Insulin resistance in the liver: Deficiency or excess of insulin?

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In insulin-resistant states (obesity, pre-diabetes, and type 2 diabetes), hepatic production of glucose and lipid synthesis are heightened in concert, implying that insulin deficiency and insulin excess coexists in this setting. The fact that insulin may be inadequate or excessive at any one point in differing organs and tissues has many biologic ramifications. In this context the concept of metabolic compartmentalization in the liver is offered herein as one perspective of this paradox. In particular, we focus on the hypothesis that insulin resistance accentuates differences in periportal and perivenous hepatocytes, namely periportal glucose production and perivenous lipid synthesis. Subsequently, excessive production of glucose and accumulation of lipids could be expected in the livers of patients with obesity and insulin resistance. Overall, in this review, we provide our integrative perspective regarding how excessive production of glucose in periportal hepatocytes and accumulation of lipids in perivenous hepatocytes interact in insulin resistant states.

Development of insulin resistance with obesity, pre-diabetes, and type 2 diabetes is a physiopathologic process where cells fail to respond normally to insulin.[1-4] Thus, suppression of glucose production in the liver is decreased and activation of GLUT-4-mediated glucose uptake does not take place, particularly in skeletal muscles and adipocytes.[5] This overall failure typically is not due to low insulin levels.[6] Instead, insulin-stimulated signal transduction pathways for peripheral glucose uptake and for hepatic glucose production are reduced, including insulin receptors and downstream mediators.[7-9]

Hyperglycemia is then driven by excessive hepatic glucose production[10,11] and reduced uptake of glucose by peripheral tissues.[12] To counteract resultant glycemic elevations, β cells of the pancreas boost insulin production, further contributing to hyperinsulinemia.[12,13] Hence, insulin resistance often is accompanied by increased circulating levels of insulin.[14-16] If this compensatory rise in insulin production is not maintained by the pancreas, causing insulin levels to drop, then type 2 diabetes ensues.[17-19]

Questions Raised by Insulin Resistance

It is well recognized that lipolysis and weight loss are accelerated in the absence of insulin, underscoring the fact that insulin stimulates lipogenesis,[20] and raising an important question: why are about 90% of patients with type 2 diabetes overweight or obese?[21] Or otherwise stated, is there a correlation between insulin deficiency and the tendency gain weight? Furthermore, given the scope of lipid deposition in liver (hepatic steatosis), skeletal muscle (intramyocytic lipid accumulation), cardiac muscle, and adipose tissues that is seen with insulin resistance,[22] one must also ask: is obesity the cause or the result of insulin resistance?

The term insulin resistance oversimplifies a highly complex physiopathologic process for which a single overarching mechanism is not easily conceived.[1-6] In addition, the paradigm that insulin resistance is pathologic at all times is simply inaccurate. For example, during late pregnancy, insulin resistance and increased glucose tolerance may be seen together as a seeming paradox.[23] Nonetheless, some degree of insulin resistance during late pregnancy is necessary for glucose

maintenance,[13] owing to substantial fetal demands for glucose.

These questions and considerations have spawned a number of questions where answers may be found or conclusions drawn.

Which Came First: The Chicken or the Egg?

Under physiologic conditions, the insulin sensitivity of various bodily tissues differs. Case in point, human skeletal muscle is more sensitive than subcutaneous fatty in terms of the effects of circulating insulin.[24]

Similarly, insulin resistance in insulin sensitive cells is not uniform but is tissue specific.[16] For example, in high fat diet fed rats, insulin resistance was found to initiate in the liver, prior to developing in skeletal muscle.[26] In addition, impairment in all steps of insulin signaling was detected in skeletal muscle, liver, hypothalamus, but not in adipose tissue of fat-rich diet treated mice.[12] Hence, even in insulin-resistant states in which glucose transport is impaired, sensitivity to insulin's antilipolytic effect is relatively preserved, resulting in maintenance or expansion of adipose stores.

