FURTHER IN VITRO STUDIES ON THE PROTECTIVE EFFECTS OF A NOVEL PEPTIDE HYDROLYSATE, SPH1, AGAINST MUSCLE ATROPHY USING PRIMARY NORMAL HUMAN SKELETAL MYOBLASTS (HSkM) & PRELIMINARY CHARACTERISATION OF GLP2 AGONIST ACTION TO SUPPORT GI FUNCTION

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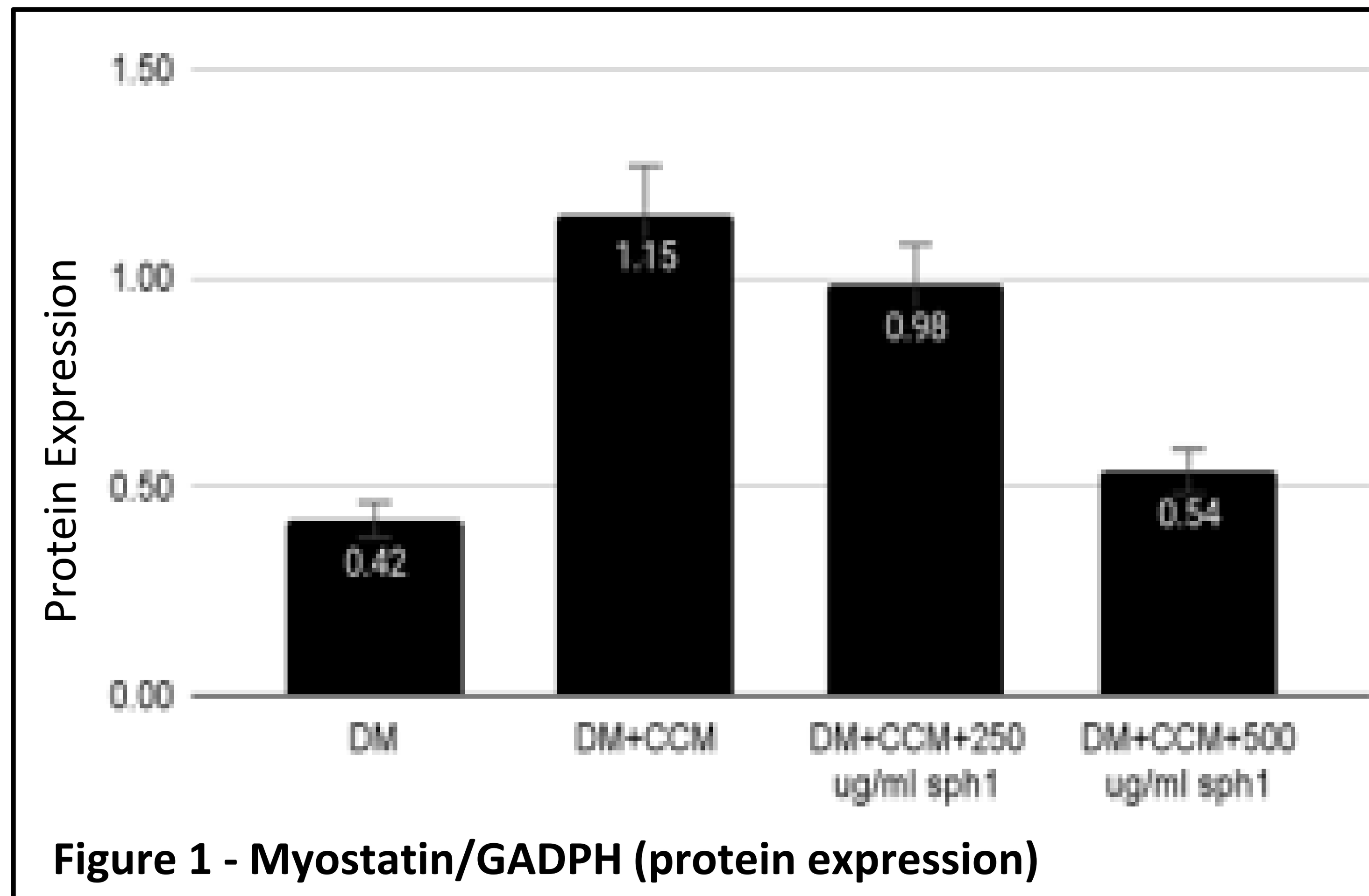

