**Title:** Cholecystokinin (CCK) as an Adipokine – Expression and secretion of the gut peptide hormone CCK in Adipocytes

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**Background:**

Today, along with conditions such as cancer, obesity is one of the biggest public health issues faced by developed countries. Over the past two decades, there has been a marked increase in the level of obesity and associated diseases, including type II diabetes, cardiovascular disease and the metabolic syndrome. According to the Global status report on non-communicable diseases 2014, published by the WHO, worldwide prevalence of obesity has nearly doubled since 1980 and in 2014, 11% of men and 15% of women aged 18 years and older were obese.

White adipose tissue, the expansion of which defines obesity, acts as the main long-term fuel store, while also acting as a thermal insulator and helping to protect other organs from mechanical damage. For a number of years white adipose tissue was considered of limited interest, however, the discovery of leptin and subsequent other proteins secreted by white adipocytes, adipokines, has led to the tissue being viewed as an important endocrine organ. Adipokines include the major adipocyte hormones leptin and adiponectin, both of which have multiple functions. A number of adipokines, including leptin as well as IL-1β, IL-6, IL-8, IL-10 and MCP-1, are linked to inflammation and their synthesis and secretion are generally increased in the obese state (Hotamisligil, 2006; Rajala and Scherer, 2003; Rosen and Spiegelman, 2006; Trayhurn and Wood, 2004). There are now well over 100 examples of adipokines, with methods such as proteomics and genomics (microarrays) being able to identify novel adipokines such as matrix metalloproteinase 1 (MMP1) (O’Hara et al 2009) and Dipeptidyl peptidase 4 (DPP4) (Lehr et al 2012).

The gut peptide hormone Cholecystokinin (CCK) is synthesized and released by enteroendocrine cells in the mucosal lining of the small intestine called I cells. CCK is released rapidly into the circulation in response to a meal and the greatest stimulator of CCK release is the presence of fatty acids and/or certain amino acids in the chyme entering the duodenum.
CCK mediates digestion in the small intestine by inhibiting gastric emptying and can mediate satiety by acting on the CCK receptors distributed widely throughout the central nervous system. The mechanism for hunger suppression is thought to be a decrease in the rate of gastric emptying (Dockray GJ 2012).

For a protein to be characterised as an adipokine, it not only has to be expressed at the mRNA level, but more importantly, it has to be secreted by white adipocytes. Previous microarray experiments examining the effect of macrophage conditioned medium (MCM) on both adipocytes and preadipocytes demonstrated a number of potential new adipokines (O’Hara 2009, 2011). The MCM simulated the inflammatory state in adipose tissue due to infiltrating macrophages. When adipocytes were exposed to MCM, there were over 5000 genes that were differentially regulated, and in preadipocytes, there were over 400 differentially expressed genes. In both cases, expression of CCK was shown to be upregulated (33 fold in adipocytes and 2.2 fold in preadipocytes).

**The Project & Research Strategy:**

This project aims initially to examine the expression of CCK in untreated (no MCM) adipocytes and preadipocytes. Utilising a preadipocyte cell line that is commercially available, we shall culture the preadipocytes and then differentiate them into mature adipocytes, at various time points throughout the differentiation process, RNA will be extracted from the cells, carry out cDNA synthesis and subsequent PCR for CCK. Once the gene expression data has been obtained, the next stage is to determine if CCK can be considered an adipokine by examining protein expression in both cellular extracts and in culture media through the use of Western Blot or ELISA.

**Skill Requirements:**

General laboratory skills are essential. Experience in cell culture and molecular biology techniques are desirable, but not essential. Training will be provided.
Approximate Costings:

Cell Culture - £500
RNA Extraction Kit - £265
cDNA Synthesis Kit - £136
PCR Primers - £30
PCR Reagents - £85.65
Total = £1016.65

References:

5. O'Hara A, Lim FL, Mazzatti DJ, Trayhurn P; Stimulation of inflammatory gene expression in human preadipocytes by macrophage-conditioned medium: upregulation of IL-6 production by macrophage-derived IL-1β. Mol Cell Endocrinol. 2012, Feb 26; 349(2), 239-247