LUDC-IRC Postdoctoral Program 2020 – Project 10

You are what you eat: A Feed-Forward study unravelling mechanisms controlling membrane lipid composition and how it affects insulin sensitivity

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Purpose and aims

In the present proposal we aim at testing the hypothesis that **adipocyte membrane lipid composition controls adipocyte size, adipose tissue expandability and function**, with a particular focus on insulin sensitivity. The ultimate aim is to identify targetable mechanisms in this metabolically important, but sometimes neglected, tissue.

State-of-the-art/background

Obesity is one of the main risk factors for type-2 diabetes (T2D). Together with liver and muscle, the adipose tissue plays a major role in regulating whole-body insulin sensitivity and energy metabolism (1). To accommodate surplus energy from over-consumption of lipids or carbohydrates, the adipose tissue rapidly expands by increasing both the adipocyte cell size (hypertrophy) and cell number (hyperplasia). The presence of enlarged, hypertrophic adipocytes is a key characteristic of impaired adipose tissue function (2), and increased adipocyte size correlates positively with impaired systemic insulin sensitivity and impaired glucose tolerance (3; 4). Obesity is commonly associated with increased circulating fatty acids levels, leading to lipid accumulation in other organs, like the pancreas and liver. For example, non-alcoholic fatty liver disease (NAFLD), is often observed in T2D. Hence, recent studies have proposed impaired adipocyte differentiation (4; 5) and limited adipocyte expansion capacity of relatively small adipocytes as factors contributing to adipose tissue dysfunction and a worsened systemic metabolic profile (6; 7). Importantly, not all obese individuals develop NAFLD and T2D at the same rate. Some individuals will never, or very slowly, develop a mild disease, a condition that has been termed “healthy obesity”. **A question that remains to be answered is why some individuals tolerate a larger surplus of energy than others before developing insulin resistance and T2D.**

Multiple biological functions in the adipocytes are linked with the plasma membrane, including nutrient uptake/release via active transport or diffusion, hormone signalling and endocytosis/exocytosis. The approximately 30 Å thick lipid bilayer constituting **the plasma membrane has for long been considered a pure barrier, in which anchored proteins mediate its properties and function**. However, in recent decades, important physiological roles of the lipid bilayer composition have been identified; this is exemplified by lipid dysregulation in the aetiology of multiple diseases, including T2D. Further emphasizing the importance of cellular membranes is the complexity and diversity of lipids found in the human body and the fact that roughly 5% of our genes to some extent are involved in lipid metabolism. Whereas the functions associated with biomolecules, such as proteins and nucleic acids, are tightly linked to an ordered structure, the function of the lipid bilayer depends both on ordered and disordered structures. On a macroscopic scale, ordered lipid domains of importance for anchoring of membrane proteins are observed, whereas on a microscopic scale a more stochastic fluid-like organisation of lipids is noticed.

Two important characteristics of the plasma membrane are its fluidity and curvature. **A high membrane fluidity, which can be obtained by unsaturated lipids, facilitates lateral diffusion of proteins and transmembrane diffusion of nutrients**. A perturbed lateral diffusion may impair protein-protein interactions in the plasma membrane and hence receptor signalling. Lipids with saturated acyls produce more crystalline and rigid membranes with lower fluidity, whereas membranes produced from unsaturated lipids have a higher fluidity. The curvature of the membrane can be described by the Israelachvili-Mitchell-Ninham packing parameter, P=v/al, where v is the average molecular volume, a is the average cross-sectional area of the head group, and l is the average length of the molecule. Lipids with P-values close to unity will create flat lipid

bilayers, those with P<1 will form micellar structures and those with P>1 inverse micellar structures. The lipid head-group, acyl carbon number and degree of unsaturation all impact on this parameter. For example, lipids showing a high v and P, such as **unsaturated lipids, promote a larger membrane curvature**, whereas lyso phospholipids show a low v and P, which associates with a smaller curvature. Notably, lysophosphatidyl choline (LPC) levels are lower in obese individuals (8). From a membrane-centric view, this is in line with the larger adipocyte sizes in the obese, and a larger unutilized lipid storage capacity in the lean, which is supported by LPCs stimulating adipocyte glucose uptake (9). **Unsaturated fatty acids have shown multiple beneficial metabolic health effects (10), in line with their capacity of increasing lipid storage by promoting a large membrane curvature and facilitating membrane fluidity-dependent processes**.

