Article

Four-stage Evolution of Diabetes or Whole-body Insulin Resistance (WBIR) Driven by Sequential Progression of Tissue-specific Glycation-induced Insulin Resistance (GIIR)

Song Jae Lee[1] and Sang Won Shin[2*]

[1] Integrated Research Institute, Seoul, South Korea; sjlee@cnu.ac.kr
[2] Korea University Hospital, Seoul, South Korea; shinsw9295@gmail.com
* Correspondence: SJ Lee, sjlee@cnu.ac.kr

Abstract: The investigation by the integrated approach newly introduced for this study indicates that insulin resistance (IR) would develop first preferentially in the muscle tissue with a relatively low cell turnover and then progress in sequence to the subcutaneous adipose tissue, to the visceral adipose tissue, and to the liver with higher cell turnovers. Moreover, metabolic disruptions due to IR vary widely from tissue to tissue, contrary to the conventional notion that IR merely impairs glucose uptake in the whole body. This warrants that IR be divided better into four distinct tissue-specific IRs: muscle insulin resistance (MIR), subcutaneous adipose insulin resistance (s-AIR), visceral adipose insulin resistance (v-AIR), and hepatic insulin resistance (HIR). Tissue-specific IRs developing in the order of MIR, s-AIR, v-AIR, and HIR – producing tissue-specific metabolic disruptions – would amount to nothing but the whole-body insulin resistance (WBIR) evolving in four distinctively insulin-resistant stages. The four-stage metabolic evolution from rapid weight gain to visceral obesity to rapid weight loss to full-blown diabetes not only complies well with the natural development history of diabetes but also resolves most of the controversies regarding obesity or diabetes, including visceral obesity, obesity paradox, and dawn phenomenon that have long remained metabolic puzzles. In addition, the four-stage WBIR evolution model refutes the entrenched notion of the lipid-induced insulin resistance (LIIR) but supports instead the glycation-induced insulin resistance (GIIR) proposed in this study. A speculation is that the glycation reaction activated ever more with WBIR evolving may serve as the common thread connecting each other a cluster of disorders or diseases that are often collectively referred to as the metabolic syndrome, such as obesity, diabetes, cardio/cerebral vasculopathy, nephropathy, neuropathy, and retinopathy.

Keywords: obesity; obesity paradox; diabetes; insulin resistance (IR); whole body insulin resistance (WBIR); tissue-specific insulin resistance; muscle insulin resistance (MIR); subcutaneous insulin resistance (s-AIR); visceral adipose insulin resistance (v-AIR); hepatic insulin resistance (HIR); lipid-induced insulin resistance (LIIIR); glycation-induced insulin resistance (GIIIR); metabolic syndrome

1. Introduction

The aim of this study is to develop a comprehensive diabetes evolution model that complies well with the natural development of diabetes(1). Insulin resistance (IR) plays a central role in development of diabetes, which appears to be at the center of association among many disorders, such as cardio/cerebral vasculopathy, nephropathy, neuropathy, and retinopathy. IR may be defined as the physiological state or condition, in which cells or tissues have been deranged enough as to be unable to respond properly to insulin, the major hormone that regulates the energy metabolism. Conventionally, IR has been considered only to merely impair glucose uptake in tissues. Given that, however, the role of insulin is very different from tissue to tissue(2), metabolic disruptions due to IR would be very wide-ranging and tissue-specific(3-5).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 14 February 2023 doi:10.20944/preprints201909.0247.v3

No less importantly, IR would not necessarily develop simultaneously over the whole body. Instead, it would, in fact, develop first preferentially in old cells over newly formed cells, simply because old ones would have been exposed longer to potential IR-inducing agents than new ones. In principle, the lower the cell turnover in a tissue, the larger the population of old cells. This would then translate into the proposition that tissues with lower cell turnovers would develop IR more readily than those with higher cell turnovers.

Adipose tissues are unique in that they differentiate preadipocytes into adult adipocytes with energy storage demand increasing – adipogenesis(6). In contrast, myogenesis in muscle tissues appears to be usually much smaller than adipogenesis(7). This implies that the average cells would be much older in muscle tissues than in adipose tissues, which translates into that muscle tissues would develop IR more readily than adipose tissues. Moreover, adipogenesis in the visceral adipose tissue is known to be much more active than in the subcutaneous adipose tissue(8), which translates into that the subcutaneous adipose tissue would develop IR more readily than the visceral adipose tissue. In the case of the liver that serves also as a major detoxification center in the body, the cells may be damaged and replaced very quickly, giving rise to a very high cell turnover. Based on this reasoning, we postulate that IR develop first preferentially in the muscle tissue and then progress in sequence to the subcutaneous adipose tissue, to the visceral adipose tissue, and to the liver. A noteworthy point here is that this postulation is contrary to the conventional notion that IR is a global parameter affecting the whole body simultaneously.

If IR indeed develop in sequence from the muscle tissue to subcutaneous adipose tissue to visceral adipose tissue to liver, producing tissue-specific metabolic disruptions, it would better be subdivided into four tissue-specific IRs: muscle insulin resistance (MIR), subcutaneous adipose insulin resistance (s-AIR), visceral adipose insulin resistance (v-AIR), and hepatic insulin resistance (HIR). Sequential development of tissue-specific IRs in the order of MIR, s-AIR, v-AIR and HIR, as depicted in Fig 1, would amount to nothing but the whole-body insulin resistance (WBIR) evolving in four distinctively insulin-resistant (IR) stages, denoted by IR-I, IR-II, IR-III, IR-IV, respectively(9).

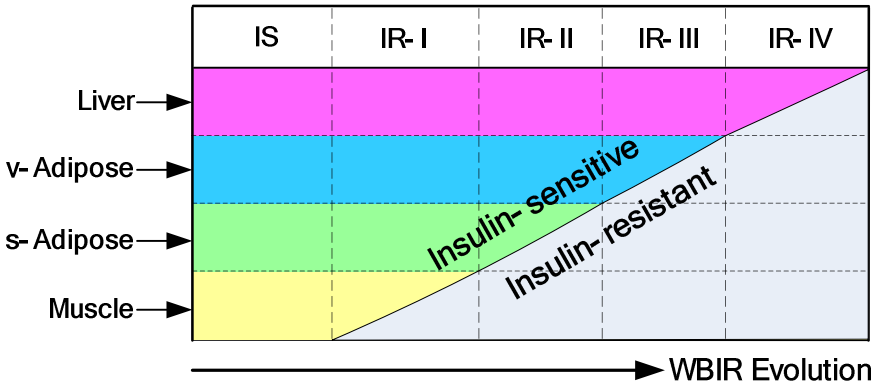


Figure 1. WBIR evolves in four distinctive stages as the tissue-specific IRs develop in the order of MIR, s-AIR, v-AIR, and HIR.

WBIR evolution would culminate, in the IR-IV stage, in the development of full-blown diabetes as glucose uptake is impaired in all of the tissues thereby elevating the plasma glucose (PG) severely enough to be diagnosed of diabetes. This suggests the four-stage WBIR evolution would, in reality, amount to the four-stage diabetes evolution.

Actually, Saad et al. proposed a two-step model for diabetes development(10). But the model lacks the concept of tissue-specific IRs developing in sequence from one tissue to others and therefore can hardly predict metabolic transition, for instance, from rapid weight gain to full-blown diabetes. By contrast, the four-stage WBIR evolution model not only complies well with the natural development history of diabetes(1) but also resolves

 doi:10.20944/preprints201909.0247.v3

most of the controversies regarding obesity or diabetes, including visceral obesity, obesity paradox, and dawn phenomenon that have long remained metabolic puzzles.

Yet the four-stage WBIR evolution model itself does not tell anything about particular IR-inducing agents or mechanisms that would be essential for WBIR to evolve. A widespread belief is that IR is induced by lipids. A core argument of the so-called the lipid-induced insulin resistance (LIIR) is that the adipose fat mass or obesity induces IR either directly or indirectly by contributing excess fatty acids (FAs) to the plasma so that they would somehow interfere the insulin action in cells or tissues(11).

However, weight gaining or obesity would have a beneficial effect of alleviating hyperglycemia that is the most prominent metabolic disturbance due to IR or diabetes. In order for the PG to be tightly regulated as in healthy individuals, various glucose disposal pathways – such as de novo lipogenesis (DNL) in the adipose tissues and liver, glycogenesis in the liver and muscles, and cellular glucose oxidation – would have to be activated in hyperglycemia. In other words, DNL would be enhanced significantly in hyperglycemia, which not only tends to alleviate hyperglycemia itself but also contributes to weight gaining or obesity. Then, how can weight gaining or obesity induce IR and simultaneously alleviate hyperglycemia that is, in fact, the most prominent metabolic disturbance resulting from IR? A noteworthy point is that newly diagnosed diabetics often lose weight very rapidly, only to gain some weight back only if treated by anti-diabetic medications, such as insulin or oral hypoglycemic agents (OHAs)(12). This may suggest that adipose tissues in diabetics can hardly respond properly to hyperglycemia in the way of enhancing DNL and thereby building up adipose fat mass. Conversely, this in turn suggests that only healthy adipose tissues are able to support weight gaining or obesity. This also explains why lean individuals often exhibit higher mortality than overweight or obese ones – obesity paradox(13). If weight gaining or obesity indeed has a beneficial effect of alleviating hyperglycemia, it would hardly be considered a risk factor per se. Nonetheless, obesity would hardly be beneficial overall for health. This suggests that behind weight gaining or obesity lies an independent risk factor, which is none other than the hyperglycemia itself.

The reasoning above largely refutes LIIR or indicates, at least, that LIIR is not compatible to the four-stage WBIR evolution model. It would then be essential to identify the IR-inducing agents or mechanisms involved in the WBIR evolution. An important aspect of the four-stage WBIR evolution is that the PG or, more accurately, time-averaged PG rises steadily with WBIR evolving. A reaction activated in hyperglycemic physiological condition is glycation or non-enzymatic glycosylation, in which glucose or its glycolytic derivatives, such as glyoxal and methylglyoxal, react non-enzymatically with proteins and form covalently bonded adducts(14, 15). Glycation adducts appear to neutralize some of the charges on the amino acid residues(16), thereby significantly changing the electrostatic forces between residues and therefore significantly altering the protein conformation as well. The protein with both the charge distribution and conformation disrupted significantly as a result of glycation can hardly function properly.

For example, insulin receptors or other proteins involved in downstream insulin signaling may also be glycated in hyperglycemia severely enough as to be rendered unable to respond properly to insulin. The cells or tissues unable to respond properly to insulin as a result of glycation would exhibit nothing but glycation-induced insulin resistance (GIIR). Importantly, GIIR would enhance hyperglycemia further – elevate PG further – by impairing glucose uptake into tissues, which would in turn enhance glycation reaction and GIIR further, thereby establishing a positive feedback loop in which hyperglycemia and GIIR enhance each other in a vicious circle. An important point regarding this vicious circle may be that PG would be elevated ever faster with the vicious circle going. Meanwhile, four-stage WBIR evolution also predicts that PG is elevated ever faster with WBIR evolving. This parallel may suggest that glycation reaction activated in the hyperglycemia is the very IR-inducing mechanism behind WBIR evolution. Another important point regarding the vicious circle may be that hyperglycemia is the cause as well as the effect of GIIR – GIIR is only the effect of hyperglycemia. This means that hyperglycemia is indeed the fundamental force driving the WBIR evolution or vicious circle.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 14 February 2023 doi:10.20944/preprints201909.0247.v3

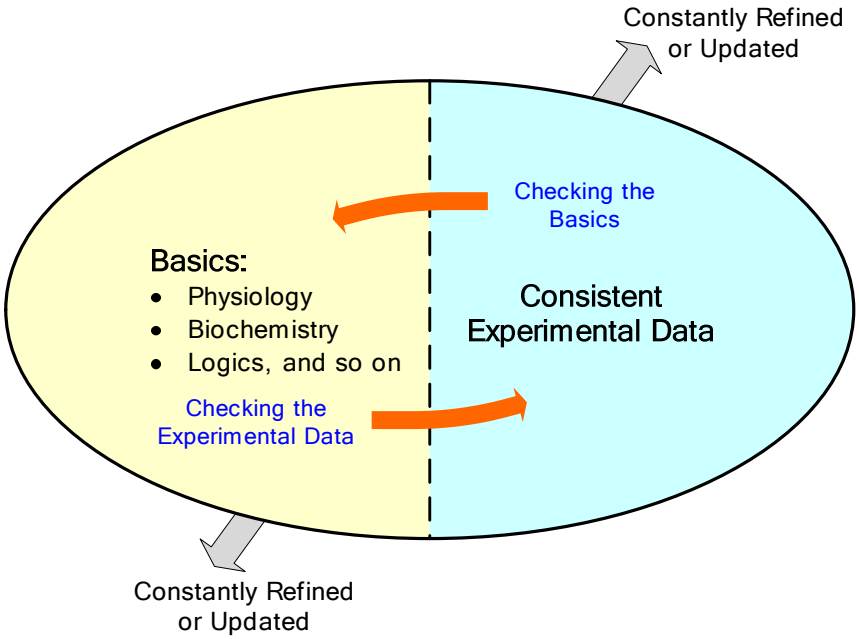
If the hyperglycemia is indeed the main driving force for WBIR evolution. Then, an obvious approach to prevent, delay, or reverse WBIR or diabetes evolution would be to alleviate hyperglycemia, which is in fact a primary goal of most diabetes management or treatment plans. And the first step to achieve alleviation of hyperglycemia would be to reduce postprandial glycemic load by curtailing carbohydrate intake.

