

Fatty acid overload to compromised oxidative phosphorylation activates inflammation in type 2 diabetes: Hidden beasts and how to find them

The epidemics of type 2 diabetes and obesity are considered to be the consequences of lifestyle changes that came with industrialization and the overconsumption of calorie-dense fatty foods accompanied by less exercise. Because obese individuals have high blood insulin levels while they maintain normal blood glucose, the ineffectiveness of insulin or insulin resistance (IR) became a key biochemical abnormality to understanding obesity. As insulin deficiency is the hallmark of diabetes, relative insulin deficiency to compensate of IR is considered to cause type 2 diabetes. In an exhaustive review of the pathophysiology of IR, Peterson and Shulman¹ emphasized that the role of tissue crosstalk in whole-body insulin action, especially between adipose lipolysis and hepatic gluconeogenesis, is essential. Regarding the molecular mechanisms, they pointed out the roles of bioactive lipids – diacylglycerol, ceramide and acylcarnitine – and consider the nutrient stresses to the endoplasmic reticulum and mitochondria, and the non-cell autonomous factors, such as inflammatory mediators, branched-chain amino acids, adipokines and various cytokines. Regarding mitochondrial (dys)function, they appreciated its association with IR, and concluded that there is no clear evidence to establish their cause–effect relationship.

Regarding lipid-induced insulin resistance, they considered the Randle cycle

or competition between glucose and fatty acids as an energy source, as increased adenosine triphosphate (ATP) production from fatty acid oxidation (FAO) would decrease glucose transporter expression among the numerous direct and indirect mechanisms. They cited the study by Koves *et al.*², entitled “Mitochondrial overload and incomplete fatty acid oxidation contribute to skeletal muscle insulin resistance high fat feeding in animal.” A high-fat diet increased FAO in muscle without increasing CO₂ production, but increased the mitochondrial acylcarnitine level and increased mitochondrial reactive oxygen species production. The authors interpreted this state to reflect an incomplete FAO, thus IR, is caused when FAO is mismatched to citrate cycle flux and induces mitochondrial stress. An elevated plasma acylcarnitine level is considered a function of incomplete FAO rates and mitochondrial dysfunction of all tissues.

Inflammatory markers, such as C-reactive protein and various cytokines (i.e., tumor necrosis factor- α), are increased in IR. For the mechanisms, T cells infiltrating adipose tissue receive special attention, as they recirculate through key metabolic regulatory tissues, produce cytokines and stimulate lipolysis. Nicholas *et al.*³ reported very interesting observations in a study entitled “Fatty acid metabolites combine with reduced β oxidation to activate Th17 inflammation in human type 2 diabetes” on this topic. Naïve CD4⁺ T cells can differentiate into various T helper cells (Th), including Th1, Th2, Th9, Th17 and regulatory T cells. They are activated when T cell receptor (TCR) is costimulated at CD3 (a signal transduction component of the

TCR complex) and CD28 (binding site of natural TCR ligands). An antibody, aCD3/aCD28, was specially developed to bind two sites and costimulate T cells to secrete cytokines. As specific subsets of T cells secrete a special set of cytokines, T cells and their specific cytokine secretion profiles could be identified using this technique. Nicholas *et al.*³ isolated peripheral blood mononuclear cells (PBMCs) and B cell-depleted PBMCs from individuals with obesity and type 2 diabetes, and stimulated them with aCD3/aCD28. By carefully comparing T-cell metabolism, as well as cytokine profiles released from the stimulated PBMCs between type 2 diabetes and obese non-diabetic people, they found the following: (i) purified CD4⁺ T cells and activated immune cells from type 2 diabetes had higher activated 5' adenosine monophosphate (AMP)-activated protein kinase (AMPK), lower mitochondrial mass, lower carnitine-acylcarnitine translocase (CACT) : carnitine-palmitoyl transferase 1A (CPT1A) ratio and evidence suggesting that PBMCs of type 2 diabetes patients use more aerobic glycolysis, compared with non-diabetic individuals; (ii) stimulated PBMCs from non-diabetic individuals and type 2 diabetes patients maintained distinct cytokine profiles in the absence of glucose, which was expected to make cells use an alternative fuel source and shift to the cytokine profile of non-diabetic individuals; (iii) PBMCs from non-diabetic individuals reduce the production of most cytokines when glucose is deprived, whereas the production of most important cytokines increased in type 2 diabetes patients; (iv) blockade of fatty acid transport and/or

