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Research paper

Lipid-load in peripheral blood mononuclear cells: Impact of food-consumption, dietary-macronutrients, extracellular lipid availability and demographic factors

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ABSTRACT

Lipid-load in peripheral blood mononuclear cells (PBMCs) has recently gained attention of the researchers working on nutritional regulation of metabolic health. Previous works have indicated that the metabolic circuitries in the circulating PBMCs are influenced by dietary-intake and macronutrient composition of diet. In the present work, we analyzed the impact of diet and dietary macronutrients on PBMCs' lipid-load. The overall analyses revealed that dietary carbohydrates and fats combinatorially induce triglyceride accumulation in PBMCs. On the other hand, dietary fats were shown to induce significant decrease in PBMCs' cholesterol-load. The effects of various demographic factors –including age, gender and body-weight– on PBMCs' lipid-load were also examined. Body-weight and age were both shown to affect PBMC's lipid-load. Our study fails to provide any direct association between extracellular lipid availability and cholesterol-load in both, freshly isolated and cultured PBMCs. The presented work significantly contributes to the current understanding of the impact of food-consumption, dietary macronutrients, extracellular lipid availability and demographic factors on lipid-load in PBMCs.

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1. Introduction

Molecular effects of the dietary macronutrients are ideally studied in the major organs involved in nutrient regulation –including brain, liver, adipose tissue, pancreas and muscles. However, often in the studies with healthy human volunteers the availability of these tissues is the major constraint. Several works have shown that peripheral blood mononuclear cells (PBMCs) could serve as a less invasive and direct alternative to the corresponding tissue biopsies, for studying the potential effects of dietary macronutrients on gene-expression [1,2]. PBMCs travel through the body and respond to various external and internal stimuli. These cells are exposed to various metabolically-relevant tissues/organs –including liver, adipose tissue, and endothelium– and are also known to cross-talk with these organs [3]. Previous studies have shown that PBMCs and hepatic cells share similarities in terms of cholesterol homeostasis [4]. Dietary cholesterol induces similar effects, on the expression of multiple metabolism-related genes, in PBMCs and hepatic cells [5–7]. In addition to that, gene expression profile of PBMCs was shown to reflect that of the skeletal muscle [8]. Therefore, previous works have recommended and utilized PBMCs as a model-system for studying diet-induced changes in expression of various genes in the metabolic tissues [7]. Several studies have shown that dietary-intake mainly affects the expression of inflammation- and metabolism-related genes in PBMCs [1,2,4,9,10]. These studies have also highlighted the interactions between metabolism and inflammation, within the context of metabolic health and nutrition [11]. The role of lipids in metabolism and activation of immune cells is also recently discussed in detail [12].

Most of the previous works, focused on nutritional regulation of metabolic pathways in PBMCs, mainly studied the diet-induced alterations in expression of lipid metabolism related genes [9,10,13,14]. However, only few studies have explored the effect of diet on lipid profile of these cells [15–18]. Nevertheless, anomalies in PBMCs' lipid-load have been reported in various pathological

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conditions [19–22]. Few studies explored the nutritional regulation of PBMCs' lipid-load and reported that postprandial triglyceride-rich lipoproteins induce lipid accumulation in these cells [23,24]. Interestingly, triglyceride-rich lipoproteins are also shown to be involved in activation of leukocytes [24–26]. This triglyceride-mediated leukocyte activation has been shown to result in the secretion of pro-inflammatory mediators that promote the infiltration of inflammatory-cells and lipoproteins into the sub-endothelium, thus contributing to the development of atherosclerotic plaques [25,27].

Some of the studies on diet-induced changes in lipid profile of PBMCs focused on fatty acid profile of PBMCs' phospholipids [17,18,28]. Nevertheless, only limited data is available regarding the effects of diet on lipid-load in circulating PBMCs.

In the present study we explored in detail the effects of diet and macronutrient composition of diet on lipid-load in PBMCs from healthy volunteers. We also studied the inter- and intra-individual differences in PBMCs' lipid deposits. The effects of various demographic factors including age, gender and body-weight on PBMCs' lipid deposits were also examined. We also compared PBMCs' lipid-load between dyslipidemic and non-dyslipidemic participants. In addition to that, the effect of extracellular lipid availability on lipid-load in PBMCs was examined both *in vivo* and *ex vivo*. The present work adds to the current understanding of nutrition-induced metabolic alterations in PBMCs.

2. Materials and methods

2.1. Human sample collection, study protocols and ethics

For the present work different experimental protocols were designed to address different research questions. The study protocols for human subjects were approved by the *Ethics Committee of School of Biological Sciences* or *Biosafety* and *Bio-resource Committee*, University of the Punjab. Informed written consent was obtained from each study-subject before sample collection. In order to obtain the basic personal information and medical history each participant was interviewed and completed a structured questionnaire.