2. Integrated Approach as the Methodology

WBIR evolution amounting, in fact, to diabetes evolution would in general proceed very slowly over years or even decades. Thus, it may not be investigated easily by simple experiments, especially conducted over a relatively short period of time. Besides, the pace of WBIR evolution in people would usually vary widely, depending on the concentration of potential IR-inducing agents. Therefore, the subjects recruited for experiments would be more likely to belong to different stages of WBIR evolution, likely making it much more difficult to analyze the data obtained.

WBIR evolution may be investigated better by integrated approach that we have newly introduced for this study. Probably, integrated approach had already been used extensively in the past. For example, nineteenth century evolutionists including Charles Darwin developed the theory of evolution based on huge amounts of data obtained from immense numbers of experiments performed by nature over an immensely long period of time, almost without conducting any experiments of their own, which would certainly have involved raising animals or growing plants for numerous generations.

The first task required for integrated approach is to build the integrated pool of resources, as depicted in Fig 2, which consists of two parts: (1) the resource of basics including fundamentals of physiology, biochemistry, logics, and so on, and (2) the resource of consistent experimental data. The resource of basics would be built initially, for instance, by consulting various literatures on physiology, biochemistry, or logics, and then constantly refined or updated by incorporating new findings. And the resource of consistent experimental data would be built by collecting only the experimental data that comply well with the basics. An important point is that the two parts would serve as a kind of guidelines for checking each other. For instance, any experimental data or results that are contrary to the basics would not be allowed into the resource of consistent experimental data. Similarly, any arguments or explanations that are not supported by the consistent experiment data would not be allowed into the resource of basics. As a consequence, the two parts of the integrated pool of resources would tend to consolidate each other so that both eventually constitute a consistent whole.



 doi:10.20944/preprints201909.0247.v3

Figure 2. Integrated approach is based on integrated pool of resources, consisting of the resource of basics and the resource of consistent experimental data.

A prevailing trend in the current medicinal or nutritional researches may be that they rely too much on experiments, largely ignoring the fundamentals of physiology, biochemistry, logics, and so on. A serious limitation with experimental studies may be that they are more likely to focus mainly on finding associations among physiologic or metabolic variables. However, association alone would not tell much about causality. This may be why largely experimental studies often fall into the fallacy of assuming association as causality or confusing cause and effect. Moreover, the results or conclusions based on experiments may not be very reliable simply because they are usually based on rather limited amounts of data obtained by a rather small number of experiments often conducted with rather narrow perspectives or mindsets familiar with particular disciplines of medicine or nutrition.

In contrast, the results or conclusions based on integrated approach would be much more reliable simply because they are supported not only by the resources of basics but also by the resource of consistent experimental data obtained from huge numbers of studies done across a wide range of disciplines. Importantly, more reliable results or conclusions based on the integrated approach may also serve as guidelines in rectifying many cause-effect confusions or misconceptions often encountered in experimental studies.

Lastly, it is believed that modern researchers are in a much better position to exploit the integrated approach considering that they have volumes of excellent textbooks on biology, biochemistry, physiology, and logics as well as virtually unlimited amounts of experimental data readily available with just a few keywords on the keyboard.

3. Tissue-Specific IRs

Metabolic disturbances due to tissue-specific IRs may easily be deduced by studying the tissue-specific roles of insulin and then verified by comparing with the results obtained from some genetically-engineered tissue-specific IR models, like MIRKO (muscle-tissue specific insulin receptor knock-out)(3), FIRKO (fat-tissue specific insulin receptor knock-out)(5), and LIRKO (liver-specific insulin receptor knock-out) mice(4). Obviously, the MIRKO, FIRKO, and LIRKO mice would be unable to initiate insulin signaling in the muscle tissue, adipose tissue, and liver, respectively, and therefore exhibit MIR, AIR, and HIR, respectively. Key aspects of tissue-specific IRs are summarized in Table 1. It is noted that metabolic disturbances due to tissue-specific IRs vary widely from tissue to tissue, refuting the common notion that IR impairs merely glucose uptake in the whole body.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 14 February 2023 doi:10.20944/preprints201909.0247.v3

Table 1. Key aspects of tissue-specific IRs.

MIR	● Disruption of insulin signaling in the muscle tissue ● Impairment of insulin-mediated muscle glucose uptake and muscle glycogenesis ■ PPG elevated significantly ■ PI (PPI and FPI) elevated significantly (as long as insulin secretory response is still intact) ● However, muscle glucose uptake mediated by muscle contraction or exercise is largely spared from MIR
s-AIR	● Disruption of insulin signaling in the subcutaneous adipose tissue ● Impairment of the subcutaneous adipose glucose uptake: ■ PPG elevated significantly ■ PI (PPI and FPI) elevated significantly (as long as insulin secretory response is still intact) ● Impairment of s-ADNL and subcutaneous fat accumulation in the fed state ● Subcutaneous adipose tissue undergoes uninhibited lipolysis and fails to entrap the FAs released from TRLs ■ Subcutaneous adipose tissue losing fat mass rapidly
v-AIR	● Disruption of insulin signaling in the visceral adipose tissue ● Impairment of visceral adipose glucose uptake: ■ PPG elevated significantly ■ PI (PPI and FPI) elevated significantly (as long as insulin secretory response is still intact) ● Impairment of v-ADNL and visceral fat accumulation in the fed state ● Visceral adipose tissue undergoes uninhibited lipolysis and fails to entrap the FAs released from TRLs ■ Visceral adipose tissue losing fat mass rapidly
HIR	● Disruption of insulin signaling in the liver ● Impairment of hepatic glucose uptake and hepatic glycogenesis: ■ PPG elevated significantly ■ PI (PPI and FPI) elevated significantly (only if insulin secretory response is still intact) ● Impairment of HDNL and VLDL generation in the fed state ● Liver undergoing unsuppressed HGP: ■ PPG elevated even more ■ FPG elevated severely as well: fasting hyperglycemia

MIR: MIR or disruption of insulin signaling in muscle tissues as observed in the MIRKO mice(3) would impair muscle glucose uptake, thereby significantly restricting muscle glucose oxidation as well as muscle glycogenesis. Consequently, postprandial plasma glucose (PPG) is elevated significantly, which in turn enhances postprandial insulin secretion significantly and subsequently leads to significantly elevated postprandial plasma insulin (PPI).

Surprisingly, however, the hyperglycemia observed in the MIR-exhibiting MIRKO mice is usually mild(3), especially considering that muscle tissues are usually responsible for more than about 70 % of glucose disposal in the whole body. This would serve as a strong evidence that muscle tissues have other glucose uptake pathway that is mediated, independently of insulin, by muscle contraction or exercise(17) and thus largely spared from MIR(18, 19).

AIR: AIR or disruption of insulin signaling in adipose tissues as observed in the FIRKO mice(5) would not only impair adipose glucose uptake – thereby elevating PPG

 doi:10.20944/preprints201909.0247.v3

and PPI significantly – but also disrupt the adipose metabolism seriously. In order to understand properly how AIR disrupts adipose metabolism, it would be essential to understand first the healthy adipose metabolism.

First, in the fed state regulated by significantly elevated PPI and PPG, healthy adipose tissues would actively take up the glucose from the plasma and then mostly convert – via adipose de novo lipogenesis (ADNL) pathway – into fatty acids (FAs)(20, 21). The amount of glucose oxidized in the adipose tissues, which are specilaized for storing energy, would usually be minimal compared to that oxidized in the muscle tissues, which usually needs a greate amount of energy for doing mechanical work. Healthy adipose tissues in the fed state would also entrap the FAs released – with the help of lipoprotein lipase (LPL) – from triglyceride-rich lipoproteins (TRLs), such as very low-density lipoproteins (VLDLs) and chylomicrons(22). The FAs either synthesized inside or fluxed from the plasma in the fed state are mostly esterified, with the help of elevated PI, into fats for storage.

Next, in the fasted state regulated by relatively low fasting plasma insulin (FPI), healthy adipose tissues would undergo lipolysis – with the help of adipose lipases, such as hormone sensitive lipase (HSL) and adipose triglyceride lipase (ATGL), that are not inhibited as strongly as in the fed state(23) – and then release the resultant FAs into the plasma, thereby elevating the fasting plasma fatty acid (FPFA) significantly. The FPFAs abundant in the plasma would then diffuse into other tissues or organs to be subsequently utilized as the substrate for oxidation(24, 25). The healthy adipose tissues that actively accumulate fat in the fed state and then undergo lipolysis in the fed state to release FAs into the plasma may better be regarded not merely as a fat storage depot but as a kind of a fatty acid (FA) reservoir.

As expected, however, AIR or disruption of adipose insulin signaling would prevent the adipose tissues from working properly as a FA reservoir. In more detail, insulin would no longer be able to inhibit adipose lipases strongly enough. Thus, the adipose tissues affected by AIR would not only undergo uninhibited lipolysis to constantly release FAs into the plasma, regardless of PI level, but also fail to entrap the FAs released from TRLs. Consequently, plasma fatty acid (PFA) would be elevated severely in the fed state as well as in the fasted state. The excess FAs either shunned by or additionally released from the AIR-affected adipose tissues would easily diffuse into other tissues or organs, notably muscle tissues and liver – a fraction of which would subsequently be esterified into ectopic fats(26). It would be quite revealing that the A-ZIP/F-1 transgenic mice, which are genetically engineered to lack white adipose tissues to work as a FA reservoir, also exhibit significant amounts of ectopic fats(27), exactly like the FIRKO mice.

In principle, PG and PFA would compete with each other to be used as the substrate for oxidation – Randle cycle or glucose-FA cycle(28, 29). This means that the severe elevation of PFA due to AIR would inevitably elevate PG severely as well. A noteworthy point is that PG appears to be inherently at a significant disadvantage in competing with PFAs. In more detail, glucose molecule is polar or hydrophilic and therefore its transport across the plasma membrane of hydrophobic lipid bilayer would require not only insulin mediation but also special plasma membrane transporters, like GLUT4 and GLUT2(30). By contrast, FAs that are nonpolar or hydrophobic would diffuse rather freely across the plasma membrane(24, 25). This may explain why PFA is preferentially utilized over PG in diabetics, in which both PG and PFA are elevated severely(31, 32). The reasoning that AIR inevitably elevates PG as well as PFA suggests conversely that healthy AIR-free adipose tissues also play essential roles for glucose homeostasis(32).

Another important point is that the AIR-associated uninhibited adipose lipolysis would inevitably lead to rapid depletion of the adipose fat mass or rapid weight loss. Conversely, this suggests only healthy, AIR-free adipose tissues are able to support weight gaining or obesity. This in turn suggests that weight gaining or obesity is an indication that at least one or both of the visceral and subcutaneous adipose tissue are working efficiently as a FA reservoir. In this sense, it would hardly be surprising that treatment of

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 14 February 2023 doi:10.20944/preprints201909.0247.v3

diabetes by anti-diabetic medications (ADMs), such as insulin or oral hypoglycemic agents (OHAs), often leads to weight gaining(12).

