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One-week high-fat diet leads to reduced toll-like receptor 2 expression and function in young healthy men

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ABSTRACT

Toll-like receptor 2 (TLR2) is implicated in inflammatory responses to high-fat diet (HFD)–induced obesity in rodents, but human HFD studies examining TLR2-mediated immune responses are lacking. Our aim was to determine whether HFD affected TLR2 function in humans. We hypothesized that a short-term HFD in humans would impair TLR2-mediated immune function. Fasting blood samples were obtained from healthy young men (N = 9) before and after a 7-day HFD. Toll-like receptor 2 function was assessed in ex vivo whole blood cultures stimulated with the TLR2 agonist *N*-palmitoyl-S-[2,3-bis[palmitoyloxy]-[2RS]-propyl]-[R]-cysteinyl-[S]-seryl-[S]-lysyl-[S]-lysyl-[S]-lysyl-[S]-lysine (Pam3-Cys-SK4). Peripheral blood mononuclear cells (PBMCs) were isolated to examine TLR2, TLR4, and p47 subunit of nicotinamide adenine dinucleotide phosphate oxidase ($p47^{phox}$) protein expression via Western blotting. Pam3-Cys-SK4–stimulated secretion of interleukin-1β (–35%, $P = .005$), interleukin-6 (–32%, $P = .01$), and tumor necrosis factor–α (–33%, $P = .06$) was reduced following the HFD. High-fat diet resulted in decreased TLR2 ($P = .049$) and $p47^{phox}$ ($P = .037$) protein expression from PBMCs. To mimic lipid overload ex vivo, follow-up experiments were performed in whole blood cultures exposed to a mixture of free fatty acids for 24 hours; and surface protein expression of TLR2 and TLR4 on CD14+ monocytes was measured by flow cytometry. Free fatty acid exposure for 24 hours ex vivo reduced monocyte TLR2 levels by about 20% ($P = .028$). A 7-day HFD in young healthy men resulted in impaired TLR2 function. Decreased TLR2 and $p47^{phox}$ protein expression in PBMCs, possibly due to excess free fatty acids, may mediate this response. Our current findings indicate that impaired TLR2 response after HFD might be partially responsible for increased risk of infection in diet-induced obesity.

Abbreviations: BSA, bovine serum albumin; ELISA, enzyme-linked immunosorbent assay; FFA, free fatty acid; HFD, high-fat diet; IL, interleukin; NADPH, nicotinamide adenine dinucleotide phosphate; $p47^{phox}$, p47 subunit of NADPH oxidase; Pam3-Cys-SK4, *N*-palmitoyl-S-[2,3-bis[palmitoyloxy]-[2RS]-propyl]-[R]-cysteinyl-[S]-seryl-[S]-lysyl-[S]-lysyl-[S]-lysyl-[S]-lysine; PBMC, peripheral blood mononuclear cell; TLR, toll-like receptor; TNF, tumor necrosis factor.

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1. Introduction

Epidemiological data suggest that obese individuals are at greater risk of opportunistic infections and have higher death rates from sepsis in hospital settings [1,2], suggesting that obesity may lead to impaired immune responses. The mechanisms contributing to this phenomenon are not fully understood.

Toll-like receptor 2 (TLR2) is a member of the TLR pattern-recognition receptor family and serves as the major transducer for bacterial lipoproteins and cell wall components of gram-positive bacteria [3]. Toll-like receptor 2 is implicated in inflammation induced by elevated free fatty acids (FFAs) in multiple cell types [4,5]. In humans, TLR2 is predominantly expressed on myelomonocytic cells [6]. Among these, peripheral blood mononuclear cells (PBMCs) circulate throughout the body and are precursors for macrophages that mediate inflammatory activation in peripheral organs upon nutritional stimuli, including changes in dietary lipids [7,8]. For example, a single high-fat meal in humans induces inflammatory responses in PBMCs [9,10]. Snodgrass et al [11] reported that palmitic acid treatment in culture can induce TLR2/1 dimerization in primary human monocytes, suggesting that TLR2 may provide a link between dietary lipids and systemic inflammation.

Despite the prevailing view that a high-fat diet (HFD) results in increased inflammation, there is accumulating evidence in mice that high-fat feeding may impair TLR2-mediated immune responses. For example, Amar et al [12] demonstrated that a 16-week HFD in mice increased susceptibility to *Porphyromonas gingivalis* infection and mortality. A follow-up study showed that high-fat feeding rendered murine immune cells less responsive to TLR2 stimulation, which was linked to reduced TLR2 expression [13]. In light of these findings, the effects of dietary fat intake on TLR2 expression and/or function appear inconclusive and controversial. Furthermore, it is currently unknown whether an HFD in humans alters TLR2 expression and/or function to possibly contribute to immune dysregulation.