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beta oxidation in PBMCs with etomoxir/trimetazidine (inhibitors of fatty acid oxidation) or CACT-specific small interfering ribonucleic acid, alone or in combination with excess palmitoyl-carnitine promoted Th17 cytokine production, independent of glucose metabolism; and (v) knockdown of CPT1A ameliorated Th17 cytokine production, consistent with the interpretation that a decrease in the CACT : CPT1A ratio promotes Th17 function. The authors interpretation of these data show that “an environment rich in long chain fatty acid metabolites” induces immune cells with “compromised fatty acid oxidation machinery” to produce the Th17 cytokines,

and it is a defining factor of inflammation in human type 2 diabetes. Glucose was not an essential factor, and glycolysis did not fuel type 2 diabetes-associated Th17 inflammation.

To understand their interpretations, we require some understanding of mitochondrial physiology and the context of their experiments (Figure 1). Glucose is normally oxidized through glycolysis, generating pyruvate, which is converted to acetyl-coenzyme A (CoA) to enter mitochondria. Acetyl-CoA condensates with oxaloacetate to generate citrate, and is then consumed to produce CO₂ reproducing oxaloacetate. Free energy in

acetyl-CoA is transferred to nicotinamide adenine dinucleotide and flavin adenine dinucleotide, then to mitochondrial electron transfer chain, building a proton-motive force gradient across the mitochondrial membranes, which is used in the synthesis of ATP. This is called oxidative phosphorylation (OXPHOS). Fatty acids are also burned to produce ATP in mitochondria. Long chain fatty acid-like palmitic acid must be carnitiny-lated by CPT to enter the mitochondria, which depends on CACT, where carnitine acts as a carrier. They are oxidized to acetyl-CoA (beta oxidation) inside of mitochondrion through the citrate cycle.