A noteworthy point in the Table 1 is that AIR is subdivided into the subcutaneous adipose insulin resistance (s-AIR) and visceral adipose insulin resistance (v-AIR), simply because we have postulated that the subcutaneous adipose tissue develop AIR earlier than the visceral adipose tissue.

HIR: HIR or disruption of hepatic insulin signaling as observed in the LIRKO mice(4) would not only impair hepatic glucose uptake – thereby elevating PPG and, as long as pancreatic insulin secretary response is still intact, PPI as well significantly – but also disrupt the hepatic metabolism seriously. Most importantly, insulin would fail not only to promote both the hepatic glycogenesis and the hepatic de novo lipogenesis (HDNL) but also to suppress hepatic glucose production (HGP). The unsuppressed HGP, regardless of PI level, would elevate PG in the fasted state as well as in fed state(33). Thus, fasting hyperglycemia may be considered a hallmark of HIR.

An evidence that HIR indeed causes fasting hyperglycemia may be found in the so-called dawn phenomenon regularly observed in diabetics treated by ADMs(34). In diabetics undergoing treatment by ADMs, the PI would wane gradually overnight, which lets HGP be elevated steadily until the next administration of ADMs usually early in the morning. On the other hand, exercise-mediated glucose uptake into skeletal muscles would usually be minimal during sleep or rest. The combination of the maximal HGP and minimal exercise-mediated skeletal muscle glucose uptake in the early morning hours would inevitably lead to severe elevation of PG in those hours – dawn phenomenon(35).

4. Metabolic Evolution in Four Stages

As schematically depicted in Fig 1, WBIB would evolve in four distinctively insulin-resistant stages denoted by IR-I, IR-II, IR-III, and IR-IV, respectively, as tissue-specific IRs develop in the order of MIR, s-AIR, v-AIR, and HIR. The net metabolism observed in each stage can be estimated by cumulatively adding up – as by referring to the Table 1 – all the metabolic disturbances resulting from the tissue-specific IRs developed thus far in that particular stage. Table 2 summarizes some important aspects of the metabolic disturbances to be observed in each stage.

Table 2. Important aspects of metabolic disturbances to be observed in each of WBIR evolution stages.

IS	● Glucose uptake not impaired in the whole body ● PPG elevated only modestly with regular postprandial glycemic load ● Postprandial pancreatic insulin secretion modest: ■Relatively low PPI and FPI ■s-ADNL, v-ADNL, and HDNL activated only modestly in the fed state ■s-lipolysis, v-lipolysis, and HGP inhibited only modestly in the fasted state ● FPFA and FPG (fasting energy substrates) relatively high: ■Intense physical activity accommodated relatively easily ■Feeling of hunger modest in the fasted state, rarely leading to overeating ● Rarely overweight or obese

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 14 February 2023 doi:10.20944/preprints201909.0247.v3

IR-I	● Glucose uptake into the muscle tissue impaired: ■Excess PG diverted to the subcutaneous and visceral adipose tissues and liver ● PPG, PPI, and FPI elevated more than in the IS stage: ■s-ADNL, v-ADNL, and HDNL enhanced more than in the IS stage ■HGP suppressed more than in the IS stage ■s-lipolysis and v-lipolysis inhibited more than in the IS stage ● FPFA and FPG (fasting energy substrates) lower than in the IS stage: ■Intense physical activity accommodated rarely ■Feeling of hunger acute in the fasted state, likely leading to overeating ● Gaining weight rapidly: ■Both the subcutaneous and visceral adipose tissue increasing fat mass rapidly
IR-II	● Glucose uptake into the muscle tissue and subcutaneous adipose tissue impaired: ■Excess PG diverted to the visceral adipose tissue and liver ● PPG, PPI, and FPI elevated more than in the IR-I stage: ■v-ADNL and HDNL enhanced more than in the IR-I stage ■HGP suppressed more than in the IR-I stage ■s-lipolysis starting to be uninhibited ■v-lipolysis inhibited more than in the IR-I stage ■FPG lower than in the IR-I stage ■FPFA starting to rise gradually ● Visceral obesity: ■Subcutaneous adipose tissue starting to lose fat mass ■Visceral adipose tissue increasing fat mass more rapidly
IR-III	● Glucose uptake into the muscle tissue, subcutaneous and visceral adipose tissue impaired: ■Excess PG diverted to the liver ● PPG elevated more than in the IR-II stage: ■Pancreatic insulin secretory response possibly starting to be impaired near the end of this stage ■HDNL enhanced more than in the IR-II stage ■HGP suppressed more than in the IR-II stage ■s-lipolysis uninhibited more than in the IR-II stage ■v-lipolysis starting to be inhibited ■FPFA elevated severely ■FPG elevated significantly as well (glucose-FA cycle: elevated PFA restricts PG utilization) ■Ectopic fat deposition enhanced in the muscle tissue and liver ● Starting to lose weight rapidly: ● Likely to be diagnosed of diabetes near the end of this stage

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 14 February 2023 doi:10.20944/preprints201909.0247.v3

IR-IV	● Glucose uptake into the muscle tissue, visceral and subcutaneous adipose tissue, and liver impaired ● PPG elevated more than in the IR-III stage ● Pancreatic insulin secretory response impaired severely: ■PPI and FPI dropped severely ● HGP unsuppressed: ■FPG as well as PPG elevated severely ● s-lipolysis and v-lipolysis uninhibited, but not sustainable with the fat mass largely depleted ■FPFA starting to drop below PPFA ■Weight loss no longer sustained ● Full-blown diabetes: ■dawn phenomenon, when treated with ADMs

With WBIR evolving, various metabolic variables would also evolve in their own unique patterns. Table 3 describes how some important metabolic variables evolve with WBIR evolving.

Table 3. General trend of WBIR-dependent variation of some important metabolic variables.

Stages	IS	IR-I	IR-II	IR-III	IR-IV
IRs developed	None	MIR	MIR, s-AIR	MIR, s-AIR, v-AIR	MIR, s-AIR, v-AIR, HIR
PPG	PPG_o	Rises steadily		Rises steeply	Rises more steeply
Insulin Sec.	Moderate	Increases steadily			Saturates then decreases
PPI	PPI_o	Rises steadily			Saturates then declines
FPI	FPI_o	Rises steadily			Saturates then declines
s-ADNL	Moderate	Increases	Decreases gradually		
v-ADNL	Moderate	Increases steadily		Decreases gradually	
PPFA	$PPFA_o$	Declines	Rises mod-estly	Rises steeply	
HDNL	Moderate	Increases steadily			Decrease gradually
FPTG	$FPTG_o$	Rises steadily		Rises steeply	Saturates gradually
HGP	Moderate	Decreases gradually			Increases steeply
FPG	FPG_o	Declines gradually		Rises gradually	Rises steeply
HbA1c	$HbA1c_o$	Rises steadily		Rises steeply	Rises more steeply
s-Lipolysis	Moderate	Decreases	Increases	Saturates	Decreases gradually
v-Lipolysis	Moderate	Decreases		Increases	Saturates then de-creases
FPFA	$FPFA_o$	Declines	Rises mod-estly	Rises steeply	Saturates then de-clines
Body Weight	Healthy lean	Increases rapidly	Saturates slowly	Decreases rapidly	Decreases slowly
Diabetes	Nondi-abetic	Prediabetic			Diabetic

 doi:10.20944/preprints201909.0247.v3

The postprandial plasma glucose (PPG) rises steadily, until the visceral adipose tissue as well as the subcutaneous adipose develops AIR in the IR-III stage and undergoes uninhibited lipolysis elevating plasma fatty acid (PFA) severely and thereby restricting plasma glucose (PG) utilization severely, and therefore it rises steeply thereafter, until the liver develops HIR in the IR-IV stage undergoing unsuppressed hepatic glucose production (HGP), and therefore it rises even more steeply thereafter; The postprandial plasma insulin (PPI) rises steadily, until pancreatic insulin secretion possibly starts to be impaired at near the end of IR-III stage, and therefore it saturates and then decreases gradually thereafter; The fasting plasma insulin (FPI) behaves similarly as PPI, but with a level attenuated significantly from that of PPI; The subcutaneous adipose de novo lipogenesis (s-ADNL) increases steadily, until the subcutaneous adipose tissue develops AIR in the IR-II stage, and therefore it decreases gradually thereafter; The visceral adipose de novo lipogenesis (v-ADNL) increases steadily, until the visceral adipose tissue develops AIR in the IR-III stage, and therefore it decreases gradually thereafter; The postprandial plasma fatty acid (PPFA) declines, until the subcutaneous adipose tissue develops AIR in the IR-II stage undergoing uninhibited lipolysis, and therefore it rises modestly thereafter, until the visceral adipose tissue also develops AIR in the IR-III stage undergoing uninhibited lipolysis, and therefore it rises steeply thereafter; The hepatic adipose de novo lipogenesis (HDNL) increases steadily, until the liver develops HIR in the IR-IV stage, and therefore it decreases gradually thereafter; The fasting plasma triglyceride (FPTG) rises steadily, until the visceral adipose tissue, in addition to the subcutaneous adipose tissue, develops AIR in the IR-III stage and fails to entrap PFAs thereby hampering VLDL delipidation, and therefore it rises steeply thereafter, until the liver develops HIR in the IR-IV stage thereby decreasing HDNL and VLDL generation and therefore it starts to saturate thereafter; The HGP decreases gradually, until the liver develops HIR in the IR-IV stage, and therefore it increases steeply thereafter; The FPG declines gradually, until the visceral adipose tissue, in addition to the subcutaneous adipose tissue, develops AIR in the IR-III stage undergoing uninhibited lipolysis elevating PFA severely and thereby restricting PG utilization severely, and therefore it rises gradually thereafter, until the liver undergoes unsuppressed HGP in the IR-IV stage, and therefore it rises steeply thereafter; The HbA1c level rises steadily, until the visceral adipose tissue as well as the subcutaneous adipose tissue develops AIR in the IR-III stage and undergoes uninhibited lipolysis elevating PFA severely and thereby restricting PG utilization severely, and therefore it rises steeply thereafter, until the liver develops HIR in the IR-IV stage undergoing unsuppressed HGP, and therefore it rises even more steeply thereafter; The s-lipolysis decreases gradually, until the subcutaneous adipose tissue develops s-AIR in the IR-II stage, and therefore it increases gradually thereafter, until the subcutaneous fat mass is largely depleted, and therefore it saturates then decreases thereafter; The v-lipolysis decreases gradually, until the visceral adipose tissue develops AIR in the IR-III stage, and therefore it increases gradually thereafter, until the visceral fat mass is largely depleted, and therefore it saturates and then decreases thereafter; The FPFA declines gradually, until the subcutaneous adipose tissue develops AIR in the IR-II stage undergoing inhibited lipolysis, and therefore it rises modestly thereafter, until even the visceral adipose develops AIR in the IR-III stage undergoing inhibited lipolysis, and therefore it increases steeply thereafter, until the visceral adipose fat mass is also largely depleted, and therefore it saturates and then decreases thereafter; The body weight increases rapidly in the IR-I stage, and then saturates slowly in the IR-II stage, and then decreases rapidly in the IR-III stage, and then decrease slowly in the IR-IV stage; Lastly, typical values for PPG, PPI, FPI, PPFA, FPTG, FPG, HbA1c, and FPFA in the IS stage may be referred to as reference values and denoted by PPG_o, PPI_o, FPI_o, $PPFA_o$, $FPTG_o$, FPG_o, $HbA1c_o$, and $FPFA_o$, respectively.