Figure 1 - Myostatin/GADPH (protein expression)

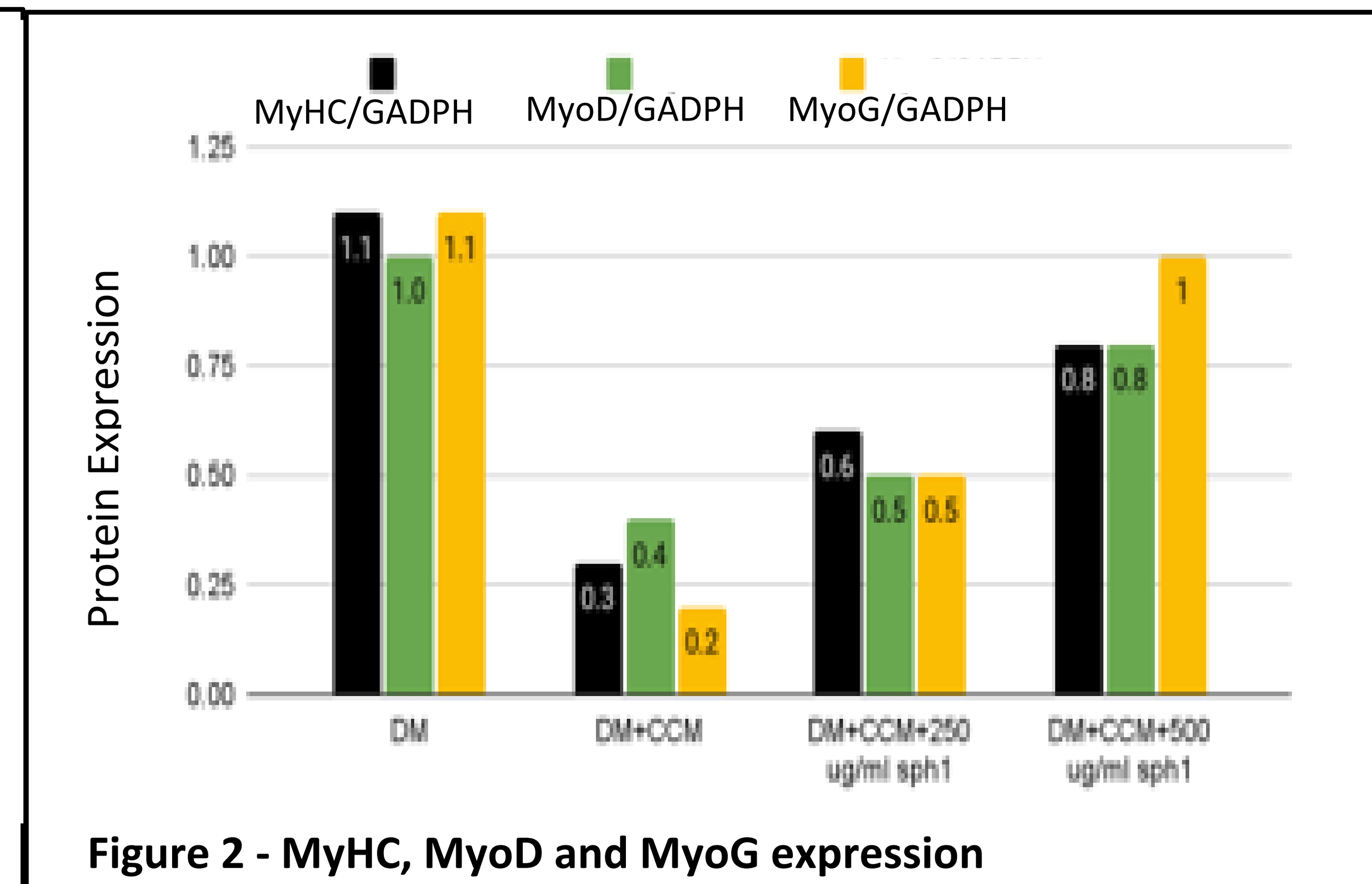

Figure 2 - MyHC, MyoD and MyoG expression

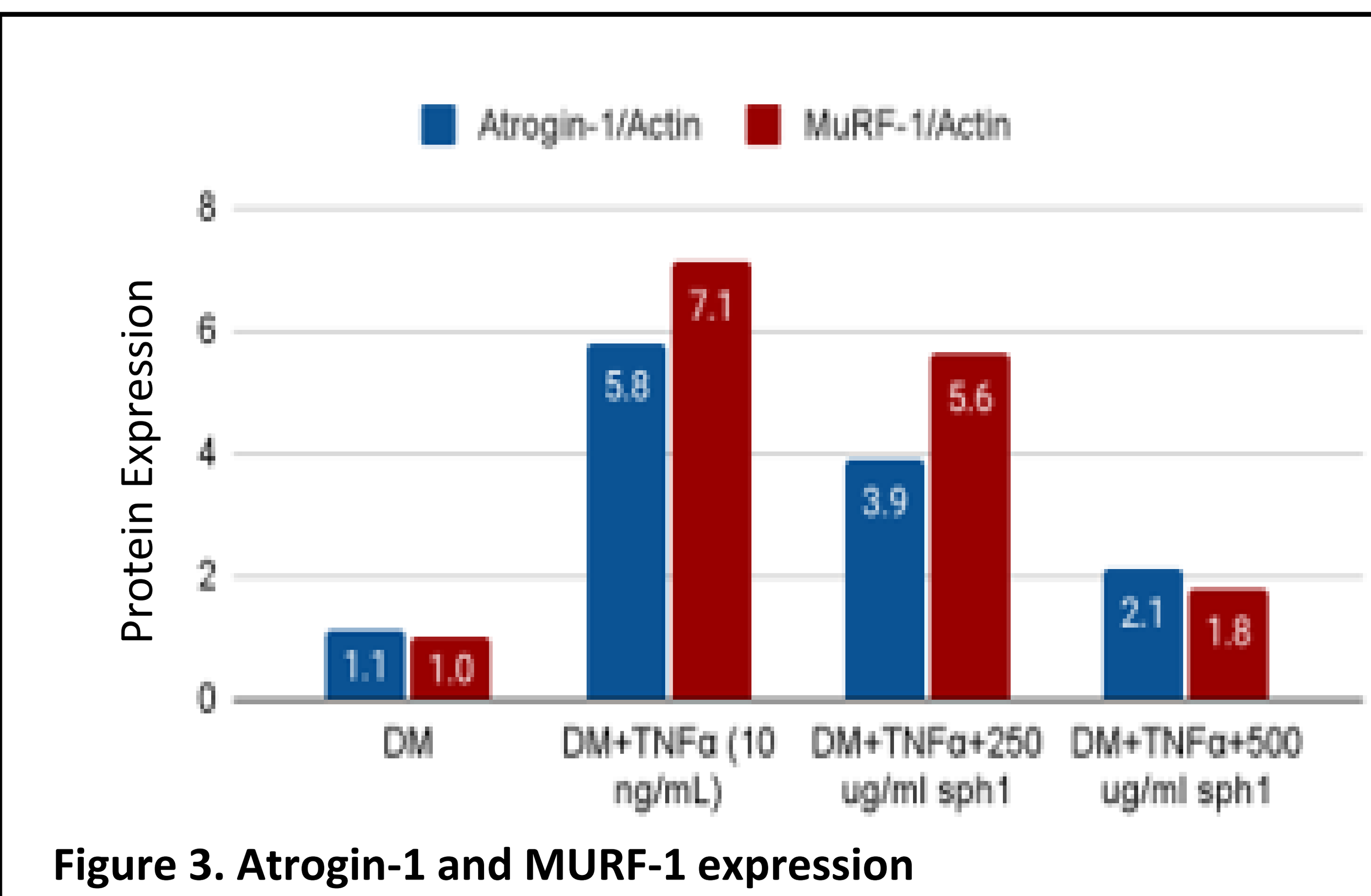

Figure 3. Atrogin-1 and MURF-1 expression

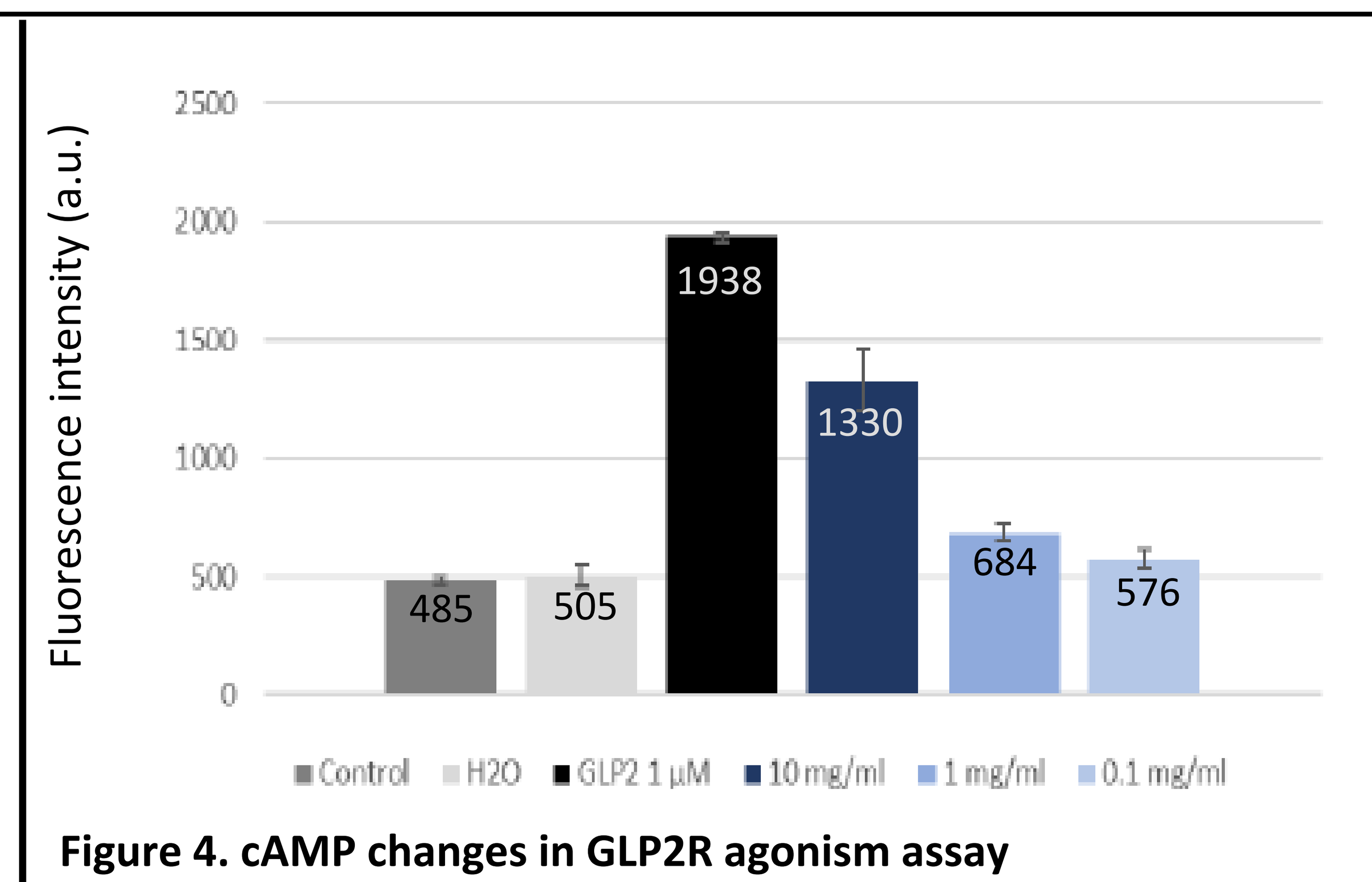

Figure 4. cAMP changes in GLP2R agonism assay

ABSTRACT

The modulation of muscle metabolism pathways is an appealing target for cachexia management. Myostatin is a key factor in inducing skeletal muscle atrophy via myotube atrophy. We previously reported that sph1 contains peptidyl inhibitors of myostatin in a HepG2 cell assay [1]. Expanding on our earlier results we show that in primary normal human skeletal myoblasts (HSkM) sph1 provides a protective effect against (i) HSkM cell myotube atrophy induced by C26 conditioned culture medium (CCM) and (ii) TNFα-induced HSkM atrophy, which we selected for models of cancer-induced muscle cell