How does insulin resistance arise, and how does it cause disease?: Human genetic lessons

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Short Title: How many Types of Insulin Resistance?

Keywords (>4): Insulin Resistance, Genetics, Lipodystrophy, Insulin Receptor, Fatty Liver, PCOS, polycystic ovaries, Diabetes, Phosphatidylinositol-3-kinase, Insulin signalling

Main Text Word Count: 5,786

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Abstract

Insulin orchestrates physiological responses to ingested nutrients, however although it elicits widely ramifying metabolic and trophic responses from diverse tissues, "insulin resistance", a pandemic metabolic derangement commonly associated with obesity, is usually defined solely by blunting of insulin's hypoglycaemic effect. Recent study of monogenic forms of insulin resistance has established that biochemical subphenotypes of insulin resistance exist, clustering into those caused by primary disorders of adipose tissue, and those caused by primary defects in proximal insulin signalling. Insulin resistance is often first recognised by virtue of its associated disorders, including type 2 diabetes, dyslipidaemia, fatty liver and polycystic ovary syndrome. Although these clinically observed associations are confirmed by cross sectional and longitudinal population-based studies, causal relationships among these phenomena have been more difficult to establish. Single gene insulin resistance is important to recognise in order to optimise clinical management, and also permits testing of causal relationships among components of the insulin resistance syndrome using the principle of Mendelian randomisation. Thus, where a precisely defined genetic defect is identified that directly produces one component of the syndrome, then phenomena that are causally linked to that component should be seen. Where this is not the case, then a simple causal link is refuted. This article summarises known forms of monogenic severe insulin resistance and considers the lessons to be learned about the pathogenic mechanisms both upstream from common insulin resistance, and those downstream linking it to disorders such as dyslipidaemia, fatty liver, polycystic ovary syndrome and cancer.

Origins of the concept of insulin resistance

The discovery and clinical introduction of purified insulin in the early 1920s transformed diabetes mellitus from a rapidly fatal childhood malady into a manageable chronic disease. However as the effort to translate therapeutic proof of principle into cheap, widely available insulin treatment gathered momentum in ensuing years, it became apparent that a subgroup of people with diabetes did not exhibit the dramatic response of the emaciated, ketotic patients first treated. Most prominent in studying and articulating this was Harold Himsworth who, in the late 1930s, discriminated between “sensitive diabetics” and “insensitive diabetics” (generally older, obese and hypertensive, commonly with arteriosclerosis, and exhibiting insidious disease onset with rare ketosis and insulin-induced hypoglycaemia)[1]. Physiological studies showed that “insulin insensitive” patients were slow to show a hypoglycaemic response to exogenous insulin[1]. Upon development of radioimmunoassays in 1960, formal proof was obtained that insulin resistant individuals do indeed have high or normal insulin levels in the face of hyperglycaemia[2, 3].

Now, nearly 100 years after the first use of insulin in diabetes, although the scourge of childhood death due to insulin deficiency is nearly vanquished, insulin resistance (IR) and the attendant hyperinsulinaemia sit at the centre of a web of burgeoning pandemic disease. They are associated strongly with obesity[4], type 2 diabetes[5], fatty liver disease[6], "metabolic" dyslipidaemia[4], polycystic ovary syndrome[7], and some cancers[8]. However despite the clear documentation of these associations, understanding of the pathogenesis of IR, and whether and how it causes all the clinical conditions with which it is associated, remains incomplete.

Mechanisms of insulin action

Teasing out how insulin exerts metabolic and growth stimulating effects on target tissues has been the subject of painstaking biochemical study for many decades. Around 40 years ago it was shown that insulin could bind competitively to hepatocyte membranes and thereby alter cellular metabolism[9], and shortly afterwards the insulin receptor was identified biochemically[10]. Sequencing of the gene for the human insulin receptor in 1985 demonstrated it to be a transmembrane

dimeric tyrosine kinase[11], a member of what is now known as a large family of receptor tyrosine kinases of immense medical significance in endocrinology, cancer, and beyond.