Actual measurement of WBIR-dependent evolution of metabolic variables would be extremely difficult, simply because WBIR evolution would generally proceed very slowly over years or even decades. Moreover, detailed aspects of WBIR evolution would vary widely among individuals. Thus, WBIR-dependent metabolic evolution profiles may better be drawn heuristically, for instance, by following the descriptions in Table 3. Important among them are the evolution profiles for PI, body weight, PFA, and PG, as depicted in Fig 3. It is noted that heuristically drawn, rule of thumb profiles would be good enough as long as they provide general idea of how metabolic variables evolve from one stage to the next stage.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 14 February 2023 doi:10.20944/preprints201909.0247.v3

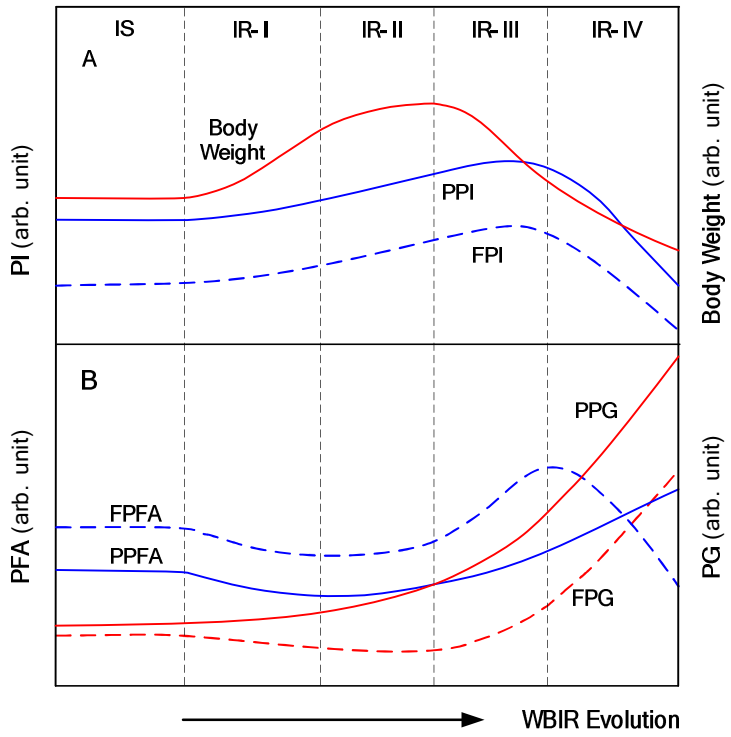


Figure 3. Heuristically drawn WBIR-dependent evolution profiles for PI and body weight (A), and for PG and PFA (B).

Insulin is the major hormone that regulates the energy metabolism. This means that the most important determinant of the metabolism would be none other than PI. As long as pancreatic insulin secretory response is largely intact as in the early stages of WBIR evolution, postprandial insulin secretion would rise proportionally to the postprandial plasma glucose (PPG) that would, in fact, rise steadily with WBIR evolving. Thus, the post prandial insulin (PPI) would also rise steadily until the pancreatic insulin secretory response may possibly start to be impaired at near the end of the IR-III stage. Given that PI concentration usually wanes gradually with metabolism progressing, the fasting plasma insulin (FPI) would be attenuated significantly from PPI, but both would evolve in similar patterns, as depicted in Fig 3(A).

Insulin plays a pro-anabolic role in the fed state(2) and then an anti-lipolytic role in the fasted state(36). This concept may be useful to understand how the body weight is affected by the WBIR-dependent evolution of PI. The MIR in the IR-I stage would not only elevate the PI (both PPI and FPI) but also effectively redistribute some of the excess PG shunned from the MIR-affected muscle tissue to the adipose tissues(37, 38). The elevated PPI would enhance, in the fed state, adipose fat accumulation by upregulating ADNL as well as entrapping more of the FAs released from TRLs in both the subcutaneous and visceral adipose tissues (pro-anabolic), whereas the elevated FPI would more or less restrict, in the fasted state, adipose lipolysis or fat mobilization (anti-lipolytic). Consequently, body weight would increase very rapidly in the IR-I stage. In the IR-II stage, the s-AIR, in addition to the MIR that would now have been matured further, would not only elevate PI further but also effectively redistribute the excess PG – shunned from the subcutaneous adipose tissue as well as the muscle tissue – to the visceral adipose tissue. Consequently, the still AIR-free visceral adipose tissue would build up the fat mass even more rapidly while the AIR-affected subcutaneous tissue would start to lose the fat mass by undergoing uninhibited lipolysis, which would lead to nothing but the visceral obesity. In the IR-III stage, even the visceral adipose tissue would develop AIR and start to undergo uninhibited lipolysis, which consequently leads to rapid weight loss. In the IR-IV stage, both the subcutaneous and visceral adipose tissue would continue to lose the fat mass until it is to be largely depleted, which would inevitably slow the pace of weight loss

 doi:10.20944/preprints201909.0247.v3

significantly. Overall, the body weight would evolve approximately in a kind of an inverted-U-shape as depicted in Fig 3(A): it would increase rapidly in the IR-I stage, then saturate slowly in the IR-II stage, then decrease rapidly in the IR-III stage, and then decrease slowly in the IR-IV stage(9).

A noteworthy point in the body weight evolution profile in Fig 3(A) is that the individuals belonging to the IR-I or IR-II stages – likely overweight or obese – would be relatively healthy metabolically simply because at least one or both of the subcutaneous and visceral adipose tissues work properly as a FA reservoir, thereby preventing PG as well as PFA from being elevated too high. By contrast, the individuals belonging to the IR-III or IR-IV stages – likely relatively lean – would be rather unhealthy simply because both the adipose tissues fail to work effectively as a FA reservoir and therefore PG as well as PFA elevates severely. This would explain why some lean individuals have higher mortality than overweight or obese individuals – obesity paradox(13).

In fact, Lee et al. conducted a prospective cohort study, in which all-cause mortality had been traced during a follow-up period of 10.5 years, for subgroups of normoglycemia, impaired fasting glucose, newly diagnosed diabetes, and prevalent diabetes, classified at the baseline(39). And they found that the all-cause mortality varies, regardless of the baseline characteristics, approximately in a U-shape with BMI. More specifically, mortality increased much more steeply with BMI decreasing below the BMI having minimum mortality than it increased with BMI increasing above the BMI having minimum mortality. Conversely, the mortality variation in a U-shape with BMI may support our assertion that the body weight evolves approximately in an inverted-U shape with WBIR evolving.

WBIR-dependent, long term variation of PI would also affect directly the evolution of plasma fatty acid (PFA). In principle, PFA concentration would depend on the intensity of adipose lipolysis and delipidation of triglyceride-rich lipoproteins (TRLs). In the fed state, the relatively high PPI would inhibit strongly the adipose lipases so that the FA concentration inside the adipocytes drops very low. Consequently, in the fed state, the PFAs would diffuse easily into adipocytes, thereby lowering the postprandial plasma fatty acid (PPFA) significantly. In the fasted state, however, the relatively low FPI would inhibit the adipose lipases less strongly so that the adipose tissues undergo lipolysis and release the resultant FAs into the plasma, thereby elevating the fasting plasma fatty acid (FPFA) significantly. This explains why the PPFA is usually lower than the FPFA, as depicted in the Fig 3(B).

An important point here is that the PI is higher in the IR-I stage than in the IS stage, which translates into that the adipose lipases are inhibited more strongly in the IR-I stage than in the IS stage, which in turn translates into that PFA is actually lower in the IR-I stage than in the IS stage, as depicted in Fig 3(B). This also explains why insulin-sensitive healthy individuals, including most children, exhibit higher FPA than somewhat insulin-resistant individuals (as in the IR-I or IR-II stage) who are more likely to be overweight or obese(40, 41). In the IR-II stage, the subcutaneous adipose tissue starts to develop AIR, not only releasing FAs into the plasma by undergoing uninhibited lipolysis but also failing to entrap the FAs released from TRLs. However, the still largely AIR-free visceral adipose tissue would entrap efficiently, especially with the help of significantly elevated PI, most of the FAs shunned or released from the AIR-affected subcutaneous adipose tissue. Consequently, PFA elevation in the IR-II stage would be rather modest, as depicted in the Fig 3(B). In the IR-III stage, however, both the subcutaneous and visceral adipose tissues are affected by AIR, and therefore they not only release FAs into the plasma but also fail to entrap the FAs released from TRLs, thereby elevating the PFA severely. Importantly, the severe elevation of PFA in the IR-III stage will inevitably restrict utilization of PG as the competing energy substrate, which elevates PG significantly as well. In the IR-IV stage, both the subcutaneous and visceral adipose tissues would no longer be able to support lipolysis as efficiently as in the IR-III stage with the fat mass largely depleted already. Consequently, FPFA in the IR-IV stage would start to saturate and then decline gradually with WBIR evolving, whereas PPFA would elevate steadily as both the adipose tissues are failing ever more to entrap the FAs released from TRLs. This would explain why both the

 doi:10.20944/preprints201909.0247.v3

FIRKO and A-ZIP/F-1 mice, lacking adipose tissues working effectively working as the FA reservoir, exhibit PPFA actually higher than FPFA(5, 27).

WBIR-dependent, long term variation of PI would also affect directly the evolution of PG, but quite differently for the postprandial plasma glucose (PPG) and fasting plasma glucose (FPG). In principle, PG would consist of two distinct components: the exogenous PG attributed to ingested carbohydrates and the endogenous PG attributed to hepatic glucose production (HGP). As long as the pancreatic insulin secretory response is largely intact as in the early stages of WBIR evolution, HGP in the fed state would be suppressed enough by relatively high PPI. This implies that PG in the fed state would consist of mostly exogenous PG, especially in the early stages of WBIR evolution. This in turn implies that PPG would rise steadily with impairment of glucose uptake being enhanced ever more or simply with WBIR evolving, as depicted in Fig 3(B).

The exogenous PG that constitutes the major component of the PPG would be mostly disposed of within the fed state by the tissues still largely IR-free, especially with the help of significantly elevated PPI. This means that in the early stage of WBIR evolution FPG would be determined primarily by HGP, which is regulated primarily by FPI – the higher FPI, the more suppressed he HGP, the more lowering FPG. As long as the pancreatic insulin secretory response is largely intact as in the early stages of WBIR evolution, higher PPG would lead to higher PPI, which in turn transition to higher FPI. This line of reasoning would then be reduced to: in the case of the early stages of WBIR evolution, FPG would decline with PPG rising, as depicted in Fig 3(B). However, the negative correlation between FPG and PPG would eventually turn positive in the IR-III and IR-IV stage as the severely elevated PFAs restrict PG utilization or HIR makes the liver undergo unsuppressed HGP, thereby elevating severely FPG as well as PPG.

In fact, Hulman et al. traced the PG evolution up to diagnosis of type 2 diabetes in the Whitehall prospective cohort study(42). We reproduced in Fig 4 the evolution of 2hPG (2-hour plasma glucose) measured by oral glucose tolerance test (OGTT) and FPG for incident diabetics who had been diagnosed of diabetes during the follow-up from 1991 to 2013. The 2hPG, which could be considered to approximately represent the PPG, rose steadily with time before diagnosis of diabetes, whereas the FPG initially declined with time and then rose steeply as it becomes closer to the diagnosis. It is also noteworthy that, far from the diagnosis, the South Asian subgroup with higher FPI than the white subgroup exhibited actually lower FPG. An important point here is that the horizontal axis of the time before diagnosis would not necessarily correspond to the WBIR evolution stages introduced in this study, considering that individuals would in general develop diabetes at widely different paces, depending on the concentration of potential IR-inducing agents. A good possibility may be that the farther way from the diagnosis, the more heterogeneous – in terms of WBIR evolution stages – the subjects.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 14 February 2023 doi:10.20944/preprints201909.0247.v3

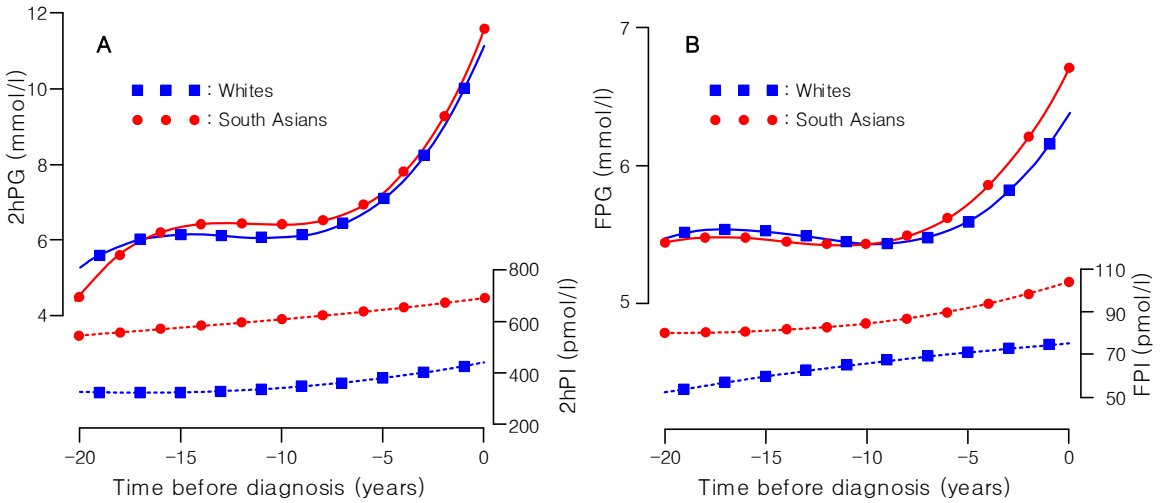


Figure 4. Variation of 2hPG and 2hPI (A), and FPG and FPI (B) before diagnosis of diabetes for the white subgroup and South Asian subgroup.