atrophy. We also show preliminary findings of screening for GLP2 agonist activity in sph1 which could provide a means to support nutrient absorption in the small intestine.

METHODS

Models of cancer-induced muscle cell atrophy

1. sph1 was produced by tailored enzymatic hydrolysis of Atlantic Salmon (*salmo salar*) offcuts.
2. HSkM cell viability was assayed by exposure to different concentrations of sph1 with sulforhodamine B at 570 nm.
3. Differentiated HSkM cells were treated with dM, dM+CCM, dM+CCM+sph1. Myostatin expression and reversal of the CCM deactivation of MyHC, MyoD, and MyoG expression was measured using western blots.
4. Differentiated HSkM cells were treated with TNFα and TNFα+sph1 and the expression of proteolysis-related Atrogin-1 and MuRF-1 was measured.

Screening for GLP2 agonist activity

1. A proprietary fluorescent biosensor assay (Innoprot) was utilised to measure cellular response in a GLP2 receptor cell line, via fluorescence intensity, mediated by cAMP signalling following activation of the GLP2 receptor by sph1 at 0.1mg/ml, 1mg/ml and10mg/ml. GLP2 at 1µm (~0.004mg/ml) was used as positive control.

RESULTS

1. HSkM cells showed 100% viability up to 500µg/ml dose of sph1.
2. CCM increased the protein level of myostatin as compared to the control group ($p < 0.05$) and sph1 reversed the protein level increase induced by CCM at 500 µg/ml dose ($p < 0.05$). **Figure 1**
3. CCM significantly decreased MyHC, MyoD and MyoG expression levels in HSkM as compared to the control ($p<0.05$). sph1 reversed the decrease in expression of all three proteins ($p<0.05$). **Figure 2**
4. TNFα significantly increased Atrogin-1 and MuRF-1 expression levels in HSkM as compared to the control ($p<0.01$). sph1 reversed the increase in expression of both proteolysis proteins ($p<0.01$). **Figure 3**
5. sph1 showed a significant increase of GLP2R activity at 1mg/ml and 10mg/ml respectively compared to negative control (with GLP2 agonist positive control showing the largest increase). **Figure 4**

CONCLUSIONS

The soluble protein hydrolysate, sph1, showed further in vitro evidence of good protection against the atrophy of primary normal human Skeletal Myoblasts (HSkM) supporting continued development of sph1 as a medical nutrition for muscle atrophy produced by cancer cachexia. Sph1 also showed significant GLP2 agonist activity which may indicate a potential to support nutrient absorption capacity of the small intestine.

REFERENCES

1. Development Of A Natural Protein Hydrolysate Supplement containing Peptidyl Inhibitors of Activin A And Myostatin To Alleviate Cancer Cachexia. Bomi Framroze, Crawford Currie and Erland Hermansen; Hofseth BioCare, Alesund,, Norway. MASCC 2023.