

Preliminary evaluation of the salivary proteome as a source of novel biomarkers of appetite

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The effect of long chain fatty acids on appetite and energy intake is not comprehensively understood⁽¹⁾. Current measurement of acute appetitive response depends upon qualitative tools that are either subjective, for example, visual analogue scales, or invasive, such as blood sampling. Saliva is increasingly recognised as a valuable resource in terms of biomarker analysis. Proteomics workflows can be used to objectively profile the protein content of saliva with relative quantification for multiplex sample comparisons; thus providing a rigorous means to identify and detect new objective surrogate markers of appetite. This study therefore aimed to assess the potential value of the salivary proteome or its components as novel biomarkers of appetite.

An initial study assessed diurnal variation of salivary protein concentrations in healthy males ($n = 9$) at 60 minute intervals, consuming a standardised diet on two occasions separated by a week. Saliva protein concentrations had over a 1 log range (0.12–1.28 mg/mL) but were consistent within subjects. Diurnal variation patterns were consistent between subjects, suggesting sampling time is important.

A randomised, controlled, crossover study examined the effects on the salivary proteome of isocaloric doses of docosahexaenoic acid (DHA) or oleic acid (OA) emulsions compared to no treatment (NT). Fasted males provided salivary samples at 08:45, 09:05 and 09:30 hrs and were dosed with DHA or OA at 08:50 hrs. Saliva samples were subject to an iTRAQ proteomic workflow which simultaneously identified and relatively quantified 118 proteins. Multiple proteins were present in significantly ($P < 0.05$) different quantities in saliva samples taken at 09:30 hrs after treatment with DHA compared to pooled fasting samples (Table 1). DHA caused specific alterations in thioredoxin and serpine relative to OA/NT. This was confirmed by western immunoblot (Figure 1). DHA caused significantly lower energy intake relative to NT and OA ($P = 0.039$).

Table 1. Protein alterations compared to pooled baseline samples

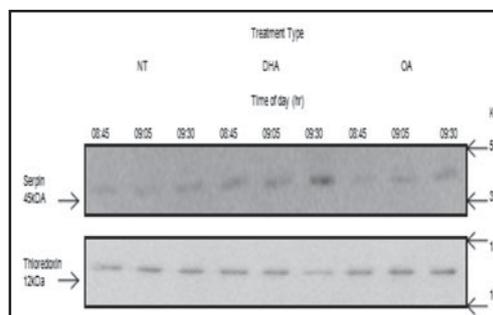
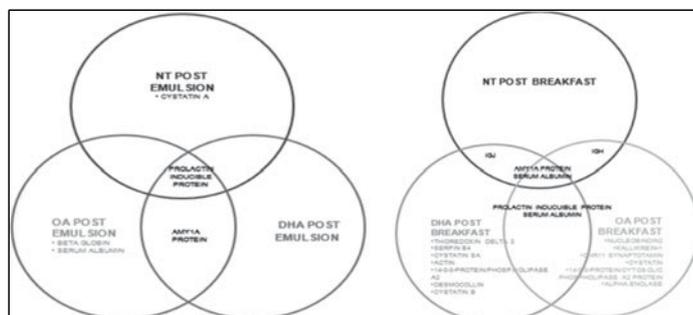


Fig. 1. Orthogonal validation of iTRAQ analysis.

The results of proteomic analysis of saliva samples taken during an acute feeding study suggest ingestion of different long chain fatty acid emulsions can alter concentrations of specific salivary proteins. Our ongoing work aims to establish whether these are a potential source of novel, non-invasive, objective indicators of appetite.

1. Li JJ, Huang CJ & Xie D (2008) *Mol Nutr Food Res* **52**, 631–645.