Triglycerides, Free Fatty Acids, and Glycemic Control: An Unresolved Puzzle

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Uncontrolled hyperglycemia is frequently accompanied by hypertriglyceridemia, and improved glucose control parallels improvement of hypertriglyceridemia [1]. It is generally assumed that insulin is a major factor causing improvement of both hypertriglyceridemia and hyperglycemia [2]. In this issue of *Israel Medical Association Journal*, Shinhar et al. [3] publish their clinical observations claiming that improvement of hypertriglyceridemia facilitated improvement in glucose control in five hospitalized patients with type 2 diabetes mellitus (T2D) [3]. The correlation between free fatty acids (FFA) and insulin secretion/glucose control has been extensively investigated in laboratory settings.

Fatty acids are carboxylic acids possessing long unbranched chain carbons and are components of fats and lipids in all organisms. There are more than 70 different known fatty acids classified according to the length of unbranched chain carbon. Short-chain fatty acids possess 1 to 5 carbon atoms, are a major product of anaerobic bacterial fermentation in the small intestine, serve as a primary energy source in mammals, and are readily absorbed into the blood stream. Medium-chain fatty acids (MCFAs) have between 6 and 12 carbon atoms. They are transported in the portal blood directly to the liver, being incorporated into chylomicron, transported to lymph, and preferentially utilized for ketogenesis. Long-chain fatty acids (LCFA), with 16 or 18 carbon atoms, may contain a few double bonds. They play an important role as an energy and cellular membrane substrate, and they are pertinent in many cellular functions such as migration, apoptosis, proliferation, survival, signaling, generation of reactive oxygen species, and insulin secretion from the pancreatic islets.

Most of the data investigating the effect of FFA on insulin secretion by the pancreatic islets originate from basic research studies. Short-term exposure to FFA will promote, or at least facilitate, insulin secretion. Long-term exposure impairs insulin secretion [4]. FAA's acute intracellular actions may be mediated through distinct isoforms of G protein-coupled receptors (e.g., GPR40, GPR41, GPR43, GPR84, and GPR120). GPR41 and GPR43 are activated by short-chain FFAs and GPR84 is activated by MCFAs, whereas GPR40 and GPR120 are activated by MCFAs and LCFAs [5]. GPR40, containing the α subunit of the Gq protein, is highly expressed in pancreatic β-cells and plays a crucial role in acute FFA-induced, calcium-dependent insulin secretion via the activation of phospholipase C [5]. This enzyme converts phosphatidylinositol-4,5-bisphosphate (PIP2) into diacylglycerol (DAG) and inositol triphosphate (IP3), which participates in calcium extrusion from the endoplasmic reticulum [6]. GPR40 activation by long-chain fatty acids is dependent on high glucose concentrations that raise malonyl-CoA levels and decrease fatty acid oxidation. This conversion leads to long chain-CoA accumulation and facilitates the fusion of secretory granules with the β-cell plasma membrane, thus promoting insulin secretion. GPR40-deficient knock out mice showed impairment of LCFA-induced insulin secretion from β cells leading to an increase in glucose levels, yet they had no symptoms of diabetes and fasting blood glucose levels and glucose tolerance was normal [7].

At least two described single nucleotide polymorphisms (SNPs) located in the coding region of the human GPR40 gene have functional impacts. One of these SNPs (amino acid substitution arginine to histidine at the position 211 in the third intracellular loop of the receptor) was reported to cause enhanced insulin secretion in Japanese men [8]. A different variation has been associated with elevated body mass index in Europeans and a reduction of insulin secretion following an oral lipid load.

Chronic exposure of β cells to palmitate impairs glucose-stimulated insulin secretion [9]. One of the mechanisms involved is excessive reactive oxygen species (ROS) generation by mitochondrial and extramitochondrial sources such as nicotinamide adenine dinucleotide phosphate-oxidase (NADPH oxidase), accompanied by reduction of antioxidant defense via glutathione depletion [4]. In addition, the RAS cascade appears to be actively involved in the decrease of FFA exposed insulin secretion, and accumulate in MIN6 cells and in mouse islets. The angiotensin receptor blockers (ARBs), candesartan and telmisartan, by opposing the RAS cascade and by activating protein kinase C and NADPH oxidase, counteract the palmitate induced decrease of intracellular insulin content [10,11].