Actually earlier, Tabak et al. had also traced PG evolution with the same Whitehall cohort study(43). However, they divided the subjects into the subgroup of incident diabetics who had been diagnosed of diabetes during the follow-up and the subgroup who had remained nondiabetic, and plotted PG evolution against the time before the end of follow-up, rather than the time before the diagnosis. While the FPG in the nondiabetic subgroup remained almost constant throughout the follow-up, the FPG in the incident diabetics rose rather slowly in the region far from the end of follow-up and then started to rise steeply as it becomes closer to the end of follow-up. The positive slope of the FPG with time before the end of the follow-up – in contrast to the negative slope with time before diagnosis – may indicate that the subjects were much more heterogeneous (in terms of WBIR evolution stages) in the horizontal axis of the time before the end of follow-up than in the horizontal axis of the time before diagnosis.

Meanwhile, Yeni-Komshian et al. conducted an insulin suppression test with nondiabetic individuals (possibly belonging to the IR-I and IR-II stages), in which steady-state plasma glucose (SSPG) concentration is measured under continuous infusion of insulin and glucose(44). And they found that FPG was negatively correlated with the SSPG, which may also be considered to represent approximately PPG. This also confirms that the FPG indeed declines with PPG in those who are more likely in the early stages of WBIR evolution. Based on these reasonings, it may be said quite safely that the FPG tends to remain relatively low until very late in the course of diabetes development(45). An important point here is that diagnosis of diabetes based on FPG would be more likely to give false sense of security when, in fact, WBIR has already advanced significantly. In other words, the practice of diagnosing diabetes based on FPG may possibly prevent early diagnosis.

The most important point regarding the evolution of FPFA and FPG depicted in Fig 3(B) may be that both the FPFA and FPG (the primary and secondary fasting energy substrate, respectively) are actually lower in the weight gaining IR-I and IR-II stages than in the IS stage. The lack of the fasting energy substrates, FPFA and FPG, in the plasma would be more likely to enhance the hunger felt in the fasted state(46) – likely leading to overeating – as well as more likely to let individuals avoid intense physical activity. The likelihood of overeating and physical inactivity would easily lead to the positive energy balance that is essential for weight gaining.

The four-stage metabolic evolution from rapid weight gain to visceral obesity to rapid weight loss to full-blown diabetes appears to comply rather well with the natural development history of diabetes(1). This may conversely validate the four-stage WBIR evolution model. In reality, the validity of the four-stage WBIR evolution model would

 doi:10.20944/preprints201909.0247.v3

rest primarily on the validity of the postulation that tissue-specific IRs develop in the order of MIR, s-AIR, v-AIR, and HIR. A simple way to check its validity may be to assume a different order of development and then to compare the predicted metabolic outcomes with the more familiar observations. For instance, if AIR develop first – as is the case with the FIRKO mice – WBIR evolution would start with rapid weight loss, rather than rapid weight gain. And if HIR develop first – as is the case with the LIRKO mice – severe fasting hyperglycemia would be observed from the early stages of WBIR evolution. And if v-AIR precede s-AIR, visceral obesity would never be observed. And if all the MIR, s-AIR, v-AIR, and HIR develop simultaneously, both PG and PFA rise steadily with WBIR evolving, whereas body weight would decrease steadily. A noteworthy point is that, in type 1 diabetics with no or little insulin secretion, insulin action would be missing in the whole body simultaneously, which would make the metabolic disruptions due to MIR, s-AIR, v-AIR, and HIR occur almost simultaneously. This may explain why untreated type 1 diabetics become emaciated quickly, possibly without showing any sign of visceral obesity.

If the four-stage WBIR evolution model were indeed valid, there would exist some surrogate measures that represent the degree of WBIR evolution fairly well(44). A good candidate would be the PPG that rises steadily with WBIR evolving(45, 47, 48). On the other hand, the FPG would be an utterly poor surrogate measure simply because it declines with WBIR in the early stages of evolution. Neither the PPI nor FPI would be a good surrogate measure – they tend to saturate or decline in the later stages of WBIR evolution(44). The FPFA would not be a good surrogate measure, either – it dips low in the IR-I and IR-II stages and starts to saturate or decline with the adipose fat mass largely depleted. The fasting plasma triglyceride (FPTG) would not be a good surrogate measure, either – it saturates in the IR-IV stage. Body weight or BMI would be an utterly poor surrogate measure – it evolves approximately in an inverted-U-shape with WBIR evolution. Probably, the best surrogate measure may be the HbA1C, which is known to reflect the time-averaged PG in the preceding 10-12 weeks and thus is considered to take into account the effect of both the fasting glycemia and postprandial glycemia(48).

5. Debunking of the lipid-induced insulin resistance (LIIR)

Even though the four-stage WBIR evolution model appears to be largely validated, it does not tell anything about IR-inducing agents or mechanisms. A widespread belief is that IR is induced by lipids. A core argument of the so-called lipid-induced insulin resistance (LIIR) is that obesity or the adipose fat mass induces IR either directly or indirectly by contributing excess FAs to the plasma so that the resultant elevated PFAs somehow interfere the insulin action in cells or tissues(11). In reality, however, elevation of PFA has an effect of effectively elevating PG – Randle cycle or glucose-FA cycle(28, 29) – lending support to LIIR.

In fact, Karpe et al. extensively reviewed literatures only to find that the correlation between the body weight and PFA is not very straightforward(11). For instance, some obese or overweight individuals exhibit PFA actually lower than the average, whereas lean individuals exhibit a wide range of PFA (from below to well above the average), which may be considered, in a sense, to validate the heuristically drawn PFA evolution depicted in Fig 3(B). Thus, the belief that obesity first elevates PFA and then induces IR would be more likely to be rather baseless.

On the other hand, if elevation of PFA indeed has a property to induce IR, insulin-sensitive individuals (as in the insulin-sensitive (IS) stage) who exhibit relatively high PFA and are more likely to be lean would develop IR more readily than already insulin-resistant individuals (as in the IR-I or IR-II stage) who exhibit relatively low PFA and are more likely to be overweight or obese. Certainly, this argument would also be utterly absurd.

A serious problem with LIIR is that it does not tell which tissue-specific IR is to be induced by obesity or lipids. From the perspective of WBIR evolution, however, MIR in the IR-I stage contributes to weight gaining or obesity – not the other way around – by not

 doi:10.20944/preprints201909.0247.v3

only elevating PI but also effectively redistributing some of the excess PG shunned from the MIR-affected muscle tissue to adipose tissues. On the other hand, the AIR, for instance, in the IR-III stage leads to rapid weight loss by letting adipose tissues undergo uninhibited lipolysis and simultaneously elevating PFA severely as well. That MIR contributes to weigh gaining whereas AIR leads to rapid weight loss can hardly be explained by LIIR.

Another branch of LIIR is the notion that ectopic fats induce IR. For instance, many researchers believe that HIR is attributed to the ectopic fats deposited in the liver(49, 50). However, basics of physiology tell that ectopic liver fat deposition appears to be more likely an effect of AIR in particular. In more detail, AIR elevates severely PFA by making the adipose tissues not only undergo uninhibited lipolysis but also unable to entrap the FAs released from TRLs. The severely elevated PFAs, shunned, in fact, by the AIR-affected adipose tissues, would easily diffuse also into the liver so that some of them are to be esterified into ectopic fat(26). A supporting evidence is that the FIRKO and A-ZIP/F-1 mice – both lacking the adipose tissue working properly as a FA reservoir and therefore exhibiting almost the same phenotype – also deposit significant amount of ectopic liver fats(5, 27).

Ectopic liver fats may also be contributed by enhancement of hepatic de novo lipogenesis (HDNL)(50). It is noted, however, that enhancement of HDNL may also be attributable to AIR. In more detail, AIR elevates not only PFA but also PG, thereby significantly enhancing hepatic glucose uptake and subsequently HDNL – only if the hepatic insulin signaling is still largely intact or the liver is not yet affected by HIR. This implies that enhancement of HDNL is, in a sense, an indication that liver is still responding properly to insulin or largely free from HIR. The enhancement of HDNL would inevitably lead to enhancement of VLDL generation and secretion to the plasma. However, the severely elevated PFAs due to AIR would hamper VLDL delipidation(22), which would in turn hamper VLDL secretion to the plasma, which would certainly contribute to ectopic liver fat deposition. Based on this reasoning, increased ectopic liver fat deposition, due to either enhancement of HDNL or increased hepatic influx of FAs, would seem to have more to do with AIR, rather than HIR. A noteworthy point is that a hallmark of HIR is, in fact, unsuppressed HGP or fasting hyperglycemia, rather than ectopic liver fat deposition.

A serious problem with the notion that ectopic fats induce IR is that even healthy individuals, who are more likely to be free from IR in general, also accumulate a considerable amount of intramyocelluar lipid (IMCL)(51) – muscle ectopic fat – especially when they fast(52), engage in prolonged exercise(53), or rely on low-carbohydrate diet (LCD)(54). Fasting would lower the FPI significantly, which would in turn enhance adipose lipolysis significantly thereby significantly elevating PFA and increasing FA flux into muscle tissues, which would in turn inevitably increase IMCL deposition significantly. Prolonged exercise would also enhance adipose lipolysis significantly, possibly with the help of elevated epinephrine, thereby elevating PFA significantly and subsequently increasing IMCL deposition significantly. LCD would minimize the postprandial insulin secretion and thus lower FPI significantly, which would in turn enhance adipose lipolysis significantly, thereby elevating PFA significantly and subsequently increasing IMCL deposition significantly.

The finding that even healthy individuals accumulate a considerable amount of ectopic fats may suggest that ectopic fat deposition is, in reality, a kind of homeostatic response of the body to prevent severe elevation of PFA. Then, in order to achieve PFA homeostasis more efficiently, the ectopic fats deposited usually in the fasted state would be more likely to be oxidized as early as possible in the ensuing fed state, which would inevitably tend to delay glucose oxidation and thereby apparently elevate PG significantly especially in the postprandial period.

Kanamori et al. conducted an interesting experiment, in which healthy subjects were fed on high-carbohydrate diet (HCD) on day 1 (D1) and D2, and switched to low-carbohydrate diet (LCD) on D3, and switched back to the same HCD on D4(55). Interestingly, however, PPG was elevated much higher on D4 than on D2, even though the subjects ingested the same HCD on those two days. Proponents of LIIR may argue that the

 doi:10.20944/preprints201909.0247.v3

apparent impairment of glucose tolerance (or elevation of PPG) on D4 serves as evidence that the elevated PFA or increased ectopic fat deposition on D3 (due to LCD) induces IR.

However, given that IR develops usually over a long span of time, the apparent impairment of glucose tolerance after ingesting LCD for only a day can hardly be attributable to IR development. If IR were induced simply by a change of diet for only a day, it could be reversed easily by correcting diet for only a few days. Similarly, the apparent impairment of glucose tolerance after exercise or fasting can hardly be attributable to IR development, considering especially that fasting had once been prescribed as an important option for treating diabetes(56) and exercise is still widely practiced for managing diabetes.