Thus, insulin resistance is not a synchronous, all-or-nothing process but rather builds in select organs or tissues amidst normal insulin response.

In parts of the body responding normally to insulin, the hyperinsulinism of insulin resistance likely is construed as a state of insulin excess. Hence, the catabolic activities of insulin resistance (increased hepatic glucose production, decreased glucose uptake, fasting hyperglycemia) may collide with complementary conditions that favor lipogenesis and obesity. So a vicious cycle can be set up with insulin resistance promoting weight gain, which promotes more insulin resistance. Subsequently, the multiple comorbidities of obesity, prediabetes, and type 2 diabetes that are attributed to insulin deficiency may actually stem in part from insulin excess. Likewise, the predisposition in patients with insulin resistance for aging,[1,18,27] cancer,[27] liver steatosis,[28] dyslipidemia,[5] atherosclerosis,[29] cardiovascular disease,[30] and why intensive insulin therapy may initially

worsen retinophaty,[31] also be at least partially explained.

Coexistent Insulin Deficiency and Insulin Excess in Organs and Tissues of the Body

Development of hyperglycemia in overweight patients,[21] is also aligned with the concept that throughout the body, both insulin deficiency and insulin excess are operant in insulin resistance. Indeed, these 2 extremes of response are even displayed by the same organ, i.e., increased hepatic production of glucose,[12,25] and parenchymal deposition of lipid,[32-36] associated with insulin resistance,[37,38] suggesting that this principle could be applied to organs and tissues separately, as well as involving the body as a whole.

Interestingly, with insulin resistance induced by a high-fat diet, a temporal sequence has been noted for each substrate during activation of hepatic gluconeogenesis, with progressive intensification for L-lactate, glycerol, and alanine on days 7, 14, and 56, respectively after dietary implementation.[25] If intensified liver gluconeogenesis then serves as a marker of insulin resistance, it appears that this process is quite specific, marked by intra-organ metabolic pathways that are unique for each substrate.

At this juncture, a new question surfaces: as with organs and tissues and the body as a whole, is the coexistence of insulin deficiency and insulin excess in insulin-resistant states applicable to isolated cells?

Metabolic Compartmentalization of Glucose Production and Lipid Synthesis in Liver

To our knowledge, a number of metabolic pathways (i.e., gluconeogenesis, lipogenesis, glycolysis, glycogenolysis, ureagenesis, ketogenesis, synthesis and catabolism of amino acids, etc.) are feasible in individual hepatocytes. Hence, gluconeogenesis (a process inhibited by insulin) and lipogenesis (which is stimulated by insulin) are achievable in the same liver cell. However, in the acini area, the hepatocytes are exposed to a spatial biochemical gradient that influences

metabolism and gene expression, so cell specialization does exist to some degree, depending on locale.[37-52]

Parenchymal acini of the liver are divisible into 2 circulatory zones, based on proximity to afferent vessels.[36] Periportal hepatocytes are supplied by blood rich in oxygen and nutrients, whereas the blood reaching perivenous hepatocytes (at the periphery of acini) is oxygen-poor and nutrient-depleted.[53,54] This distinctive microvascular arrangement encourages metabolic heterogeneity. Periportal hepatocytes, harboring an abundance of mitochondria and sympathetic nerves are ideally suited for oxidative metabolism or glucose production, and perivenous hepatocytes are optimally configured for anaerobic metabolism and lipid synthesis (**Fig. 1**).