Both phospholipid type and degree of unsaturation, via desaturases, may be linked to a polygenic component. However, **the prevalence of obesity is escalating, posing life-style, and particularly both the quantity and quality of the diet, as the key driver**. In line with this, increased consumption of diets rich in saturated fatty acids, or glucose, have been shown to result in accumulation of saturated lipids in and reduced fluidity of erythrocyte plasma membranes. On the contrary, consumption of fish-oil, rich in polyunsaturated fat, produced higher erythrocyte plasma membrane fluidity, which remained for at least 42 days, at which time circulating lipid profiles were normalized (11).

The duration of the postprandial state depends on the macronutrient composition, lasting for roughly 2h after a carbohydrate-rich meal and several hours for a lipid-rich meal. Moreover, de novo lipogenesis is tightly linked with desaturase activity, which is also increased in adipose tissue from insulin resistant individuals, likely in an attempt to prevent an increase in membrane rigidity, but with the downside of increasing production of proinflammatory arachidonic acid (12). Hence, **saturated lipids produced by de novo lipid synthesis from glucose may exert physiological effects that are different from those elicited by dietary saturated fats**.

Here we propose a membrane-centred study on the effect of diets on adipocyte function. Whereas a convincing line of evidence indicates that the plasma membrane composition is vital for adipocyte function, a number of key questions remain to be answered:

- What are the functional consequences of adipocyte plasma membrane lipid remodelling?
- At which concentrations do the dietary supply of saturated lipids overcome the endogenous desaturase machinery?
- Do dietary fatty acids and fatty acids derived from glucose via de novo lipid synthesis differentially impact on membrane fluidity?
- How fast are changes in dietary patterns translated into lipid remodelling in the plasma membrane?
- For how long does a diet-elicited plasma membrane lipid remodelling last?

Significance and scientific novelty

Insulin resistance is a major driver of T2D development. The outlined project aims at producing detailed knowledge on the interaction between exogenously and endogenously supplied lipids and adipocyte function. The experimental approach, including a careful characterization of hormonal responses and substrate fluxes in live primary human and rodent adipocytes, in combination with sophisticated lipidomic profiling and accurate cell size distribution and plasma membrane fluidity analyses, will provide ground-breaking knowledge that is required to **resolve the relation between diet, plasma membrane composition, adipocyte function and systemic glucose homeostasis**. This knowledge may be exploited in dietary interventions aiming at improving adipocyte lipid storage capacity and thereby ameliorating insulin sensitivity.

Preliminary and previous results

In this project we will take advantage of protocols and methods already established in the applicants' labs. Previous work in the lab, using methods established by the applicants, have resulted in the discovery of strong correlations between the insulin response in isolated adipocytes and BMI and ISI of the donor subject (13; 14), which justify the effort, and strengthen the feasibility, of the project. The Coulter counter instrument (new instrument installed at BMC in 2019) has proven superior for detailed analyses of human adipocyte cell size distribution over a large cell size range (6; 7), and accurately measures the frequency of very small cells that are difficult to detect with other techniques. Metabolomic and lipidomic methods developed in the lab have enabled detailed studies on metabolism in multiple tissues. Using these platforms, we have

characterized lipid remodulation in insulin secreting beta-cells and alterations in the circulating lipidome elicited by dietary interventions.

Research plan

Stage 1 - In vitro studies: Primary mature adipocytes will be collected from C57bl/6J mice (epididymal and inguinal fat depots). Cells will be cultured in media supplemented with 0-1 mM saturated or unsaturated fatty acids and 2.8-16.7 mM glucose over 1-48 hours, following a face-centred central composite design blocked for fatty acid type. Cells will be analysed as outlined below (Methods). The experiments aim at establishing the impact of time, and de novo lipogenesis, fatty acid incorporation, and their interaction, on lipid desaturation and plasma membrane lipid composition, fluidity, adipocyte size and function.

Stage 2 - Animal studies: C57Bl6/J mice will be subjected to a diet rich in poly unsaturated fatty acids (PUFA) or low PUFA, for 4+4 weeks in a cross-over design. Blood samples will be procured and adipocyte size, lipid composition, membrane fluidity and function, will be examined after 0, 2, 4, 6, and 8 weeks (see Methods). These experiments aim at examining the kinetics of diet-induced effects on plasma membrane lipid composition, fluidity and adipocyte function.

