

RESEARCH ON TYPE 2 DIABETES, INSULIN RESISTANCE, AND THE IMMUNE SYSTEM

Prof. Eddy Karnieli, MD

Rappaport Faculty of Medicine

Introduction

Type 2 diabetes mellitus (DM2) is a disease that occurs in all populations and age groups and affects more than 10% of the population in the Western world. Over the past few decades, as dietary habits have changed and people have become less active, the number of people with diabetes mellitus has more than doubled globally, making it one of the most important public health challenges to all nations. Concomitantly, a recent study suggests that the prevalence of diabetes in Japanese adults aged 55-74 has also increased, now reaching 8.2% (1). Epidemiological studies confirm that obesity and DM2 are closely linked and this condition is often termed the metabolic syndrome (2-4). Life-style modification, diet, and exercise have been shown to prevent the development of diabetes in overweight patients with impaired glucose tolerance (5).

DM2 is a heterogeneous, polygenic disorder characterized by defects in insulin action in tissues (insulin resistance) and/or defects in pancreatic insulin secretion (beta cell dysfunction), which eventually includes loss of pancreatic insulin-secreting cells. DM2 is a progressive and complex disorder that is difficult to treat effectively in the long term. Diabetes complications include myocardial infarction (MI) and strokes, diabetic neuropathy, diabetic nephropathy, and diabetic retinopathy, and result in increasing disability, reduced life expectancy, and enormous health costs. The development of these diabetes-related complications can be prevented and retarded by maintaining blood glucose levels as close to normal as possible (6-9). However, in spite of the current knowledge and new treatment protocols, currently many patients do not reach the desired treatment goals.

The purpose of this manuscript is to briefly overview the link between inflammation processes and DM2. Investigating the molecular mechanism(s) underlying DM2 pathophysiology has the potential to find new molecular targets for the prevention, treatment, and, hopefully, the cure for DM2.

Inflammation Linking Obesity and Diabetes

The potential role of the immune system in the pathogenesis of reduced insulin sensitivity (insulin resistance) and type 2 diabetes was observed many years ago (10). For example, when diabetic patients with rheumatoid disease were treated with high doses of salicylic acid (aspirin) their overall glucose homeostasis improved (11, 12). Furthermore, increased levels of markers and mediators of inflammation, oxidative stress components, correlate with impaired insulin action (13). For example, while markers of inflammation and coagulation are reduced with intensive lifestyle intervention, studies show that in omental fat tissue of obese patients, macrophage migration accumulation increases (14). The overproduction of macrophage-derived cytokines such as TNF α (an important inflammatory factor) activates the IKK-NF κ B pathway leading to serine phosphorylation of IRS-1 and results in insulin resistance (15, 16). This phosphorylation is mediated by IKK- β (17), which can be inhibited by salicylates, and thus improve insulin action (18). The NF κ B pathway leads also to impaired GLUT4 translocation machinery and concomitant reduced cellular glucose uptake (19, 20). In an as yet unpublished work, we studied GLUT4 regulation by NF κ B, demonstrating that in adipose cells and L6 myotubes, beyond its effect on the insulin signaling cascade, NF κ B: 1) interferes with insulin stimulation of cellular Glut4 function in cellular glucose uptake, and 2) represses Glut4 gene expression at transcription and post-transcription level, in a dose-dependent and tissue-specific manner. Thus, obesity and type 2 diabetes are considered as low-scale chronic inflammatory diseases.