Hepatocytes are also subject to differential regulatory control, due to gradients in oxygen, substrate, and hormone levels. Notably, O_2 partial pressure of approximately 65 mmHg in periportal areas drops to 35 mmHg in perivenous zones.[52] The O_2 partial pressure regulates the expression of genes encoding glucose-metabolizing enzymes, (for example: pyruvate carboxykinase, glucokinase and pyruvate kinase), through O_2-responsive transcription factors, such as hypoxia-inducible factor (HIF).[53,54] Moreover, liver metabolism is controlled among others by nuclear receptors,[36] mammalian target of rapamycin (mTOR) pathway,[37] and sirtuin family of proteins.[38]

The model of metabolic zonation assumes a functional specialization by each hepatic zone (**Fig. 1**). In periportal areas, gluconeogenesis, glycogenolysis, β-oxidation, amino acid metabolism, ureagenesis, and uric acid production predominate,[55-61] reserving lipogenesis, glycolysis, glutaminogenesis, and biotransformation for perivenous areas.[62-72] Accordingly, periportal and perivenous acinar zones differ in content of many key enzymes and subcellular constituents (**Table 1A** and **Table 1B**), all of which serve to optimize liver function for a central role in metabolic homeostasis.[36]

It must be emphasized that functional specialization of this nature is also quite flexible. For example, the periportal-to-perivenous ratios for mitochondrial palmitate oxidation in fed, starved, re-fed, and
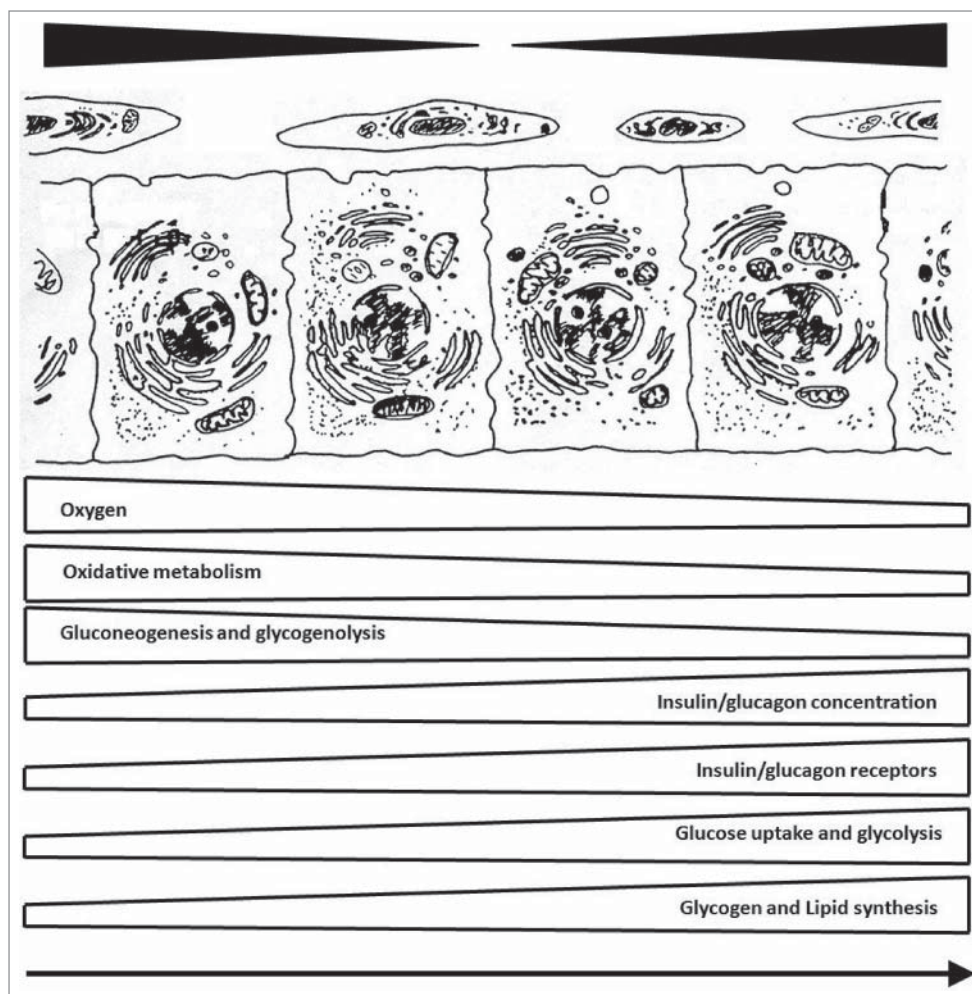
Figure 1. Functional compartmentalization of liver: periportal hepatocytes on left and perivenous hepatocytes on right, with arrow in direction of blood flow.