6. Proposing of Glycation-induced insulin resistance (GIIR)

If the LIIR is hardly supportable – especially in the perspective of the four-stage WBIR evolution model – it would be essential to find an appropriate IR-inducing model or mechanism that is compatible to the WBIR evolution. A prominent aspect of WBIR evolution is that PPG rises ever faster with WBIR evolving. A speculation is that hyperglycemia may have an inherent property to induce or worsen IR. For instance, even type 1 diabetics, who have little or no insulin secretion and therefore are inevitably exposed to severe hyperglycemia, also develop severe IR(57, 58). The A-ZIP/F-1 mice, lacking adipose tissues working as a FA reservoir and therefore inevitably exposed to severe elevation of PG as well as PFA, eventually develop diabetes, but surgical implantation of wild-type adipose tissue in the transgenic mice not only normalizes PG but also reverses the diabetes(59). Moreover, most diabetes management or treatment plans, including exercise and administration of anti-diabetic medications (ADMs), make a point of lowering PG.

A reaction activated especially in hyperglycemic physiological condition is glycation or non-enzymatic glycosylation, in which glucose and its glycolytic derivatives, such as glyoxal and methylglyoxal, react non-enzymatically with proteins and form covalently bonded adducts(14, 15). If hyperglycemia were sustained long enough, the glycation adducts would rearrange themselves into nonreversible advanced glycation end-products (AGEs).

An important aspect of the glycation reaction is that glycation adducts are formed preferentially on the positively charged (of either polarized or ionic) constituents of proteins, such as N-terminus amino group and residues of lysine, arginine, and cysteine. No less importantly, glycation adducts formed on the positively charged constituents appear to effectively neutralize the positive charges(16). In this case, the attractive forces between the neutralized (originally positively charged) residues and the nearby negatively charged residues would disappear, which would inevitably unfold locally the polypeptide chain. Importantly, the proteins with both the charge distribution and conformation disrupted significantly can hardly function properly.

Another important aspect of the protein glycation is that cellular proteins in general are glycated more readily than extracellular proteins in the plasma or the interstitial fluid(60). Plasma membrane proteins on the surface of the cells in tissues, certainly a category of cellular proteins, may particularly be susceptible to glycation, simply because they would be exposed not only to glucose in the interstitial fluid but also to the very potent glycating agents, such as glyoxal and methylglyoxal that may be produced as glycolytic byproducts inside the cells (61).

An example of the plasma membrane proteins is the insulin receptor that mediates insulin action in the cell. If the insulin receptor protein is glycated enough so that its charge distribution and conformation are hardly compatible to those of insulin, insulin can hardly bind properly to glycated insulin receptors and thus fail to initiate insulin signaling. The failure of insulin to initiate insulin signaling, as is the case with the mice with insulin receptors knocked-out(3-5), as a result of glycation of the insulin receptor or other proteins directly involved in downstream insulin signaling would give rise to nothing but the glycation-induced insulin resistance (GIIR). Actually, the authors had proposed this IR-induction mechanism elsewhere(62).

 doi:10.20944/preprints201909.0247.v3

An important point is that glycation reaction is a process that proceeds rather slowly over a relatively long span of time, exactly like WBIR evolution, whereas the glucose-FA cycle proceeds rather quickly(51). Thus, the possibility for the glycation reaction to be involved in IR development or WBIR evolution is much higher than that for the glucose-FA cycle to be involved. Another revealing observation is that metformin – probably the most widely prescribed oral hypoglycemic agent (OHA) – is known to inhibit glycation process(63-65). Conversely, this may support that glycation reaction is indeed involved in IR development or WBIR evolution.

Importantly, GIIR would enhance hyperglycemia further by impairing glucose uptake, which would in turn enhance GIIR further, establishing a positive feedback loop in which hyperglycemia and GIIR reinforce each other in a vicious circle. This means PG is elevated ever faster with the vicious circle going. Meanwhile, the four-stage WBIR evolution model also predicts that PG or time-averaged PG rises ever faster with WBIR evolving. This parallel may suggest that GIIR is the very IR-inducing mechanism behind the WBIR evolution. An important point regarding the vicious circle may be that hyperglycemia is the cause as well as the effect of GIIR – GIIR is only the effect of hyperglycemia. This means that hyperglycemia is indeed the fundamental force driving the WBIR evolution or vicious circle. The four-stage WBIR evolution driven by hyperglycemia could be described as below.

Hyperglycemia, the fundamental driving force for WBIR evolution, can easily be achieved, at least in the postprandial period, even in the insulin-sensitive (IS) stage by increasing postprandial glycemic load or increasing carbohydrate intake. With postprandial hyperglycemia regularly achieved for a period long enough, for instance, by relying on high-carbohydrate diet (HCD), GIIR would start to develop first preferentially in the muscle tissue with a relatively low cell turnover, in which average cells would have been exposed longer to hyperglycemia. With the muscle tissue gradually developing IR (more specifically MIR), the IS stage would gradually transition to IR-I stage. Meanwhile, MIR in the IR-I stage would impair the muscle glucose uptake and therefore enhance postprandial hyperglycemia further, thereby accelerating WBIR evolution further. The hyperglycemia enhanced by MIR would eventually make even the subcutaneous adipose tissue start to develop gradually GIIR (more specifically s-AIR). With s-AIR developing gradually, the IR-I stage would gradually transition to the IR-II stage, in which MIR and s-AIR would enhance hyperglycemia further, thereby accelerating WBIR evolution further. Consequently, even the visceral adipose tissue would start to develop gradually GIIR (more specifically v-AIR). With v-AIR developing gradually, the IR-II stage would gradually transition to the IR-III stage, in which severe elevation of PFA would enhance hyperglycemia even more severely by restricting PG utilization. Consequently, even the liver with a very high cell turnover would start to develop gradually GIIR (more specifically HIR). With HIR developing gradually, the IR-III stage would gradually transition to IR-IV stage, in which HIR would make the liver undergo unsuppressed HGP, thereby enhancing hyperglycemia even more and accelerating WBIR evolution further.

An indisputable evidence for glycation reaction being active in hyperglycemia would be a disproportionately high plasma level of glycation free adducts – glycated amino acids or short peptides circulating freely in the plasma – observed in diabetics(66, 67). Glycation free adducts that are broken off the glycated proteins that are undergoing proteolysis may first enter the interstitial fluid that bathes the cells in tissues and then eventually be collected in the blood plasma. On the other hand, the glycation adduct residues that are still constituents of the working proteins in tissues or organs would not be easily detected. Nevertheless, we speculate, the glycation adduct residues may be extremely harmful or toxic pathologically simply because they could disable the host proteins in cells or tissues. By contrast, we speculate against the common belief, glycation free adducts may be considered only risk markers, rather than risk factors, at least because they would no longer interfere directly the function of the cells or tissues and at the same time they would be mostly excreted in urine(66, 67).

 doi:10.20944/preprints201909.0247.v3

A speculation is that the glycation reaction activated in hyperglycemia may possibly serve as the common thread connecting the various disorders related with diabetes or hyperglycemia. If MIR, s-AIR, v-AIR, and HIR were indeed due to glycation of the proteins involved in insulin action in the muscle tissue, subcutaneous adipose tissue, visceral adipose tissue, and liver, respectively, it would hardly be surprising for them to appear to be associated with each other and then with hyperglycemia. An important point here is that even though MIR, s-AIR, v-AIR, and HIR are associated with each other, they would not necessarily develop simultaneously.

If the proteins involved in insulin action in the muscle tissue, adipose tissue, and liver were glycated severely enough in hyperglycemia as to give rise to impairment of insulin action in the respective tissues or organs, some of the proteins involved in insulin generation or secretion in the pancreas could also be glycated severely enough as to give rise to impairment of insulin generation or secretion. A revealing observation is that chronic hyperglycemia is known to trigger loss of pancreatic beta cell differentiation, consequently impairing insulin generation or secretion(68). This may explain why impairment of pancreatic insulin secretory response is also associated with IR in general or hyperglycemia. By the same token, under sustained hyperglycemia, some critical proteins in still other tissues or organs, such as cardio/cerebral endothelia, nephrons, neurons, and retina can also be glycated severely so that they are deteriorated enough functionally or structurally as to make them exhibit particular symptoms associated with cardio/cerebral vasculopathy, nephropathy, neuropathy, and retinopathy, respectively. This may explain why a cluster of disorders or diseases that are often collectively referred to as the metabolic syndrome(69), such as obesity, diabetes, cardio/cerebral vasculopathy, nephropathy, neuropathy, and retinopathy appear to be associated with each other and then with hyperglycemia.

Perhaps one of the best-known examples of glycated proteins may be the glycated version of hemoglobin, often referred to as HbA1c, which is formed inside the red blood cells during the life span of three to four months(70). As well known, the level of HbA1c represents the time-averaged PG concentration in the preceding 10-12 weeks. Certainly, glycation reaction is behind the formation of glycated hemoglobin HbA1c, as it is insisted (in this study) to be behind the WBIR evolution. It would then hardly be incidental that the HbA1c level is one of the best surrogate measures of WBIR or diabetes evolution.

Lastly, the concept of GIIR can also be applicable to type 1 diabetes(57, 58), in which insulin secretory response is largely missing. In the case of untreated type 1 diabetics, metabolic disruptions due to MIR, s-AIR, v-AIR, and HIR would occur simultaneously – no insulin action in the whole body, exactly as in the IR-IV stage – thereby elevating PG severely and enhancing GIIR severely. This explains why delay of insulin injection therapy in type 1 diabetes would rapidly aggravate the disease, only to increase the dose of insulin required.

7. Conclusion and Discussion

We have developed four-stage WBIR or diabetes evolution model based on integrated approach that we have introduced for this study. The four-stage metabolic evolution from rapid weight gain to visceral obesity to rapid weight loss to full-blown diabetes complies well with the natural development history of diabetes, and resolves most of the controversies regarding obesity or diabetes, including visceral obesity, obesity paradox, and dawn phenomenon that have long remained metabolic puzzles. In addition, the model refutes the LIIR but supports, instead, the GIIR proposed in this study.

The single most important conclusion in this study is that hyperglycemia is the main driving force or primary risk factor for WBIR evolution. Hyperglycemia and GIIR reinforce each other in a vicious circle, consequently driving WBIR to evolve steadily forward and enhancing glycation reaction ever more. A speculation is that the glycation reaction activated in hyperglycemia may serve as the common thread connecting each other a cluster of diseases or disorders that are collectively referred to as the metabolic syndrome,

 doi:10.20944/preprints201909.0247.v3

such as obesity, diabetes, cardio/cerebral vasculopathy, nephropathy, neuropathy, and retinopathy. Glycation reaction activated ever more with WBIR evolving would deform ever more critical proteins in various organs or tissues, thereby letting ever more symptoms or disorders associated with hyperglycemia or diabetes be manifested with WBIR evolving. With WBIR being driven basically by hyperglycemia, the most obvious way to delay or reverse WBIR evolution would be to alleviate hyperglycemia, which is in fact the primary goal of diabetes management or treatment. And the most fundamental approach to alleviate hyperglycemia is to reduce postprandial glycemic load by curtailing carbohydrate intake.

A serious problem with current researches on obesity or diabetes may be that they largely ignore the basics of physiology or metabolism and therefore are more likely to focus mainly on finding associations among important metabolic variables. However, the association alone would not tell much about the causality and thus it often leads to the fallacy of assuming association as causality, thereby giving rise to many cause-effect confusions. For example, simple measurement in the IR-I stage of the association between weight gaining and IR – more accurately MIR – would easily let researchers claim that weight gaining induces IR. By the way, it would not be a simple task to measure tissue-specific IRs in general(71) – the gold standard techniques for measuring insulin sensitivity/resistance, such as hyperinsulinemic euglycemic clamp (HEC) and oral glucose tolerance test (OGTT), would measure only the average values in the whole body, rather than tissue-specific values. However, the association, for instance, between weight gaining and IR in the IR-I stage would not necessarily mean the former causes the latter or vice versa but more likely mean that the two have a common risk factor, i.e., hyperglycemia. More specifically, hyperglycemia in the IR-I stage induces MIR via glycation reaction. And hyperglycemia in the IR-I stage also causes weight gaining by inducing hyperinsulinemia and enhancing ADNL, as explained already. Certainly, metabolic variables caused by a common risk factor would inevitably appear to be associated with each other.