Based on previous findings in mice [12,13], we hypothesized that short-term high-fat feeding in humans would reduce TLR2 expression and impair cytokine secretion from immune cells in response to TLR2 agonism. Peripheral blood mononuclear cells travel and interact with every organ in the body and are sensitive to nutritional challenges, including changes in dietary fatty acids [8]. Human whole blood culture is a valid ex vivo technique to measure monocyte cytokine production that maintains physiologically relevant intercellular communication [14]. Consequently, the aims of the current study were to determine whether short-term HFD impairs TLR2-mediated immune function using human whole blood cultures and to explore potential mechanisms by characterizing the expression of TLR2 and associated proteins from isolated PBMCs. To explore the potential mechanisms involved in HFD-associated TLR2 downregulation, follow-up experiments in cultured whole blood treated with fatty acids were also performed.

2. Methods and materials

2.1. Participants

Healthy male subjects (N = 9, 21 ± 3 years, 76 ± 4 kg, 181 ± 9 cm) volunteered to participate. Participants in this report were part of a larger study examining the impact of HFD on vascular, metabolic, and cognitive function. Subjects completed a 3-day food record, and total energy intake and macronutrient profile of their usual diet were calculated using FoodWorks Diet Analysis software (The Nutrition Company, Long Valley, NJ). Individualized HFDs (supplying 70% energy from fat, 20% energy from protein, and 10% energy from carbohydrates) isocaloric to usual intake were designed and prepared for each subject. On day 1 of the study, subjects reported to the laboratory after an overnight fast; and a baseline blood sample (pre-HFD) was obtained by venipuncture from an antecubital vein and collected into 10-mL sodium heparin tubes (BD, Mississauga, ON, Canada). Subjects were then provided with an individualized meal plan and prepackaged food to consume their HFD over the next 7 days. Subjects were instructed to record any deviations from the meal plan, and these records were analyzed after the HFD intervention to confirm compliance. Subjects were also instructed to maintain their usual physical activity habits for the study. Fasting blood samples (post-HFD) were obtained on day 8 after 7 days of consuming the HFD. Procedures were approved by the University of British Columbia Clinical Research Ethics Board, and written informed consent was obtained.

2.2. Materials

RPMI-1640; Histopaque-1077; fatty acid–free endotoxin-free bovine serum albumin (BSA; catalog no. A8806); and sodium salts of palmitate, linoleate, and oleate were from Sigma-Aldrich (Oakville, ON). *N*-palmitoyl-S-[2,3-bis[palmitoyloxy]-[2RS]-propyl]-[R]-cysteinyl-[S]-seryl-[S]-lysyl-[S]-lysyl-[S]-lysyl-[S]-lysine (Pam3-Cys-SK4) (L2000) was from EMC microcollections GmbH (Tubingen, Germany). Tumor necrosis factor (TNF)–α, interleukin (IL)-6, and IL-1β DuoSet enzyme-linked immunosorbent assay (ELISA) kits and TLR4 antibody were from R&D Systems (Minneapolis, MN). Toll-like receptor 2, p47 subunit of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase ($p47^{phox}$) and tubulin antibodies, protease/phosphatase inhibitor cocktail, phenylmethylsulfonyl fluoride, Signal Fire ECL Reagent, and cell lysis buffer were from Cell Signaling Technologies (Danvers, MA). Antibodies against CD284 (TLR4)-APC (catalog no. 130-096-236, clone HTA125), CD282 (TLR2)-PE (catalog no. 130-099-016, clone REA109), CD14-VioBlue (catalog no. 130-094-364, clone TUK4), and CD45-APC-Vio770 (catalog no. 130-096-609, clone 5B1), along with human FcR blocking reagent (catalog no. 130-059-901), were from Miltenyi Biotec (Auburn, CA). NEFA-HR kit was purchased from Wako Chemicals (Richmond, VA).

2.3. Whole blood cultures

Whole blood was diluted 1:10 with serum-free RPMI-1640 medium (penicillin 100 U/mL, streptomycin 100 μg/mL) (ie, 1 mL whole blood diluted in 9.0 mL RPMI-1640 medium), plated in duplicate on 24-well plates at a final volume of 600 μL, and cultured at 37°C in a humidified incubator (5% CO_2) as described by Elsasser-Beile et al [15]. Samples were cultured unstimulated and stimulated with the TLR2 agonist Pam3-Cys-SK4 (100 ng/mL). Supernatants were harvested after 12 hours via centrifuge at 2000*g* for 15 minutes at 4°C. Samples were stored at –80°C before batch analysis of TNF-α, IL-6, and IL-1β via ELISA according to the manufacturer's instructions.