cold-exposed animals were 1.5, 2.0, 1.0, and 0.4, respectively.[73]

Given that insulin released by β cells of the pancreas reaches periportal zones first, greater inhibition of glucose production in periportal area and lesser activation of lipogenesis perivenous area are anticipated.[74] However, insulin receptors are predominantly found in the perivenous zone, where their expression is enhanced by high glucose concentration and decreased venous partial pressure.[52] By contrast, glucagon receptors predominate in the periportal zone,[75] where glucose release from glycogenolysis and gluconeogenesis preferentially takes place. In addition, due to biotransformation, glucagon and insulin concentrations decline during a single passage of blood through the liver by approximately 50% and 15%, respectively, resulting in proportionately higher perivenous concentrations of insulin.[50,75]

Therefore, under physiologic conditions, glucagon and insulin (from pancreatic α and β cells, respectively) first reach periportal zones, where glucagon receptors predominate for glycogenolysis and gluconeogenesis.[76] Because after meal the biotransformation of glucagon is comparatively more rapid,[75] the insulin/glucagon ratio increases as blood circulates to the periphery of liver acini. The higher insulin/glucagon ratio of perivenous zones, in conjunction with their preponderance of insulin receptors, is then favorable for lipid synthesis.

Here again, a question can be raised: is the paradox of insulin resistance, namely the hyperproduction of glucose in liver alongside steatosis, adequately explained by the concept of metabolic compartmentalization?

Hepatic Glucose Hyperproduction and Steatosis in Insulin Resistance

Liver metabolism comprises an immense spectrum of interrelated anabolic and catabolic functions which are performed simultaneously without futile cycles. Therefore, functional compartmentalization of the liver, as shown in **Fig. 1**, implies coexistence of an anabolic liver in a fed state (with perivenous insulin effects predominating) and a catabolic liver in a fasted state (with predominance of periportal glucagon effects).

Interestingly, decreasing periportal-perivenous gradients of oxygen tension and increasing periportal-perivenous gradients of insulin: glucagon ratio appear to be major factors in the zonation,[50] but how is functional compartmentalization of the liver affected by insulin resistance?

A schematic of our theories is shown in **Fig. 2**, starting with an increased basal rate of lipolysis as a consequence of augmented visceral adiposity.[22,77]

Because insulin stimulates expansion of visceral fat, this pathologic process functions as a self-sustaining closed-loop system that will only be interrupted by weight loss (diet and/or exercise) and /or use of drugs to increase insulin sensitivity.

The excess of free fatty acids (FFA) from splanchnic lipolysis is taken up by periportal hepatocytes (**Fig. 2**) and oxidized as a source of energy, all of which biochemically increases oxygen consumption.[78]

In spite of the fact that FFA cannot be used as substrates for gluconeogenesis, their oxidation furnishes energy to increase glucose production via gluconeogenesis. In addition, the excess of FFA delivery to the liver, results in

Table 1A. Zonation of cells, receptors, metabolism and biotransformation in liver. Key: [+++] predominant localization in periportal or perivenous zone.