Stage 3 - Human studies: Fasting blood samples and primary adipocytes will be procured from subcutaneous and visceral adipose tissue excised from morbidly obese subjects, with or without T2D, during gastric bypass surgery using our established pipeline (15; 16) (ethical protocol approved 2013/580). Blood lipid profiles, and adipocyte size, lipid composition, membrane fluidity, and function will be assessed using our established pipelines (see Methods). Clinical parameters collected include BMI, ISI, HbA1c, HOMA, diabetes duration and serum adiponectin/leptin. Associations between lipid composition and clinical variables, and differences between non-diabetic and diabetes subtypes will be assessed in models adjusted for relevant confounders.

Methods

Lipidomics: Lipidomic analyses will be conducted on blood and cell lysates using ultrahigh performance liquid chromatography quadrupole time of flight mass spectrometry (UHPLC/QTOF-MS). Lipid classes and fatty acid composition will be quantified using ultrahigh performance supercritical fluid chromatography (UHPSFC)/QTOF-MS.

Plasma membrane fluidity and cell-size distribution: Plasma membrane fluidity will be measured using a fluorescent lipophilic pyrene probe. The cell size distribution will be determined in osmium-fixed adipose tissue samples (3*12 mg/individual) by a Multisizer Coulter counter instrument which accurately measures particle sizes of a wide range (<20-500 µm diameter) (17). Histology will be done to verify the size distribution of the very large adipocyte population. Data will be processed using Multisizer software 4.1.

Cellular characterization in respect to hormonal response, lipid and glucose transport: To determine the cellular insulin response, mature adipocytes isolated from adipose tissue will be subjected to glucose-14C-tracer uptake assay and the anti-lipolytic effect analysed by measuring glycerol and fatty acid release from cells co-incubated with insulin and isoproterenol (β-adrenergic stimulus). Total protein level and phosphorylation of specific insulin signalling targets involved in glucose- and lipid metabolism will be examined by western blot analysis, and the cytosolic volume quantified by the 3-O-methyl glucose assay. Live cell imaging analysis of lipid uptake and glucose transport at a single cell level will complement the analyses measuring average cell population responses.

Characterization of different adipocyte cell populations: Due to difficulties in separating adipocytes based on cellular size, almost nothing is known about metabolism in different cell size populations. We aim at establishing a technique to sort and collect adipocytes of varying size (small (<40 µm diameter) and large (>100 µm diameter)) using dielectrophoretic field-flow fractionation (DEP-FFF) (17) or gravitational (Gr) FFF. In short, adipocytes are separated in a microfluidic device based on size-dependent partitioning of cells in a parabolic laminar flow profile. This will allow us to resolve if the lipidome, hormonal response and fatty acid efflux in cells of varying sizes correlate with systemic insulin sensitivity and BMI of the donor subject.

Time plan and implementation

Stages 1-2 will be conducted in consecutive order, while collection of samples for Stage 3 will be initiated immediately at project start. The number of patients analysed in Stage 3 will depend on accessibility to adipose tissue from patients classified as SIRD or MOD. Our clinical partners operate approximately 200 diabetes patients per year.

Project organization

A postdoc candidate will be responsible for setting up the adipocyte isolation facility, functional studies and preparation of samples for lipidomic analyses. Peter Spégel: Oksana Rogova (PhD student, metabolomics/lipidomics), Klinsmann Carolo dos Santos (islet function, alpha- and beta-cells). O.R. will assist in the lipidomics analyses. Karin Stenkula: Maria Lindahl (lab technician), Claes Fryklund (PhD stud., functional cell analysis), Björn Morén (postdoc, imaging). M.L. and C.F. will assist in adipocyte handling and analyses, and B.M. will perform image analysis and assist cell size distribution analysis. Nils Wierup has a broad clinical network of private and public care providers performing Roux-en-Y gastric bypass surgery (RYGB) and is participating in the IMI2 project Rhapsody. Johan Berggren (PhD-student and RYGB surgeon), Jan Hedenbro (Associate Prof and experienced RYGB surgeon), senior postdocs Andreas Lindqvist and Michael Miskelly will be responsible for biopsy sampling and logistics using our established pipeline.

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