While systemic insulin resistance in obesity can be related to macrophage-mediated tissue inflammation, the signals underlying macrophage attraction to the adipose depot during obesity are still unknown. In a recent review by Osborn and Olefsky (21), the authors suggest that over-nutrition causes adipocytes to secrete chemokines such as chemotactic protein-1 (MCP-1), leukotriene B4 (LTB4) and others, attracting the monocytes into the adipose tissue. Indeed, recent work by Olefsky's research group showed that by inhibiting the LTB4 receptor, insulin sensitivity can be restored to normalcy (22, 23). Further, PPAR γ agonists have also been shown to enhance insulin sensitivity by reducing inflammation stimulation. Recently, Barbara Kahn's group identified endogenous branched fatty acid esters of hydroxy-fatty acids (FAHFAs) that relate to metabolism and the immune system. These novel lipids have both anti-diabetic and anti-inflammatory effects improving insulin sensitivity in animal models (24). Additional support for the view that these products have clinical importance as potential therapeutics has recently been demonstrated in an animal model for colitis (25).

Nutrients, Gut Microbiomes, Immunomodulation and Metabolism

Certain grains, mushrooms, and microbes, including bacteria and yeast, contain polysaccharides like beta-glucans. Beta-glucans are primary components of the cell wall of certain bacteria, yeast, and more. These components are recognized by specific receptors (such as Dectin-1) on immune cells leading to immune stimulatory action against bacteria or the fungi, for example (26). The discovery that these compounds are able to interact with and modulate the activity of specific receptors on immune cells suggested that they might have a role as anti-tumor adjunct products (27). On the other hand, the increase in the incidence of type 1 diabetes in developed countries with better sanitary conditions suggests a role for microbial exposure in preventing type 1 diabetes (28). Supporting evidence recently demonstrated that systemic treatment of pre-diabetic NOD mice with β -glucan can delay the appearance of diabetes (28). Further, oral administration of the β -glucan effectively prevented the development of high-fat-diet-induced fatty liver in mice as well as ameliorating the development of atherosclerosis in susceptible mice.

The discovery by Gordon's group that gut microbiota affect obesity and metabolism (29) led to increased efforts to understand the underlying causes of the various microbiota on glucose homeostasis. For example, artificial sweeteners affect microbiota content, resulting in impaired glucose tolerance. Recent evidence demonstrated that the microbiota induce increased levels of branched-chain amino acids (BCAAs) leading to insulin resistance (30). Understanding the relationship between nutrients, specific microbiota host, gut barrier function, and intestinal immunity response is yet not fully understood (31).

In conclusion, the modern life style, high-fat diet, and lack of exercise have been shown to trigger the development of DM2 in overweight patients with impaired glucose tolerance. The increased levels of markers and mediators of inflammation and oxidative stress components correlate with impaired insulin action (20, 32). Recent findings suggest that aberrations in the communication between the innate immune system and the gut microbiota might contribute to complex diseases as well as to obesity and diabetes (33). Taken together, this knowledge is important for personalized nutrition to predict and prevent diabetes (34, 35).

Technion Diabetes Research Activity

Several projects are currently being conducted by various investigators at the Technion. A few examples of such projects are outlined below.

A. Schneur Center for Diabetes Research:

1. Studies focused on understanding glucose transporters GLUT4 regulation in diabetes and obesity. PI: Prof. Eddy Karnieli. The recent projects focus on body weight regulation and glucose homeostasis by a CYP2E1 - NRF2 pathway, and by the ANAHK protein. The studies are conducted in tissue culture human adipose cells as well as in specific KO mice models.
2. Studies utilizing tissue engineering techniques and biodegradable 3D constructs to treat diabetes. PI: Prof. Shulamit Levenberg. The studies focus on: a) engineering functional insulin secreting islets aiming to cure type 1 diabetes; b) overcoming peripheral tissue insulin resistance in a long-term manner by producing an engineered vascularized tissue. This construct will be implanted into an animal model of DM2 to enhance the overall glucose uptake, therefore restoring normal blood glucose levels (Co-PI: Prof. Eddy Karnieli)

B. Dr. Mogher Khamaisi (PI) is conducting research aiming at exploring the molecular mechanisms for impaired wound healing in long-standing diabetes by combining *in vivo* and *in vitro* studies and using human stem cells and other differentiated cells.

