning

Animals grouped based on non-fasting blood glucose and weight

Animals acclimatized for 6 days orally with physiological saline prior to treatments

Treatments commenced and given orally by gavage twice daily

Weight, food intake, blood glucose and plasma analysed every 3 days

DEXA + Terminal analysis commenced post 18 day treatment

Results & Grouping

Fig 1

A

Panel A/B – Post-treatment screening of animals consisted of group matched, based upon non-fasting blood glucose (Panel A) and fasting (8 h) blood glucose (Panel B). Both fasting and non-fasting blood glucose were significantly elevated versus pre-administration of STZ. Results are Mean $\pm$ SEM for 7-8 observations per group ***p<0.001 change versus starting time point.

B

Panel C/D. Effects of twice-daily administration of palmaria palmata hydrolysate and metformin on non-fasting blood glucose in diabetes induced STZ mice (**Panel C**). Parameters were measured 21 days prior with treatment commencing at day 0 to day 18. Twice daily treatment with palmaria palmata hydrolysates (50 mg/kg bw) and metformin (200 mg/kg bw) or saline vehicle (0.9% (w/v) NaCl) in both a STZ and non-STZ control groups. Values represent mean $\pm$ SEM for 7-8 mice. *p<0.01, ***p<0.001 vs STZ saline control. (**Panel D**) represents Mean $\pm$ SEM blood glucose area under the curve (AUC). ***p<0.001 versus STZ saline control, respectively.

Panel E/F - Effects of twice-daily administration of palmaria palmata hydrolysate and metformin on non-fasting plasma insulin concentration in diabetes induced STZ mice (**Panel E**). Parameters were measured 6 days prior with treatment commencing at day 0 to day 18. Twice daily treatment with palmaria palmata hydrolysates (50 mg/kg bw) and metformin (200 mg/kg bw) or saline vehicle (0.9% (w/v) NaCl) in both a STZ and non-STZ control groups. Values represent mean $\pm$ SEM for 8 mice. *p<0.05, **p<0.01 vs ob/ob saline, **Panel F** represents Mean $\pm$ SEM plasma insulin area above the curve (AAC). ***p<0.001, versus STZ saline control.

Panel G - Effects of twice-daily administration of marine hydrolysates on fasting blood glucose. Mice were fasted for 12 hours prior to measurement of glucose. Values represent mean $\pm$ SEM for 7-8 mice. *p<0.05 versus STZ saline control group.

Panel H - Effects of twice-daily administration of palmaria palmata hydrolysate on terminal oral glucose tolerance. Parameters were measured prior to (t=0) and 15, 30, 60, 90 and 120 min after administration of glucose alone (18 mmol/kg body weight; IP Injection). *p<0.05, and **p<0.01, ***p<0.001 compared to STZ saline controls.

Panel I - Effects of twice-daily administration of palmaria palmata hydrolysate on percentage weight change post-treatment. Analysed using mean weight at day 0 and day 18 with percentage change analysed. Values represent mean $\pm$ SEM for 7-8 mice. *p<0.05, **p<0.01 versus STZ saline control group.

Panel J - Effects of twice-daily administration of marine hydrolysates on terminal glycated haemoglobin (HbA1c). Values represent mean $\pm$ SEM for 8 mice. *p<0.05, **p<0.01, ***p<0.001 versus STZ saline control group.

Panel K - Effects of twice-daily administration of marine hydrolysates on terminal total plasma GLP-1 concentration. Values represent mean $\pm$ SEM for 8 mice. *p<0.01, ***p<0.001 versus STZ saline control group.

Panel L - Effects of twice-daily administration of palmaria palmata on terminal plasma triglyceride concentration. Values represent mean $\pm$ SEM for 8 mice. *p<0.01, ***p<0.001 versus STZ saline control group.

Panel M - Effects of twice-daily administration of palmaria palmata on terminal plasma cholesterol concentration. Values represent mean $\pm$ SEM for 8 mice. *p<0.01, ***p<0.001 versus STZ saline control group.

Panel N - Effects of twice-daily administration of marine hydrolysates on anthropometric analysis assessed via PIXImus DEXA. Graph represents total body fat (%). Values represent mean $\pm$ SEM for 7-8 mice.

Panel O - Effects of twice-daily administration of palmaria palmata hydrolysate on anthropometric analysis (BMC) assessed via PIXImus DEXA. Graph represents total body bone mineral content (BMD g/cm²). Values represent mean $\pm$ SEM for 8 mice. *p<0.05, **p<0.01, versus STZ saline control group and Δ p<0.05 versus non-STZ saline control, respectively.

Panel P - Effects of twice-daily administration of palmaria palmata hydrolysate on anthropometric analysis (Femur ROI BMC) assessed via PIXImus DEXA. Values represent mean $\pm$ SEM for 7-8 mice. *p<0.05, versus STZ saline control group, respectively

SUMMARY

- Several vital improvements in glucose homeostasis were noted in both hydrolysate and metformin treated groups.
- Significant reduction in non-fasting blood glucose levels evident from treatment day 9 through 18 in both *P. palmata* and metformin treatment groups.
- Circulating plasma insulin was elevated from day 6 in the *P. palmata* hydrolysate group, however no increase in insulin secretion was noted until day 18 in the metformin group.
- Terminal fasting blood glucose was significantly improved in all treatment groups, interestingly *P. palmata* hydrolysate group presented with almost normoglycaemia compared to the supra-physiological non-treated animals.
- Improvement in oral glucose tolerance, circulating lipids and significantly lower HbA_{1c} levels evident in both treatment groups.
- DEXA analysis has highlighted improvement in total BMC and ROI BMC of the femur in the *P. palmata* hydrolysate group warranting further investigations.
- The research has highlighted oral peptides or liberated amino acids play a vital role in glucose regulation and improvement of diabetic status in STZ-induced diabetic mice and should be further investigated in diet induced obesity rodent models.