	Periportal zone	Perivenous zone
Oxygen gradient[53,54]	+++	
Cell size[41]	15-20 μm	30-40 μm
Kupffer cells[39,40]	Phagocytosis	Cytotoxicity
Endothelial cells - Fenestrae[39,40]	Larger	Smaller
Stellate cells and Pit cells[39,40]	+++	
Sympathetic nerves[50]	+++	
Glucagon receptors[75]	+++	
Insulin receptors[52]		+++
Insulin/glucagon levels[76]		+++
Mitochondria and aerobic metabolism[36]	+++	
Glucose uptake and glycolysis[50]		+++
Glucose release: gluconeogenesis[36]	+++	
Glucose release: glycogenolysis[56]	+++	
ß-oxidation and ketogenesis[50]	+++	
Peroxisomal lipid oxidation[70]		+++
Triglycerides[59] and VLDL synthesis[83]		+++
Cholesterol and bile synthesis[71]	+++	
Glycogen synthesis from glucose[59]		+++
Glycogen synthesis from pyruvate and lactate[50]	+++	
Uptake of the majority of amino acids[70]	+++	+++
Uptake of glutamate and aspartate[70]		+++
Uptake of α-ketoglutarate and malate[70]	+++	+++
Glutamine synthesis and release[60]		
Amino acid catabolism and urea synthesis[59]	+++	
Uric acid synthesis from adenosine[57]	+++	
Glutation peroxidase and ROS detoxification[50]	+++	

Table 1B. Zonation of enzyme activity and protein synthesis in liver. Key: [+++] predominant localization in periportal or perivenous zone.

|	Periportal zone	Perivenous zone
Phosphoenolpyruvate carboxykinase[50]	+++	
Pyruvate carboxykinase[41]	+++	
Fructose-1,6-biphosphatase[72]	+++	
Glucose-6-Phosphatase[58]	+++	
Pyruvate kinase type L[59] and glukokinase[76]		+++
Acetyl-CoA carboxylase[83]		+++
Suppressor of cytokine signaling 2 (SOCS-2)[68]		+++
Glutaminase[60]	+++	
Glutamine synthetase[48]		+++
Succinate dehydrogenase[58]	+++	
Hydroximethylglutaryl-Coa-reductase[59]	+++	
Alanine[48] and tyrosine aminotransferase[59]	+++	
Carbamoyl phosphate synthetase[59]	+++	
UDP-glucoronosyltransferase[50]		+++
Cytocrome P-450[45]		+++
Serine dehydratase[61]	+++	
Fibrinogen and laminin synthesis[50]	+++	
α2-macroglobulin and conexin 26 synthesis[50]	+++	
Collagen IV and V synthesis[41]	+++	
Collagen I, III and VI synthesis[41]		+++
α1-antitrypsin and fibronectin synthesis[50]		+++
α-fetoprotein and angiotensinogen synthesis[50]		+++
Lectin binding[62]		+++
Heme synthesis[46]		+++
Gene expression of albumin[48]		+++
Xenobiotic metabolism[67]		+++
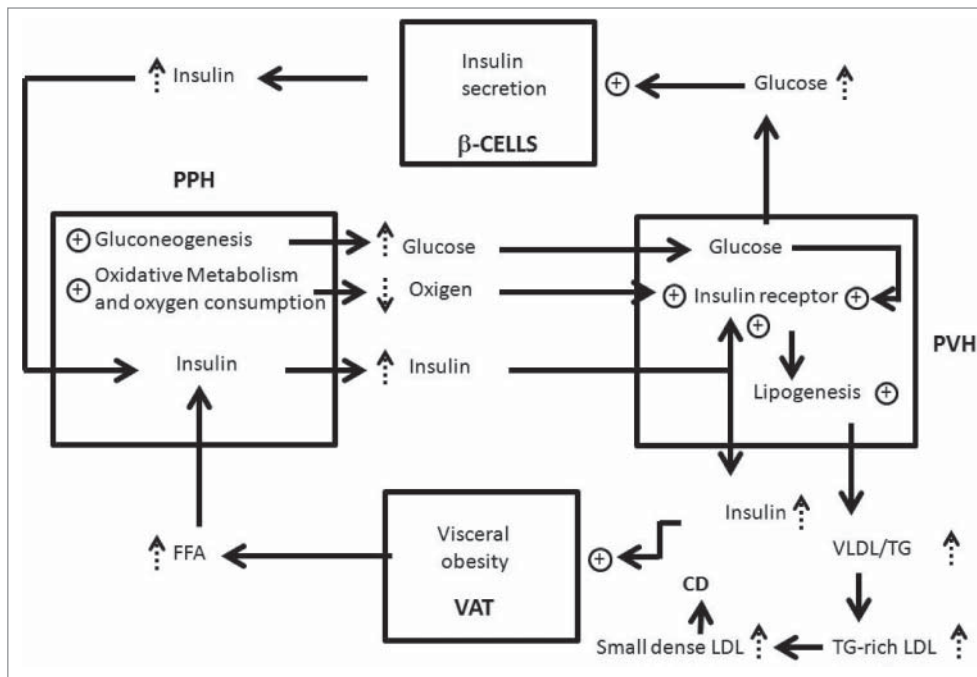
Figure 2. Relationship between visceral obesity, insulin resistance, functional compartmentalization and liver steatosis. Visceral adipocytes (VAT), periportal hepatocytes (PPH), perivenous hepatocytes (PVH) and β cells of pancreas (β-CELLS) are represented by squares. Key:↑augmentation; ⊕ stimulation; cardiovascular disease (CV); low-density lipoproteins (LDL); very low density lipoprotein (VLDL); triglyceride (TG).