C. Prof. Andrew Levy (PI) is conducting long-standing research in pharmacogenetics investigating the role of haptoglobin subtypes in predicating diabetes complications and the molecular mechanism(s) involved. In addition, his team is studying the role of vitamin E to mitigate diabetes complications based on haptoglobin subtypes.

References

1. Kabeya Y, Kato M, Isogawa A, Takahashi Y, Matsushita Y, Goto A, Iso H, Inoue M, Mizoue T, Tsugane S, et al. Descriptive epidemiology of diabetes prevalence and HbA1c distributions based on a self-reported questionnaire and a health checkup in the JPHC diabetes study. *J Epidemiol.* 2014;24(6):460-8.
2. Haffner SM. The metabolic syndrome: inflammation, diabetes mellitus, and cardiovascular disease. *Am J Cardiol.* 2006;97(2A):3A-11A.
3. Haffner S. Diabetes and the metabolic syndrome--when is it best to intervene to prevent? *Atherosclerosis.* 2006;7(1):3-10.
4. Alberti KG, Zimmet P, and Shaw J. Metabolic syndrome—a new world-wide definition. A Consensus Statement from the International Diabetes Federation. *Diabet Med.* 2006;23(5):469-80.
5. Knowler WC, Barrett-Connor E, Fowler SE, Hamman RF, Lachin JM, Walker EA, and Nathan DM. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *The New England journal of medicine.* 2002;346(6):393-403.
6. DCCT. Hypoglycemia in the Diabetes Control and Complications Trial. The Diabetes Control and Complications Trial Research Group. *Diabetes.* 1997;46(2):271-86.
7. UKPDS. Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). UK Prospective Diabetes Study (UKPDS) Group. *Lancet.* 1998;352(9131):854-65.
8. UKPDS. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). UK Prospective Diabetes Study (UKPDS) Group. *Lancet.* 1998;352(9131):837-53.
9. Nathan DM, and Group DER. The diabetes control and complications trial/epidemiology of diabetes interventions and complications study at 30 years: overview. *Diabetes care.* 2014;37(1):9-16.
10. Shoelson SE, Lee J, and Goldfine AB. Inflammation and insulin resistance. *J Clin Invest.* 2006;116(7):1793-801.
11. Gilgore S. The influence of salicylate on hyperglycemia. *Diabetes.* 1960;9(392-3).
12. Reid J, Macdougall AI, and Andrews MM. Aspirin and diabetes mellitus. *Br Med J.* 1957;2(5053):1071-4.
13. Hotamisligil GS. Inflammation and metabolic disorders. *Nature.* 2006;444(7121):860-7.
14. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, and Ferrante AW, Jr. Obesity is associated with macrophage accumulation in adipose tissue. *J Clin Invest.* 2003;112(12):1796-808.
15. Kanety H, Hemi R, Papa MZ, and Karasik A. Sphingomyelinase and ceramide