One of the most significant findings in this study may be that weight gaining or obesity has a beneficial effect of alleviating hyperglycemia, the main driving force for WBIR evolution. Once again in the IR-I stage, enhancement of ADNL in the postprandial hyperglycemia would not only contribute to weight gaining or obesity but also alleviate hyperglycemia simply because it is potentially the most important glucose disposal pathway in the IR-I stage – glucose disposal in the muscle tissue would be restricted significantly by MIR and the glucose disposal in the liver would be rather minimal given that the capacity to store glycogen in the liver is rather limited. Thus, weight gaining or obesity in the IR-I stage would have an effect of alleviating hyperglycemia. It would then be quite absurd to argue that weight gaining or obesity induces IR and simultaneously has an effect of alleviating hyperglycemia that is, in fact, the most prominent metabolic disturbance due to IR. A revealing observation is that even the visceral obesity is known to have protective effect(72).

On the other hand, in the IR-III or IR-IV stage already affected by AIR, the glucose disposal pathway, ADNL is significantly inhibited, thus barely alleviating hyperglycemia. In the meantime, AIR-associated uninhibited adipose lipolysis leads to rapid weight loss as well as elevates PFA severely, thereby effectively elevating PG as well – Randle cycle or glucose-FA cycle. This means that weight loss in the IR-III or IR-IV stage, in fact, has an effect of aggravating hyperglycemia. That weight gaining has an effect of alleviating hyperglycemia (thereby delaying WBIR evolution) whereas weight losing has an effect of aggravating hyperglycemia (thereby promoting WBIR evolution) would hardly support LIIIR.

Weight gaining or obesity that has an effect of alleviating hyperglycemia and thus delaying WBIR evolution can hardly be considered a risk factor per se. However, it would still hardly be beneficial overall for health. This suggests strongly that behind the weight gaining or obesity lies an independent risk factor, which is none other than the hyperglycemia. This in turn suggests that healthy weight loss can be achieved easily by alleviating hyperglycemia. For instance, the rapid weight gaining in the IR-I stage can be reversed by

 doi:10.20944/preprints201909.0247.v3

alleviating postprandial hyperglycemia or by reducing postprandial glycemic load. The alleviation of postprandial hyperglycemia would decrease postprandial insulin secretion, which subsequently alleviates hyperinsulinemia. The alleviation of hyperinsulinemia coupled with the alleviation of postprandial hyperglycemia would restrict significantly ADNL in the fed state but enhance more or less adipose lipolysis in the fasted state, thereby likely leading to weight loss. Moreover, enhancement of adipose lipolysis and HGP due to alleviation of hyperinsulinemia would respectively lead to significant elevation of FPFA and FPG, the fasting energy substrates. The relative abundance of the fasting energy substrate in the plasma would be more likely to relax the hunger felt in the fasted state(46, 73) – rarely leading to overeating – as well as let individuals relatively easily accommodate intense physical activity. The likelihood of moderate eating and intense physical activity would lead to negative energy balance essential for weight loss.

Alleviation of postprandial hyperglycemia in the IR-I stage for long enough to reduce the body weight to the level expected in the IS stage could also reverse WBIR from the IR-I stage to the IS stage. This would be in line with the previous argument that sustaining postprandial hyperglycemia for long enough in the IS stage, for instance, by relying on high-carbohydrate diet (HCD) would not only promote WBIR to the IR-I stage but also increase the body weight to the level expected in the IR-I stage. An important point here is that the weight loss achieved by alleviating hyperglycemia would be of a healthy kind since it would help to secure more of the storage capacity of the FA reservoir of adipose tissues. By contrast, the weight loss associated with AIR, as observed in the IR-III or IR-IV stage, is of a very unhealthy kind since it would shrink further the storage capacity of the FA reservoir as well as effectively enhance hyperglycemia further.

If weight loss could be either achieved healthily by alleviating hyperglycemia or incurred very unhealthily by being afflicted by AIR, body weight or body mass index (BMI) can hardly be a good surrogate measure of healthiness in general. This suggests that it would not be a good idea to plot health-related variables against BMI put in the horizontal axis, as can be seen so often in literatures. For instance, when we plot mortality against BMI put in the horizontal axis, the graph would in general have approximately a U-shape or J-shape(39, 74-77), which confirms nothing but the obesity paradox.

Notwithstanding the obesity paradox so commonly observed, however, many researchers may still firmly believe that weight gaining or obesity per se is an absolute risk factor, rather than a risk marker. This ill-founded belief could easily let some researches be biased in interpreting their experimental results. For instance, researchers, probably from the same institute, had made conflicting assessments of the AIR model of FIRKO mice in their studies reported in 2002, 2003, and 2016. In more detail, the 2002 study insisted that FIRKO mice were protected against obesity and obesity-related glucose intolerance(78), and the 2003 study insisted that the mice could extend longevity(79), whereas the 2016 study found that the FIRCO mice quickly develop diabetes as well as nonalcoholic fatty liver disease (NFLD) (5). According to our understanding, however, the AIR in the FIRKO mice is a very lethal disorder that elevates severely PG as well as PFA and eventually let the mice develop diabetes as well as NFLD. Thus, the assessment in the 2016 study is much closer to the truth, which is basically why we referred only the 2016 study and, at the same time, we could not allow the arguments made in the 2002 and 2003 studies into the integrated pool of resources for the integrated approach.

This study may be quite unique in that we have not needed any experiments of our own and are still able to resolve most of the controversies regarding obesity or diabetes. Conversely, this may confirm that the integrated approach has a great potential as a methodology to conduct research. No less importantly, integrated approach also helps expedite sharing of important findings across wide range of disciplines. For instance, if the finding that surgical implantation of wild-type adipose tissue into the A-ZIP/F-1 mice, exhibiting almost the same phenotype as the FIRKO mice, not only normalizes PG but also reverses the diabetes(59) were shared broadly, such a claim that weight gaining or obesity induces IR would no longer be sustained. By the same token, such ludicrous claims that FIRKO mice are protected against the obesity-related glucose intolerance(78) or they can extend

longevity(79) would not have been published in the first place, especially in prestigious journals.

Acknowledgement: The authors appreciate partial support of Chungnam National University for this study.

References

1. Weyer C, Bogardus C, Mott DM, Pratley RE. The natural history of insulin secretory dysfunction and insulin resistance in the pathogenesis of type 2 diabetes mellitus. J Clin Invest. 1999;104(6):787-94.
2. Dimitriadis G, Mitrou P, Lambadiari V, Maratou E, Raptis SA. Insulin effects in muscle and adipose tissue. Diabetes Res Clin Pract. 2011;93 Suppl 1:S52-9.
3. Bruning JC, Michael MD, Winnay JN, Hayashi T, Horsch D, Accili D, et al. A muscle-specific insulin receptor knockout exhibits features of the metabolic syndrome of NIDDM without altering glucose tolerance. Mol Cell. 1998;2(5):559-69.
4. Michael MD, Kulkarni RN, Postic C, Previs SF, Shulman GI, Magnuson MA, et al. Loss of insulin signaling in hepatocytes leads to severe insulin resistance and progressive hepatic dysfunction. Mol Cell. 2000;6(1):87-97.
5. Softic S, Boucher J, Solheim MH, Fujisaka S, Haering MF, Homan EP, et al. Lipodystrophy Due to Adipose Tissue-Specific Insulin Receptor Knockout Results in Progressive NAFLD. Diabetes. 2016;65(8):2187-200.
6. Rosen ED, MacDougald OA. Adipocyte differentiation from the inside out. Nat Rev Mol Cell Biol. 2006;7(12):885-96.
7. Milo R, Phillips R. Cell Biology by the numbers. New York: Garland Science; 2016.
8. Kim SM, Lun M, Wang M, Senyo SE, Guillermier C, Patwari P, et al. Loss of white adipose hyperplastic potential is associated with enhanced susceptibility to insulin resistance. Cell Metab. 2014;20(6):1049-58.
9. Lee S, Kim W, Shin S. Whole-body insulin resistance (WBIR) evolving in four stages and its evolutionary effect on the body weight. ECE 2019; May 18-21, 2019; Lyon, France2019.
10. Saad MF, Knowler WC, Pettitt DJ, Nelson RG, Charles MA, Bennett PH. A two-step model for development of non-insulin-dependent diabetes. Am J Med. 1991;90(2):229-35.
11. Karpe F, Dickmann JR, Frayn KN. Fatty acids, obesity, and insulin resistance: time for a reevaluation. Diabetes. 2011;60(10):2441-9.
12. McFarlane SI. Antidiabetic medications and weight gain: implications for the practicing physician. Curr Diab Rep. 2009;9(3):249-54.
13. Hainer V, Aldhoon-Hainerova I. Obesity paradox does exist. Diabetes Care. 2013;36 Suppl 2(Suppl 2):S276-81.
14. Rabbani N, Thornalley PJ. Dicarbonyls linked to damage in the powerhouse: glycation of mitochondrial proteins and oxidative stress. Biochem Soc Trans. 2008;36(Pt 5):1045-50.
15. Zhang Q, Ames JM, Smith RD, Baynes JW, Metz TO. A perspective on the Maillard reaction and the analysis of protein glycation by mass spectrometry: probing the pathogenesis of chronic disease. J Proteome Res. 2009;8(2):754-69.
16. Pun PB, Murphy MP. Pathological significance of mitochondrial glycation. Int J Cell Biol. 2012;2012:843505.
17. Richter EA, Hargreaves M. Exercise, GLUT4, and skeletal muscle glucose uptake. Physiol Rev. 2013;93(3):993-1017.
18. Slimani L, Oikonen V, Hallsten K, Savisto N, Knuuti J, Nuutila P, et al. Exercise restores skeletal muscle glucose delivery but not insulin-mediated glucose transport and phosphorylation in obese subjects. J Clin Endocrinol Metab. 2006;91(9):3394-403.
19. Wojtaszewski JF, Higaki Y, Hirshman MF, Michael MD, Dufresne SD, Kahn CR, et al. Exercise modulates postreceptor insulin signaling and glucose transport in muscle-specific insulin receptor knockout mice. J Clin Invest. 1999;104(9):1257-64.
20. Aarsland A, Chinkes D, Wolfe RR. Hepatic and whole-body fat synthesis in humans during carbohydrate overfeeding. Am J Clin Nutr. 1997;65(6):1774-82.
21. Solinas G, Boren J, Dulloo AG. De novo lipogenesis in metabolic homeostasis: More friend than foe? Mol Metab. 2015;4(5):367-77.
22. Karpe F, Bickerton AS, Hodson L, Fielding BA, Tan GD, Frayn KN. Removal of triacylglycerols from chylomicrons and VLDL by capillary beds: the basis of lipoprotein remnant formation. Biochem Soc Trans. 2007;35(Pt 3):472-6.
23. Schweiger M, Schreiber R, Haemmerle G, Lass A, Fledelius C, Jacobsen P, et al. Adipose triglyceride lipase and hormone-sensitive lipase are the major enzymes in adipose tissue triacylglycerol catabolism. J Biol Chem. 2006;281(52):40236-41.
24. Kamp F, Hamilton JA. How fatty acids of different chain length enter and leave cells by free diffusion. Prostaglandins Leukot Essent Fatty Acids. 2006;75(3):149-59.
25. Hamilton JA, Johnson RA, Corkey B, Kamp F. Fatty acid transport: the diffusion mechanism in model and biological membranes. J Mol Neurosci. 2001;16(2-3):99-108; discussion 51-7.
26. McQuaid SE, Hodson L, Neville MJ, Dennis AL, Cheeseman J, Humphreys SM, et al. Downregulation of adipose tissue fatty acid trafficking in obesity: a driver for ectopic fat deposition? Diabetes. 2011;60(1):47-55.
27. Moitra J, Mason MM, Olive M, Krylov D, Gavrilova O, Marcus-Samuels B, et al. Life without white fat: a transgenic mouse. Genes Dev. 1998;12(20):3168-81.
28. Roden M. How free fatty acids inhibit glucose utilization in human skeletal muscle. News Physiol Sci. 2004;19:92-6.
29. Randle PJ. Regulatory interactions between lipids and carbohydrates: the glucose fatty acid cycle after 35 years. Diabetes Metab Rev. 1998;14(4):263-83.

