Understanding the insulin-sensitising role of the lipokine palmitoleate: investigations using human *in vivo* and *in vitro* cellular models

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Background

Understanding the mechanistic underpinning of crosstalk between insulin-sensitive tissues is a way to explore complications of insulin resistance. Findings from *in vivo* and *in vitro* rodent studies has suggested that adipose tissue-derived palmitoleate (16:1n-7) acts as an insulin-sensitising 'lipokine', improving insulin signalling in muscle and adipose tissue[1]. Of the limited human studies undertaken some[2-4], but not all[5], have reported a strong positive relationship between the proportion of 16:1n-7 in the plasma non-esterified fatty acid (NEFA) pool and markers of insulin-sensitivity in humans. We have previously demonstrated that gluteofemoral adipose tissue specifically generates 16:1n-7, which is released by the tissue and potentially signals to liver and skeletal muscle. This release is greater in insulin-sensitive compared to insulin-resistant individuals[6]. Palmitoleate is not ubiquitously found in foods. Although macadamia nut oil and mackerel are enriched food sources the majority of palmitoleate in the human body originates from endogenous synthesis via the desaturation of the very abundant fatty acid palmitate (16:0) by the enzyme stearoyl-CoA desaturase-1 (SCD1)[7]. Within adipose tissue, palmitoleate and its endogenous precursor, palmitate, will be stored in inert triacylglycerol or become incorporated into ceramide esters which can act as important signalling molecules, the functional effects of which will depend on the fatty acyl composition (e.g. palmitoyl-ceramide is pro-apoptotic whereas palmitoleoyl-ceramide is anti-apoptotic in adipocytes).

To date only a very limited number of dietary intervention studies have been undertaken to assess the action of palmitoleate and the focus of these studies has been on plasma lipids; LDL-cholesterol was significantly decreased after short-term exposure to a palmitoleate-enriched diet[8].

This fellowship program will combine *in vitro* studies characterising the potential insulin-sensitising properties of palmitoleate with a mechanistically oriented dietary intervention study exploring *in vivo* effects in humans.

Hypothesis

Inhibition of palmitoleate production will cause a pro-inflammatory and insulin resistant phenotype, which will be more evident in cells from gluteofemoral adipose tissue than other adipose depots (e.g. subcutaneous abdominal) due to channelling saturated palmitate through the ceramide pathways.

Aims

1. Determine if the same factors affect and regulate palmitoleate production in subcutaneous abdominal and gluteofemoral adipose tissue.
2. Determine if palmitoleate is an insulin-sensitising lipokine in non-adipose tissue organs using *in vitro* cellular models.
3. Using a dietary intervention, determine if an increase in plasma palmitoleate, achieved via consumption of a diet enriched with palmitoleate increases insulin-sensitivity *in vivo* in humans.

Description of work to be undertaken

The proposed project will utilise human *in vivo* and *in vitro* models.

- Use genetic and nutritional manipulation to alter palmitoleate production (via SCD1) within cells derived from subcutaneous abdominal and gluteofemoral adipose tissue to investigate changes in cellular insulin sensitivity, gene expression, adipokine and pro-inflammatory cytokine release.
- Investigate effects of exogenous palmitoleate on non-adipose tissue cellular models (liver and skeletal muscle).
- Conduct a dietary intervention where non-diabetic men and women (n=16) with a BMI of 27-30 kg/m^2 consume a diet enriched with palmitoleate, which will be achieved through the consumption of macadamia nut oil for 4 weeks, to investigate if insulin-sensitivity improves.
- Insulin sensitivity and metabolic features will be assessed before and after the dietary intervention using a combination of metabolic substrates labelled with stable-isotope tracers[9-11] after a mixed test meal challenge[9-11].
- Biopsies will be taken from subcutaneous abdominal and gluteal adipose tissue before and after the dietary intervention, for transcriptional and fatty acid profiling as well as protein expression to investigate the effects of palmitoleate in these depots.
- Body composition will be assessed by DEXA and ectopic fat content in liver, skeletal muscle and pancreas will be measured by magnetic resonance spectroscopy (MRS) at OCMR, University of Oxford.

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