The modulation of high density lipoprotein structure and function as a mechanism of cardio-metabolic protection after RYGB

General situation of the research: Severe obesity is a worldwide epidemic associated with co-morbidities that significantly increase cardiovascular (CV) disease risk and mortality (1). Obesity is associated with increased levels of pro-atherosclerotic low-density lipoprotein and a decrease in vaso-protective high-density lipoprotein (HDL) levels (1). Low circulating HDL are a well-established independent risk factor for CV disease (1). Recent studies show that HDL function is more informative to evaluate the real CV risk than the HDL concentration alone (1,2). Therapeutic interventions able to improve HDL vaso-protective properties rather than merely increasing HDL levels have the potential to impact CV outcome. Increasing evidence suggests that HDL size and molecular composition are crucial contributors to HDL cardiovascular protective effects (2-4). Roux-en-Y gastric bypass (RYGB) is currently the most effective therapy to achieve sustained and substantial body weight (BW) loss in morbidly obese subjects, improving co-morbidities like dyslipidemia and CV outcome.

Personal contribution: We showed that RYGB rapidly improves HDL vascular-protective properties not only in obese rats, but also in patients in a weight loss-independent manner (5). These benefits were associated to the modified release of gut hormones after RYGB, rather than simply being a consequence of weight loss (5). Taken together, these and previous results suggest that RYGB increases HDL concentrations in the long term, but also leads to a rapid and improvement of HDL function. However, the underlying mechanism is largely unknown. Interestingly, we observed that the cholesterol distribution profile in rats after RYGB shifted towards smaller HDL particles compared to the sham-operated group, as shown by fast protein liquid chromatography (FPLC) analysis (5). In the project submitted now, we will study and compare the changes in HDL size and composition after RYGB in patients before and 1 year after surgery and correlate structural HDL changes with their functionality. This will allow to determine which HDL particles are mainly responsible for the improved HDL functionality seen after RYGB and to assess whether the rapid improvement of HDL function (5) is maintained in the long-term follow-up in the same patients.

Hypothesis: In patients after RYGB, we hypothesize that cholesterol distribution profile will be shifted towards smaller and potentially more protective HDL particles, as we recently observed in a rat model of obesity undergoing RYGB. To date no thorough analysis has been performed on the changes in HDL size and composition that might occur after RYGB surgery and that might contribute to its beneficial CV effects.

Significance. Understanding how RYGB modulates HDL composition and function will pave the way for future less invasive HDL-targeted therapies to treat obesity-associated comorbidities and high cardiovascular disease risk.

Research plan

Group size: We have collected serum samples from fasting patients before and 1 year after RYGB in collaboration with the University Hospital Zurich (5). In addition, we will include also two control groups: group 1 includes patients who followed a diet-induced body weight loss program and are matched for body weight loss to RYGB patients. In these patients we will assess the relevance of food restriction or weight loss dependent effects after bariatric surgery. Control group 2 includes healthy subjects matched for age and sex. **Schedule:** RYGB and Control group 1 samples were collected at the time of enrollment (D0) and we are currently completing the 1-year follow-up.

Aim 1: to study the correlation between HDL size and functionality, we will:

1. Investigate the cholesterol particles distribution profile by FPLC as previously described (5)
2. Perform *in vitro* cholesterol efflux experiments with J774 macrophages by stimulating them with HDL obtained from individual FPLC fractions in order to study the relationship between HDL size and cholesterol efflux capacity. More specifically, we will test only selected fractions containing large, medium and small HDL particles in order to correlate HDL size and functionality (6).
3. Assess other HDL functional properties of selected FPLC fractions in endothelial cells, e.g. anti-oxidative, anti-apoptotic, anti-inflammatory properties as previously described (5).

This experimental approach will allow us to directly correlate HDL size, as determined by FPLC analysis, with HDL functional cholesterol efflux capacity and other HDL properties of selected individual FPLC fractions.

Aim 2: We will study and compare changes in HDL molecular lipid and protein composition before and 1 year after RYGB surgery or diet-induced weight loss and in healthy subjects, and assess whether there is a relationship between specific molecular components and HDL size, cholesterol efflux capacity and other HDL properties.
We will perform lipidomic and proteomic analysis of isolated HDL by mass spectrometry (in collaboration with the research unit of Internal Medicine of the University Hospital of Zurich and the Functional Genomics Center, University of Zurich). In parallel, with the same method, we will perform a selective phospholipid analysis of differently sized HDL obtained from the FPLC fractions mentioned in Aim 1, in order to study the association between HDL size and specific phospholipid content. This experimental approach will allow us to 1) study the changes in HDL composition over time and 2) correlate HDL phospholipid content with HDL size and HDL-stimulated cholesterol efflux capacity of macrophages after RYGB surgery and in the control groups.

Finances: This prestigious prize will be crucial to cover the costs for laboratory consumables, for example reagents for cell culture experiments, FPLC, cholesterol efflux assay, HDL functionality tests, lipidomic and proteomic analysis.

Infrastructure: The equipment and instruments required for the project are available at the Laboratory of Translational Nutrition Biology, Institute of Food, Nutrition and Health- ETH Zurich. Moreover, the Applicant is affiliated with the Center for molecular Cardiology at the University of Zurich and also has access to lab space, equipment, instruments and infrastructures of this Centre.

Personnel: Fahmida Jahan is a first year PhD student who works under the supervision of Dr. Osto and will be involved in this project.

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