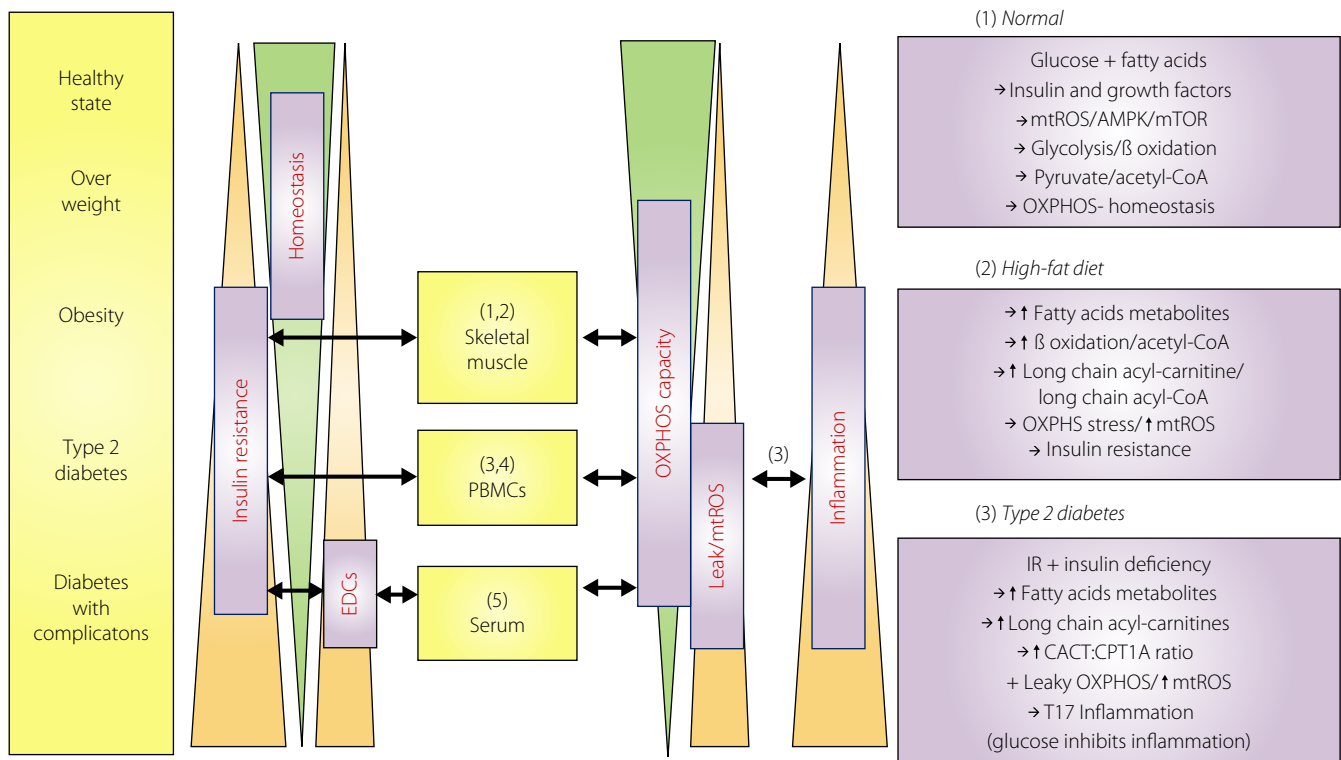


Figure 1 | Interrelations between various conceptual states: diseases, insulin resistance, mitochondrial function, effect of endocrine disrupting chemicals (EDCs) and inflammation. The numbers indicate the cited article numbers and the arrows indicate interrelations shown in the articles. In the healthy state, glucose and fatty acids are burned by the oxidative phosphorylation system (OXPHOS) in mitochondria, producing just enough adenosine triphosphate to meet the energy need. Insulin and growth factors play pivotal roles in the homeostasis and biogenesis of mitochondrion, mediated by various metabolites, mitochondrial reactive oxygen species (mtROS), adenosine monophosphate activated protein kinase (AMPK), mammalian target of rapamycin (mTOR) and others. Koves *et al.*² showed when a high-fat diet is fed, mitochondrion (of skeletal muscle) is stressed and becomes insulin resistant. Nicholas *et al.*³ showed that peripheral blood mononuclear cells (PBMCs) of individuals with type 2 diabetes have smaller and leaky mitochondria, and secrete T17 cytokines when challenged with lipid-derived metabolites (decreased carnitine-acylcarnitine translocase [CACT] : carnitine-palmitoyl transferase 1A [CPT1A] ratio), inducing inflammation. Li *et al.*⁴ reported quantitative relations between OXPHOS function parameters of PBMCs and the degree of insulin resistance of the whole body. Park *et al.*⁵ showed that EDCs could damage mitochondria, and the serum levels of mitochondrial inhibiting substances are quantitatively related with the degree of insulin resistance and inflammation.

Citrate can be transported back to the cytosol, and re-converted to acetyl-CoA and oxaloacetate, depending on cellular needs. Acetyl-CoA is carboxylated by acetyl CoA carboxylase (ACC) to form malonyl-CoA, the starting material of long chain fatty acids. This synthetic process is catalyzed by a multi-enzyme complex, fatty acid synthase (FAS), and occurs typically in a nutrient excess state.

Naïve T cells are quiescent cells and usually use ATP generated from OXPHOS. When stimulated, CD4⁺ naïve T cells become proliferative and differentiate into T helper cells (Th), which require metabolic reprogramming, shifting the energy supply to glycolysis. However, OXPHOS is also indispensable for T-cell activation and proliferation. Oligomycin, an ATP synthase inhibitor, can completely abrogate the proliferation of TCR-activated T cells, and N-acetylcysteine, an inhibitor of mitochondrial reactive oxygen species production, can drastically reduce Th17 differentiation. When cells are exposed to hypoxia or their mitochondria are dysfunctional, cells produce more ROS and use more glucose and produce pyruvate. However, pyruvate could not enter mitochondria, so they are converted to lactate (as in fermentation), making the cell environment acidic. In this condition, adenosine diphosphate and its precursor, AMP, accumulates (making AMP : ATP ratio increase), activating AMPK.