accumulation of intracellular diacyglycerols which, in turn, leads to activation of protein kinase C (PKC). PKC induce insulin resistance by inhibiting insulin-stimulated phosphorylation of IRS proteins. Furthermore the excess of FFA results in activation of inflammatory toll-like receptors (TLR) signaling leading to increased *de novo* ceramide synthesis. Thus, accumulation of ceramides and ceramide-mediated inhibition induce insulin resistance in the liver by inhibition of Akt phosphorylation.[79] Yet, FFA can cause activation of Akt phosphatase protein phosphatase 2A (PP2A). PP2A induce insulin resistance in the liver by dephosphorilation and inactivation of Akt that in turn acts to phosphorylate and inactivate the transcription factor Forkhead Box 01 (FOX-01), which induces transcription of the key enzymes of gluconeogenesis.[80]

As consequence of these metabolic changes,[73] perivenous hepatocytes exposed to increased glucose concentration and reduced levels of oxygen,[53] intensifying their expression of insulin receptors.[52]

The enhanced FFA also increase the synthesis of triglyceride (TG) and very-low-density lipoprotein (VLDL) production in the perivenous zone,[81-83] thereby promoting hypertriglyceridemia. Furthermore, the TG in VLDL is exchanged for cholesteryl esters from low-density lipoproteins (LDL) and high-density lipoproteins (HDL) by the cholesteryl ester transport protein, producing TG-rich LDL and TG-rich HDL. The TG in the TG-rich LDL and TG-rich HDL is then hydrolyzed by hepatic lipase, producing small dense LDL and small dense HDL. The formation of these particles are linked to a higher risk of cardiovascular disease.[5] In fact, patients die more often from cardiovascular disease than from direct consequences of liver steatosis.[84]

Concluding Remarks

All aspects of the human body must be considered to better understand the mechanisms of insulin resistance and to appreciate how insulin deficiency and insulin excess may coexist in this pathologic condition. The fact that insulin may be inadequate or excessive at any one point in

differing organs and tissues has many biologic ramifications. Ultimately, attention must be paid to specific defects of insulin signaling in isolated cells and to subcellular compartments affected by the increased circulating insulin that accompanies insulin resistance. However, despite the enthusiasm for molecular aspects, we must never lose sight of the isolated organ and *in vivo* situation.

One key message is that insulin resistance accentuates differences in periportal and perivenous hepatocytes, namely periportal glucose production and perivenous lipogenesis. Subsequently, excessive production of glucose and accumulation of lipids are to be expected in the livers of patients with obesity and insulin resistance.

However, there is still a great deal to be learned about the mechanisms linking insulin resistance and metabolic compartmentalization in the liver. Therefore, future studies on the regulation of zonal gene expression in parenchymal and nonparenchymal liver cells will provide advances in our understanding of the impact of insulin resistance on metabolic compartmentalization in the liver.

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