For the experiments aimed to demonstrate the intra/inter-individual differences or diet-induced changes in PBMCs' lipid-load, the studies were designed as single-blind, randomized, within subject-crossover, for which the participants were recruited from University of the Punjab, Lahore. Exclusion criteria were as follows: body mass index (BMI; in kg/m^2), <18 or >30, fasting blood glucose outside the normal range, tobacco smoking, regular use of prescribed medication, diagnosis of long-term medical condition (e.g., diabetes, hemophilia, cardiovascular disease, anemia, or gastrointestinal disease), symptoms of allergy or vegetarianism.

For studying the inter- and intra-individual differences in cellular cholesterol and triglyceride content in PBMCs, 3 fasting peripheral blood samples were obtained from nine study subjects, with each sampling event separated by at least 1 week (Fig. 1a).

For all diet-based experiments the subjects came to the University in a fasted condition (10–12 h) on morning of the study-day. First, intravenous blood (3 ml) was collected from all the subjects in vials containing EDTA-anticoagulant agent (BD Biosciences). Immediately after the first blood sampling, study-subjects consumed the experimental-diet within 15 min. Subsequently, the second blood sample (3 ml) was drawn 4 h after consumption of the respective diet (Fig. 1b–c).

To study the impact of diet-intake on PBMCs' lipid-load, fourteen healthy volunteers were recruited. For these initial experiments we used typical cafeteria-diet that included highly palatable and energy dense food-items with high-carb and -fat and content (see Supplementary Table 1 for relative distribution of macronutrients and caloric values). Fig. 1b displays the design of this experiment.

For studying the impact of macronutrient composition of diet on lipid load in PBMCs 5 volunteers were recruited for 3 separate experiments (Fig. 1c). Here, the participants were provided with different experimental-diets that varied by emphasis on carbohydrate (High-Carb), fat (High-Fat), or protein (High-Protein) but had similar energy densities (Supplementary Table 1). Each experiment was separated by at least 1 week.

For studying the combinatorial effect of high-carbohydrates and -fats, we designed another experimental-diet namely, the High-Fat-High-Carb (HFHC) diet. This diet combined the fat and carbohydrates contents of the High-Fat and High-Carb diets, respectively (Supplementary Table 1). For this experiment ten healthy volunteers were recruited.

For studying the impact of various demographic factors and dyslipidemia on PBMCs' lipid content, fasting (10–12 h) blood samples were collected from different participants. Except for the BMI range, the exclusion criteria were same as mentioned above for the experiments aimed at studying intra/inter-individual differences or diet-induced changes in PBMC's lipid-load. For BMI-based analysis the study groups were categorized into two major BMI groups; Normal weight (BMI; 18–24.9) and Over-weight (BMI$\geq$25), according to the current *National Institutes of Health* (NIH) guidelines [29].

Blood samples were used for plasma collection and peripheral blood mononuclear cells (PBMCs) isolation. All samples were processed within 30 min of collection. Plasma was promptly separated. PBMCs were isolated from whole blood using Ficoll-based Lymphocyte Separation Medium (Biowest, L0560). PBMCs were pelleted for further processing.

2.2. Cell culture and treatments

The human non-adherent monocytic THP-1 cell line was purchased from the American Type Culture Collection (ATCC). THP-1 or peripheral blood mononuclear cells (PBMCs) were cultivated in RPMI 1640 (ATCC, 30–2001) supplemented with 10% FBS (ATCC, 30–2021) and penicillin-streptomycin solution (ATCC, 30–2300). PBMCs were stimulated with 10 μg/ml phytohaemagglutinin (PHA) (HiMedia, TC209) for 24–48 h, where stated. Lipoprotein deficient serum (LPDS) was purchased from Merck (LP4) and used according to the manufacturer's guidelines. For different experiments, cells were cultivated in LPDS containing media for 24–48 h. Cell cultures were maintained in the atmosphere of 5% CO_2 and 37 °C.

2.3. Determination of cellular cholesterol and triglyceride content

Lipids were extracted from cell pellets using a methanol/chloroform extraction method [30]. Total cholesterol content in the lipid extracts was spectrophotometrically determined using commercially available kit (Analyticon Biotechnologies AG, 4046) against a calibration-curve generated using known concentrations of cholesterol standard (SUPELCO, 47127-U). Total triglyceride content in the lipid extracts was spectrophotometrically determined using commercially available kit (Analyticon Biotechnologies AG, Catalogue # 5052) against a calibration-curve generated using known concentrations of triglyceride standard (SUPELCO, 17811-1AMP).

2.4. Determination of plasma lipid levels

Plasma total cholesterol levels were spectrophotometrically determined using commercially available kit (Analyticon Biotechnologies AG, 4046). For the estimation of high-density

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