30. Navale AM, Paranjape AN. Glucose transporters: physiological and pathological roles. Biophys Rev. 2016;8(1):5-9.
31. Felber JP, Magnenat G, Casthelaz M, Geser CA, Muller-Hess R, de Kalbermatten N, et al. Carbohydrate and lipid oxidation in normal and diabetic subjects. Diabetes. 1977;26(7):693-9.
32. Rosen ED, Spiegelman BM. Adipocytes as regulators of energy balance and glucose homeostasis. Nature. 2006;444(7121):847-53.
33. Fisher SJ, Kahn CR. Insulin signaling is required for insulin's direct and indirect action on hepatic glucose production. J Clin Invest. 2003;111(4):463-8.
34. Porcellati F, Lucidi P, Bolli GB, Fanelli CG. Thirty years of research on the dawn phenomenon: lessons to optimize blood glucose control in diabetes. Diabetes Care. 2013;36(12):3860-2.
35. LEE S, Shin S. The Energy Metabolism behind Dawn Phenomeon and Exercise-Associated Hypoglycemia in Diabetics. WCPD10; July 15-18, 2018; Edinburgh, United Kingdom2018.
36. Morimoto C, Tsujita T, Okuda H. Antilipolytic actions of insulin on basal and hormone-induced lipolysis in rat adipocytes. J Lipid Res. 1998;39(5):957-62.
37. Flannery C, Dufour S, Rabol R, Shulman GI, Petersen KF. Skeletal muscle insulin resistance promotes increased hepatic de novo lipogenesis, hyperlipidemia, and hepatic steatosis in the elderly. Diabetes. 2012;61(11):2711-7.
38. Kim JK, Michael MD, Previs SF, Peroni OD, Mauvais-Jarvis F, Neschen S, et al. Redistribution of substrates to adipose tissue promotes obesity in mice with selective insulin resistance in muscle. J Clin Invest. 2000;105(12):1791-7.
39. Lee EY, Lee YH, Yi SW, Shin SA, Yi JJ. BMI and All-Cause Mortality in Normoglycemia, Impaired Fasting Glucose, Newly Diagnosed Diabetes, and Prevalent Diabetes: A Cohort Study. Diabetes Care. 2017;40(8):1026-33.
40. Salgin B, Ong KK, Thankamony A, Emmett P, Wareham NJ, Dunger DB. Higher fasting plasma free fatty acid levels are associated with lower insulin secretion in children and adults and a higher incidence of type 2 diabetes. J Clin Endocrinol Metab. 2012;97(9):3302-9.
41. Allard P, Delvin EE, Paradis G, Hanley JA, O'Loughlin J, Lavallee C, et al. Distribution of fasting plasma insulin, free fatty acids, and glucose concentrations and of homeostasis model assessment of insulin resistance in a representative sample of Quebec children and adolescents. Clin Chem. 2003;49(4):644-9.
42. Hulman A, Simmons RK, Brunner EJ, Witte DR, Faerch K, Vistisen D, et al. Trajectories of glycaemia, insulin sensitivity and insulin secretion in South Asian and white individuals before diagnosis of type 2 diabetes: a longitudinal analysis from the Whitehall II cohort study. Diabetologia. 2017;60(7):1252-60.
43. Tabak AG, Jokela M, Akbaraly TN, Brunner EJ, Kivimaki M, Witte DR. Trajectories of glycaemia, insulin sensitivity, and insulin secretion before diagnosis of type 2 diabetes: an analysis from the Whitehall II study. Lancet. 2009;373(9682):2215-21.
44. Yeni-Komshian H, Carantoni M, Abbasi F, Reaven GM. Relationship between several surrogate estimates of insulin resistance and quantification of insulin-mediated glucose disposal in 490 healthy nondiabetic volunteers. Diabetes Care. 2000;23(2):171-5.
45. Soonthornpun S, Rattarasarn C, Leelawattana R, Setasuban W. Postprandial plasma glucose: a good index of glycemic control in type 2 diabetic patients having near-normal fasting glucose levels. Diabetes Res Clin Pract. 1999;46(1):23-7.
46. Lee SJ, Shin SW. Mechanisms, Pathophysiology, and Management of Obesity. N Engl J Med. 2017;376(15):1491-2.
47. Avignon A, Radauceanu A, Monnier L. Nonfasting plasma glucose is a better marker of diabetic control than fasting plasma glucose in type 2 diabetes. Diabetes Care. 1997;20(12):1822-6.
48. Ketema EB, Kibret KT. Correlation of fasting and postprandial plasma glucose with HbA1c in assessing glycemic control; systematic review and meta-analysis. Arch Public Health. 2015;73:43.
49. Jelenik T, Kaul K, Sequaris G, Flogel U, Phielix E, Kotzka J, et al. Mechanisms of Insulin Resistance in Primary and Secondary Nonalcoholic Fatty Liver. Diabetes. 2017;66(8):2241-53.
50. Sanders FW, Griffin JL. De novo lipogenesis in the liver in health and disease: more than just a shunting yard for glucose. Biol Rev Camb Philos Soc. 2016;91(2):452-68.
51. Boden G, Lebed B, Schatz M, Homko C, Lemieux S. Effects of acute changes of plasma free fatty acids on intramyocellular fat content and insulin resistance in healthy subjects. Diabetes. 2001;50(7):1612-7.
52. Stannard SR, Thompson MW, Fairbairn K, Huard B, Sachinwalla T, Thompson CH. Fasting for 72 h increases intramyocellular lipid content in nondiabetic, physically fit men. Am J Physiol Endocrinol Metab. 2002;283(6):E1185-91.
53. Goodpaster BH, He J, Watkins S, Kelley DE. Skeletal muscle lipid content and insulin resistance: evidence for a paradox in endurance-trained athletes. J Clin Endocrinol Metab. 2001;86(12):5755-61.
54. Johnson NA, Stannard SR, Rowlands DS, Chapman PG, Thompson CH, O'Connor H, et al. Effect of short-term starvation versus high-fat diet on intramyocellular triglyceride accumulation and insulin resistance in physically fit men. Exp Physiol. 2006;91(4):693-703.
55. Kanamori K, Ihana-Sugiyama N, Yamamoto-Honda R, Nakamura T, Sobe C, Kamiya S, et al. Postprandial Glucose Surges after Extremely Low Carbohydrate Diet in Healthy Adults. Tohoku J Exp Med. 2017;243(1):35-9.
56. Westman EC, Yancy WS, Jr., Humphreys M. Dietary treatment of diabetes mellitus in the pre-insulin era (1914-1922). Perspect Biol Med. 2006;49(1):77-83.
57. Vuorinen-Markkola H, Koivisto VA, Yki-Jarvinen H. Mechanisms of hyperglycemia-induced insulin resistance in whole body and skeletal muscle of type I diabetic patients. Diabetes. 1992;41(5):571-80.
58. DeFronzo RA, Hendler R, Simonson D. Insulin resistance is a prominent feature of insulin-dependent diabetes. Diabetes. 1982;31(9):795-801.

59. Gavrilova O, Marcus-Samuels B, Graham D, Kim JK, Shulman GI, Castle AL, et al. Surgical implantation of adipose tissue reverses diabetes in lipoatrophic mice. J Clin Invest. 2000;105(3):271-8.
60. Thornalley PJ, Battah S, Ahmed N, Karachalias N, Agalou S, Babaei-Jadidi R, et al. Quantitative screening of advanced glycation endproducts in cellular and extracellular proteins by tandem mass spectrometry. Biochem J. 2003;375(Pt 3):581-92.
61. Brownlee M. Biochemistry and molecular cell biology of diabetic complications. Nature. 2001;414(6865):813-20.
62. Shin SW, Lee SJ. Ectopic fat in insulin resistance, dyslipidemia, and cardiometabolic disease. N Engl J Med. 2014;371(23):2236.
63. Beisswenger P, Ruggiero-Lopez D. Metformin inhibition of glycation processes. Diabetes Metab. 2003;29(4 Pt 2):6S95-103.
64. Zhou Z, Tang Y, Jin X, Chen C, Lu Y, Liu L, et al. Metformin Inhibits Advanced Glycation End Products-Induced Inflammatory Response in Murine Macrophages Partly through AMPK Activation and RAGE/NFkappaB Pathway Suppression. J Diabetes Res. 2016;2016:4847812.
65. Ahmad S, Shahab U, Baig MH, Khan MS, Khan MS, Srivastava AK, et al. Inhibitory effect of metformin and pyridoxamine in the formation of early, intermediate and advanced glycation end-products. PLoS One. 2013;8(9):e72128.
66. Ahmed N, Babaei-Jadidi R, Howell SK, Beisswenger PJ, Thornalley PJ. Degradation products of proteins damaged by glycation, oxidation and nitration in clinical type 1 diabetes. Diabetologia. 2005;48(8):1590-603.
67. Thornalley PJ. Measurement of protein glycation, glycated peptides, and glycation free adducts. Perit Dial Int. 2005;25(6):522-33.
68. Jonas JC, Sharma A, Hasenkamp W, Ilkova H, Patane G, Laybutt R, et al. Chronic hyperglycemia triggers loss of pancreatic beta cell differentiation in an animal model of diabetes. J Biol Chem. 1999;274(20):14112-21.
69. Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, et al. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute scientific statement: Executive Summary. Crit Pathw Cardiol. 2005;4(4):198-203.
70. Schernthaner G, Guerci B, Gallwitz B, Rose L, Nicolay C, Kraus P, et al. Impact of postprandial and fasting glucose concentrations on HbA1c in patients with type 2 diabetes. Diabetes Metab. 2010;36(5):389-94.
71. Johansson E, Lubberink M, Heurling K, Eriksson JW, Skrtic S, Ahlstrom H, et al. Whole-Body Imaging of Tissue-specific Insulin Sensitivity and Body Composition by Using an Integrated PET/MR System: A Feasibility Study. Radiology. 2018;286(1):271-8.
72. Bechlioulis A, Vakalis K, Naka KK, Bourantas CV, Papamichael ND, Kotsia A, et al. Paradoxical protective effect of central obesity in patients with suspected stable coronary artery disease. Obesity (Silver Spring). 2013;21(3):E314-21.
73. Nickols-Richardson SM, Coleman MD, Volpe JJ, Hosig KW. Perceived hunger is lower and weight loss is greater in overweight premenopausal women consuming a low-carbohydrate/high-protein vs high-carbohydrate/low-fat diet. J Am Diet Assoc. 2005;105(9):1433-7.
74. Logue J, Walker JJ, Leese G, Lindsay R, McKnight J, Morris A, et al. Association between BMI measured within a year after diagnosis of type 2 diabetes and mortality. Diabetes Care. 2013;36(4):887-93.
75. Aune D, Sen A, Prasad M, Norat T, Janszky I, Tonstad S, et al. BMI and all cause mortality: systematic review and non-linear dose-response meta-analysis of 230 cohort studies with 3.74 million deaths among 30.3 million participants. BMJ. 2016;353:i2156.
76. Bhaskaran K, Dos-Santos-Silva I, Leon DA, Douglas IJ, Smeeth L. Association of BMI with overall and cause-specific mortality: a population-based cohort study of 3.6 million adults in the UK. Lancet Diabetes Endocrinol. 2018;6(12):944-53.
77. Kwon Y, Kim HJ, Park S, Park YG, Cho KH. Body Mass Index-Related Mortality in Patients with Type 2 Diabetes and Heterogeneity in Obesity Paradox Studies: A Dose-Response Meta-Analysis. PLoS One. 2017;12(1):e0168247.
78. Bluher M, Michael MD, Peroni OD, Ueki K, Carter N, Kahn BB, et al. Adipose tissue selective insulin receptor knockout protects against obesity and obesity-related glucose intolerance. Dev Cell. 2002;3(1):25-38.
79. Bluher M, Kahn BB, Kahn CR. Extended longevity in mice lacking the insulin receptor in adipose tissue. Science. 2003;299(5606):572-4.