Cellular and animal studies have subsequently delineated many of the signalling events that pass the insulin signal from its receptor on the cell surface in order to change cellular metabolism and growth. A detailed account of these, which have been comprehensively reviewed elsewhere[12, 13], is beyond the scope of this article, however a simplified schematic is shown in **Figure 1**. In brief, insulin binding to the receptor induces phosphorylation of its intracellular domains, leading to recruitment of several insulin receptor substrates, the most important of which are Insulin Receptor Substrates (IRS) 1 and 2. These large phosphoproteins serve as a "platform" that initiates downstream signalling pathways, among which the phosphatidylinositol-3-kinase(PI3K)/AKT pathway and the MEK/ERK (formerly MAP Kinase) pathway have received most attention. The PI3K pathway has traditionally been viewed as the key metabolic effector arm of the insulin signalling response, exerting critical metabolic actions in particular through AKT2, one of three isoforms of the serine-threonine kinase AKT that is enriched in insulin-responsive tissues[14].

Several general features of the insulin signalling "pathway" bear particular note. First, although signalling downstream from the receptor is often conceptualised as a series of branching pathways emanating from the receptor or IRS proteins, there is both extensive crosstalk among branches of the signalling pathway and also negative feedback within them, so that they function as a rather more complex network than often implied by simple schematics. Moreover, most of the signalling pathways downstream from the insulin receptor are shared by other receptor tyrosine kinases, and in particular the IGF1 receptor, whose signalling network is nearly indistinguishable from that of insulin. How distinct biological responses are exerted by different growth factors and RTK ligands is not fully understood, though this is likely to depend in part on different patterns of ligand and receptor expression, and probably on different subcellular location of signalling molecules. Second, it has previously been pointed out that at several key points of insulin signal transduction the signal may be transmitted by several different homologous proteins, some of which function as dimeric products of more than one gene[12]. This introduces the

potential for huge combinatorial signalling complexity. Finally, cellular studies are beginning to unpick the importance of different temporal sequences of signalling events after receptor stimulation, which, allied to different thresholds for activation of metabolic endpoints, introduces yet more tiers of signalling complexity[15].

All these considerations mean that, even at the refined level of cellular signalling, it is difficult to formulate one comprehensive, biologically meaningful definition of "insulin resistance", and doing so with reference to only one or even a handful of downstream readouts may mask a more complex and patchy perturbation of cellular signalling. As will be discussed, this may well be important for common diseases related to insulin resistance

Quantitative Definitions of Insulin Resistance

The catastrophic short term consequences of severe hypoglycaemia, and the damaging longer term consequences of hyperglycaemia, have meant that it is insulin's actions on blood glucose, rather than on other aspects of intermediary metabolism or growth, that have been by far the major focus of attention over the past century[16]. Thus it is by reference to insulin's ability to lower blood glucose that insulin sensitivity, and thus resistance, is generally defined[17]. The simplest definitions rely on measurement of fasting plasma insulin together with blood glucose. When blood glucose is in the normal range, IR may simply be defined using an arbitrary threshold established with reference to fasting insulin in a reference population. Where beta cell decompensation and hence hyperglycaemia have arisen, use of an empirical index such as the HOMA-IR index is of use. More complicated dynamic testing may also be used, including oral or intravenous glucose tolerance testing with derivation of one of a variety of indices, while determination of the amount of insulin required in the face of a fixed infusion of glucose to maintain normal blood glucose (so-called hyperinsulinaemic euglycaemic clamping) is generally held to be the gold standard. However although each of these approaches has utility, and while several permit assessment of different aspects of insulin's glucose lowering action, each neglects the effect of insulin on other processes such as amino acid and lipid metabolism. Although efforts have been made to develop

surrogate indices of IR based on other plasma analytes[18, 19], none of these has yet been widely clinically adopted.

As insulin sensitivity is a continuous trait, thresholds for diagnosis of “severe” IR are arbitrary, and ideally should be defined with reference to appropriate controls, taking into account such influences on insulin sensitivity as age, sex, pubertal stage, obesity and concomitant medical illness. Operational examples of thresholds for defining severe IR are a requirement for exogenous insulin of greater than 3 units/kg/day to maintain normoglycaemia in those with absolute insulin deficiency, or a fasting insulin greater than 150 pmol/l in lean adults without diabetes. However given the complexity of setting valid numerical thresholds, especially once diabetes has developed, clinical evidence of severe IR from history and examination are of tremendous importance in timely diagnosis.