Hence, based on data from animal models and cell culture systems, it has been

shown that FFAs play a significant role in glucose homeostasis. Short exposure of islet cells to FFA promote or facilitate insulin secretion while chronic exposure results in decreased insulin secretion.

Substantial evidence correlates T2D with preceding and/or concurrent hypertriglyceridemia in human patients. Most of these data derive from large epidemiological studies demonstrating that the risk of developing T2D is significantly increased in patients with prior elevation of triglycerides [12]. Moreover, the vast majority of patients with established T2D are also characterized by hypertriglyceridemia, underscoring this association that is attributed to a shared genetic basis as well as underlying insulin resistance as a mediating common pathophysiology. It is of interest that similar, albeit much weaker, epidemiological data support the association between NEFA and T2D. However, although these epidemiological data may suggest that elevated triglycerides by themselves can disrupt glucose homeostasis, it is also possible that both hypertriglyceridemia and dysglycemia are the result of an unhealthy life style rather than causally related.

In several clinical studies [13], short-term infusions of lipid emulsions and administration of a high fat diet were performed to shed light on the intriguing interaction between hypertriglyceridemia and glucose homeostasis. The net effect of an acute increase in plasma triglycerides concentration was achieved by intravenous administration of lipids and resulted in a mild increase in glucose and reduced glucose tolerance in spite of higher insulin concentrations. These effects were mostly observed for elevated triglycerides and to a much lesser extent when NEFA were administered. The data are less clear for the interaction between high fat diets and glucose control. The acute effects of lipid ingestion on glucose-related parameters are highly variable and depend on the type, timing, and composition of the ingested lipids and/or lipids containing mixed meal. By the same token, multiple confounding factors, including gender, ethnicity, duration of diet, as well as calories and lipid content of different diets, modulate the effects of dietary lipids on glucose homeostasis. These differences underscore the results of different studies and limit the ability to derive clear conclusions as to the effects of short- and long-term chronic lipid enriched diets on glucose homeostasis. As opposed to the previously mentioned research on the uncertainty regarding the effects of dietary lipids on glucose homeostasis, multiple short- and long-term studies confirmed the beneficial effect of treatment with bezafibrate in both non-diabetic and T2D patients with elevated triglycerides. Bezafibrate administration reduced the incidence and delayed time to onset of T2D in non-diabetic subjects as well as lowered the levels of fasting glucose in T2D patients. Bezafibrate, which is different from other fibrates, most notably fenofibrate, proved itself more likely to reduce T2D incidence.

Taken together, epidemiological and experimental human data strongly support a significant association between hypertriglyceridemia and various parameters of glucose homeostasis. While the net effect of acute and/or chronic modulations of lipid administration and consumption on glucose homeostasis is not clear, treatment with bezafibrate was found to reduce T2D incidence and may ameliorate glycemia in patients with established diabetes.

Five T2D patients with hypertriglyceridemia and hyperglycemia hospitalized for various reasons are described by Shinhar et al. [3] in this issue of *IMAJ*. Based on the previously reported results, all of these patients were treated with bezafibrate in addition to insulin, assuming that lowering triglyceride levels would result in reduced lipid toxicity and better glycemic control, which in fact did occur. However, the small number of patients, and most importantly the lack of a matched control group, do not safely show that this combined and simultaneous treatment is by any clinical or other way superior to insulin-mediated glycemic control of hospitalized patients, which is currently the gold standard adopted by the American Diabetes Association [13] and recommended by the Israeli Society of Internal Medicine. These and other guidelines do not recommend lipid lowering therapy in hospitalized T2D patients as a part of glycemic control protocols. It should also be noted that β-cell function, namely insulin secretion and or insulin sensitivity/resistance before and after the administration of lipid lowering treatment were not performed in these patients and FFA levels were not recorded. Thus, in the absence of solid clinical and basic research evidence demonstrating the efficacy of the combined lipid and glucose lowering treatments, it seems too early to adopt this approach in hospitalized patients. Nevertheless, the data presented by Shinhar and colleagues [3] are intriguing. Moreover, the relative proportion of patients with T2D in the general wards is ever growing, reaching approximately 40% at any given moment, and a much higher percentage in specialized units such as hemodialysis and intensive coronary care units.