- suppress insulin-induced tyrosine phosphorylation of the insulin receptor substrate-1. *J Biol Chem.* 1996;271(9895-7).
16. Hotamisligil GS, Peraldi P, Budavari A, Ellis R, White MF, and Spiegelman BM. IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF- α - and obesity-induced insulin resistance. *Science.* 1996;271(665-8).
 17. Yuan M, Konstantopoulos N, Lee J, Hansen L, Li ZW, Karin M, and Shoelson SE. Reversal of obesity- and diet-induced insulin resistance with salicylates or targeted disruption of Ikk β . *Science.* 2001;293(5535):1673-7.
 18. Kim JK, Kim YJ, Fillmore JJ, Chen Y, Moore I, Lee J, Yuan M, Li ZW, Karin M, Perret P, et al. Prevention of fat-induced insulin resistance by salicylate. *J Clin Invest.* 2001;108(437-46).
 19. de Alvaro C, Teruel T, Hernandez R, and Lorenzo M. Tumor necrosis factor α produces insulin resistance in skeletal muscle by activation of inhibitor κ B kinase in a p38 MAPK-dependent manner. *J Biol Chem.* 2004;279(17070-8).
 20. Wellen K, and Hotamisligil G. Inflammation, stress, and diabetes. *J Clin Invest* 2005;115(1111-9).
 21. Osborn O, and Olefsky JM. The cellular and signaling networks linking the immune system and metabolism in disease. *Nat Med.* 2012;18(3):363-74.
 22. Li P, Oh da Y, Bandyopadhyay G, Lagakos WS, Talukdar S, Osborn O, Johnson A, Chung H, Mayoral R, Maris M, et al. LTB $_4$ promotes insulin resistance in obese mice by acting on macrophages, hepatocytes and myocytes. *Nat Med.* 2015;21(3):239-47.
 23. Esmaili S, and George J. Ltb4r1 inhibitor: A pivotal insulin sensitizer? *Trends Endocrinol Metab.* 2015;26(5):221-2.
 24. Moraes-Vieira PM, Saghatelian A, and Kahn BB. GLUT4 Expression in Adipocytes Regulates De Novo Lipogenesis and Levels of a Novel Class of Lipids With Antidiabetic and Anti-inflammatory Effects. *Diabetes.* 2016;65(7):1808-15.
 25. Lee J, Moraes-Vieira PM, Castoldi A, Aryal P, Yee EU, Vickers C, Parnas O, Donaldson CJ, Saghatelian A, and Kahn BB. Branched fatty acid esters of hydroxy fatty acids (FAHFAs) protect against colitis by regulating the gut innate and adaptive immune systems. *J Biol Chem.* 2016.
 26. van den Berg TK, Yoder JA, and Litman GW. On the origins of adaptive immunity: innate immune receptors join the tale. *Trends Immunol.* 2004;25(1):11-6.
 27. Vannucci L, Krizan J, Sima P, Stakheev D, Caja F, Rajsiglova L, Horak V, and Saieh M. Immunostimulatory properties and antitumor activities of glucans (Review). *Int J Oncol.* 2013;43(2):357-64.
 28. Karumuthil-Meilethil S, Gudi R, Johnson BM, Perez N, and Vasu C. Fungal β -glucan, a Dectin-1 ligand, promotes protection from type 1 diabetes by inducing regulatory innate immune response. *J Immunol.* 2014;193(7):3308-21.
 29. Ridaura VK, Faith JJ, Rey FE, Cheng J, Duncan AE, Kau AL, Griffin NW,

- Lombard V, Henrissat B, Bain JR, et al. Gut microbiota from twins discordant for obesity modulate metabolism in mice. *Science*. 2013;341(6150):1241214.
30. Pedersen HK, Gudmundsdottir V, Nielsen HB, Hyotylainen T, Nielsen T, Jensen BA, Forslund K, Hildebrand F, Prifti E, Falony G, et al. Human gut microbes impact host serum metabolome and insulin sensitivity. *Nature*. 2016;535(7612):376-81.
 31. McPhee JB, and Schertzer JD. Immunometabolism of obesity and diabetes: microbiota link compartmentalized immunity in the gut to metabolic tissue inflammation. *Clin Sci (Lond)*. 2015;129(12):1083-96.
 32. Hotamisligil G. Inflammation and metabolic disorders. *Nature*. 2006;444(860-7).
 33. Thaiss CA, Zmora N, Levy M, and Elinav E. The microbiome and innate immunity. *Nature*. 2016;535(7610):65-74.
 34. Glauber HS, Rishe N, and Karnieli E. Introduction to personalized medicine in diabetes mellitus. *Rambam Maimonides Med J*. 2014;5(1):e0002.
 35. Zeevi D, Korem T, Zmora N, Israeli D, Rothschild D, Weinberger A, Ben-Yacov O, Lador D, Avnit-Sagi T, Lotan-Pompan M, et al. Personalized Nutrition by Prediction of Glycemic Responses. *Cell*. 2015;163(5):1079-94.