Insulin is the main anabolic hormone and stimulates mitochondrial respiration, as well as its biogenesis. Insulin stimulates mammalian target of rapamycin, which controls a wide spectrum of cellular processes, including cell growth and response to stress. These processes are orchestrated with various hormones, growth factors, cytokines and metabolites. Mammalian target of rapamycin stimulates Th17 differentiation through promotion of hypoxia-inducible factor-1 α , a master transcriptional regulator of the adaptive response to hypoxia. In the hypoxic condition, cells rely more on glycolysis for their energy, as OXPHOS requires oxygen to produce ATP. Many enzymes involved in fatty acids oxidation and synthesis are also

regulated by the activation of AMPK, including ACC and CPT I. Cell energy level is sensed by the AMP : ATP ratio, which limits what cells could do, whereas mammalian target of rapamycin determines what cells should do.

What Nicholas *et al.*³ did was an interrogation of PBMCs from obesity and type 2 diabetes with various stimulants and inhibitors of mitochondrial metabolism. They found lipid metabolites drive inflammation when the mitochondrial state is dysfunctional, leading them to the conclusion that control of lipid metabolism will be better for the prevention of diabetic complications. Although the authors' conclusion is undeniable, they failed to mention insulin deficiency, which should have contributed to both "an environment rich in long chain fatty acid metabolites" and "compromised fatty acid oxidation machinery."

The "Mitochondrial overload and incomplete fatty oxidation" state reported by Koves *et al.*² is very similar to "Fatty acid metabolites combine with reduced β oxidation" described by Nicholas *et al.*³ The two studies are not directly comparable, but demonstrate the fact that common alterations in lipid metabolism and mitochondrial state control both immune response and insulin sensitivity, as explained in Figure 1.

I was surprised to see PBMCs of obesity or type 2 diabetes maintain the characteristics of mitochondria function. Then, I found that Li *et al.*⁴ tested the mitochondrial function of PBMCs with high-resolution respirometry (Oxygraph-2 k; Oroboros Instruments, Innsbruck, Austria) in 24 patients with early-stage heart failure with (cardio-)metabolic syndrome and compared this with 25 healthy controls. Mitochondrial respiratory functional parameters; that is, routine respiration and activities of electron transfer chain complex 1 and 2, were significantly lower in heart failure patients. Most importantly, those parameters correlated with the degree of inflammation and anti-oxidant capacity of participants quantitatively. Furthermore, metabolic risk factors themselves, such as salt intake and blood pressure, were quantitatively

related to the mitochondrial dysfunctions. Li *et al.* concluded that "cardiometabolic risk factors cause chronic inflammation and ROS production" and "cardiometabolic risk factor-mediated mitochondrial respiratory dysfunctions of PBMCs link with the cellular inflammation/oxidative stress." How could there be such correlations, if they are not determined by the mitochondrial state?

I had been advocating that environment-polluting chemicals, particularly the roles of persistent organic pollutants in a high-fat diet, are important determinants of IR. My colleagues developed a novel cell-based aryl-hydrocarbon receptor (AhR) bioassay system for human serum AhR agonists, and found serum bioactivity closely correlated with AhR agonist bioactivity and parameters of metabolic syndrome, including bodyweight, suggesting that circulating AhR ligands directly reduce mitochondrial function, leading to IR⁵.

In summary, endocrine-disrupting chemicals, especially persistent organic pollutants, inhibit mitochondrial OXPHOS, and combined with high blood lipids, start inflammation by releasing mitochondrial reactive oxygen species. They disrupt insulin signaling and other endocrine systems, causing IR. In this state, hyperglycemia is beneficial, as Nicholas *et al.* showed. Now we have tools to test this hypothesis.

DISCLOSURE

The author owns a patent on the AhR ligands bioassay in part.

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