Glucose levels in many of these patients, who are usually characterized by multiple chronic diseases, are also poorly controlled during their hospital stay in spite of various modalities of glycemic control. Therefore, targeting and treating lipid toxicity as a possible glycemia aggravating factor during acute illness consists of a novel and mind opening approach that may ultimately provide a solution to a growing need. Larger and randomized control clinical studies in diverse cohorts of T2D hospitalized patients are warranted to clarify the role of lipid lowering treatment in these complex patients.

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Capsule

Semaphorin 6D reverse signaling controls macrophage lipid metabolism and anti-inflammatory polarization

Polarization of macrophages into pro-inflammatory or anti-inflammatory states has distinct metabolic requirements, with mechanistic target of rapamycin (mTOR) kinase signaling playing a critical role. However, it remains unclear how mTOR regulates metabolic status to promote polarization of these cells. **Kang** et al. showed that an mTOR-Semaphorin 6D (Sema6D)-Peroxisome proliferator receptor γ (PPARγ) axis plays a critical roles in macrophage polarization. Inhibition of mTOR or loss of Sema6D blocked anti-inflammatory macrophage polarization, concomitant with severe impairments in PPARγ expression, uptake of fatty acids, and lipid metabolic reprogramming. Macrophage expression of the receptor Plexin-A4 is responsible for Sema6D-mediated anti-inflammatory polarization. The authors found that a tyrosine kinase, c-Abl, which associates with the cytoplasmic region of Sema6D, is required for PPARγ expression. Furthermore, Sema6D is important for generation of intestinal resident CX3CR1^{hi} macrophages and prevents development of colitis. Collectively, these findings highlight crucial roles for Sema6D reverse signaling in macrophage polarization, coupling immunity, and metabolism via PPARγ.

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Eitan Israeli

Capsule

A single injection of crystallizable fragment domain-modified antibodies elicits durable protection from SHIV infection

In the absence of an effective and safe vaccine against HIV-1, the administration of broadly neutralizing antibodies (bNAbs) represents a logical alternative approach to prevent virus transmission. **Gautoam** and colleagues introduced two mutations encoding amino acid substitutions (M428L and N434S, collectively referred to as 'LS') into the genes encoding the crystallizable fragment domains of the highly potent HIV-specific 3BNC117 and 10-1074 bNAbs to increase their half-lives. The authors evaluated their efficacy in blocking infection following repeated low-dose mucosal challenges of rhesus macaques (*Macaca mulatta*) with the tier 2 SHIV$_{AD8-EO}$. A single intravenous infusion of 10-1074-LS monoclonal antibodies markedly delayed virus acquisition for 18 to 37 weeks (median 27 weeks), whereas the protective effect of the 3BNC117-LS bNAb was more modest (provided protection for 11–23 weeks, median 17 weeks). Serum concentrations of the 10-1074-LS monoclonal antibody gradually declined and became undetectable in all recipients between weeks 26 and 41, whereas the 3BNC117-LS bNAb exhibited a shorter half-life. To model immunoprophylaxis against genetically diverse and/or neutralization-resistant HIV-1 strains, a combination of the 3BNC117-LS plus 10-1074-LS monoclonal antibodies was injected into macaques via the more clinically relevant subcutaneous route. Even though the administered mixture contained an amount of each bNAb that was nearly threefold less than the quantity of the single monoclonal antibody in the intravenous injections, the monoclonal antibody combination still protected macaques for a median of 20 weeks. The extended period of protection observed in macaques for the 3BNC117-LS plus 10-1074-LS combination could translate into an effective semiannual or annual immunoprophylaxis regimen for preventing HIV-1 infections in humans.

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