Mendelian Randomisation to Test Causality in Human Disease

A key challenge in studies of IR-associated disease in humans arises from the difficulty in discerning cause and effect, if any, in observed associations. Causality may be implied though not proven from the sequence of appearance of clinical phenomena, however the most direct test of whether a disease is caused by an associated disorder in humans is to directly introduce that perturbation, and to observe whether the disease ensues. In most cases, however, even where this might technically be possible, the postulated deleterious nature of the first perturbation, and/or the time course over which the disease of interest develops render this impractical or unethical. Natural genetic variation may sometimes be used opportunistically to undertake equivalent studies, where a primary genetic cause of a candidate mediator of disease can be identified. An often cited paradigm for this so-called "Mendelian randomisation" approach lies in Brown's and Goldstein's use of patients with LDL receptor mutations and thus primary elevation of LDL cholesterol to provide strong evidence for the causal link between high LDL cholesterol and atherosclerosis[20]. The principle of Mendelian randomisation is illustrated in **Figure 2.**

Mendelian randomisation would also be of value in principle in testing which of the IR-associated diseases are indeed caused by IR, as long as primary genetic

causes of IR, with no independent link to the diseases being studied, could be identified. Such groups of patients with primary monogenic IR are indeed now known, and continue to be discovered, and their value in informing studies of common IR-related disease will be the main focus of the rest of this article.

An Analytic "Toolkit": Subtypes of Human Monogenic Insulin Resistance

It is 27 years since the first single gene form of severe IR, caused by mutations in the gene encoding the insulin receptor, was discovered[21, 22], and in the past 17 years further disorders have been added to this, latterly with increasing rapidity as modern genetic technologies have been brought to bear[23]. As the number of known monogenic disorders has increased, so it has become apparent that IR is not monomorphic, and indeed that most single gene causes of severe IR do not directly affect insulin cellular signalling pathways. Instead, many defects do directly affect the development or function of adipose tissue, with secondary effects on insulin action *in vivo*. Single gene causes of severe IR may thus be divided into groups loosely defined by the tissue or cellular process affected. It is these groupings which are lending increasing power to Mendelian randomisation.

Primary insulin signalling defects: The first human loss-of-function mutations were reported in the insulin receptor in 1988, both in Donohue Syndrome, an extreme infantile form of failure to thrive with IR[22] or with the less severe “type A” form of IR[21], which usually becomes manifest peripubertally. Since this time more than a 150 different mutations within the insulin receptor have been discovered, with relatively few recurring mutations, and the phenotypic spectrum has been well described elsewhere[23-25]. A single family with four members affected by severe IR due to a loss-of-function mutation in AKT2 was also described in 2004, and was the second example of a highly penetrant Mendelian form of severe IR due to mutation of a canonical insulin signalling gene[26]. Mutations in TBC1D4, involved in regulating exocytosis of glucose-transporting GLUT4 vesicles in response to insulin, were described also described in association with an apparently muscle-selective form of severe IR[27], and this was later confirmed in a Greenland population, where a similar genetic defect accounts for a significant part of the variance of insulin sensitivity[28].

The latest form of severe IR established to be caused by mutation of an insulin signalling gene is seen in the context of SHORT syndrome, denoted by Short Stature, joint Hyperextensibility and Hernias, Ocular depression, and Teething delay. Although not part of the acronym, severe IR has been shown to be common in this disorder, and in 2012 the underlying cause was established to be mutations in the *PIK3R1* gene[29-31]. *PIK3R1* encodes three of the regulatory subunits of type 1A phosphatidylinositol-3-kinase, which are critical for the coupling of insulin binding to its receptor to activation of AKT2 and other downstream signalling molecules[12]. Primary insulin signalling disorders are schematised in **Figure 1**.

Primary Lipodystrophies

Inherited disorders of adipose tissue development, or lipodystrophies, were described clinically from at least the 1920s[32], however it has only been in this century that they have yielded to genetic study, with many different causal genetic defects now identified. Inherited lipodystrophies are most commonly divided into those that feature complete, or near complete absence of all adipose tissue, called congenital generalised lipodystrophy (CGL), and those in which only some adipose tissue depots are affected, called familial partial lipodystrophy.

CGL, often known as Berardinelli–Seip congenital lipodystrophy, is caused in around 95% of cases by biallelic mutations in one of two genes, namely AGPAT2, encoding 1-acylglycerol-3-phosphate O-acyltransferase 2 (AGPAT2)[33, 34], and BSCL2, which encodes a transmembrane endoplasmic reticulum protein named seipin[35]. Seipin plays a role in preadipocyte differentiation[36] and in the regulation of lipid droplet synthesis and function[37, 38]. Smaller numbers of patients with CGL have also been reported with loss-of-function mutations in CAV1[39] or PTRF[40], the latter associated also with myopathy. Both these genes encode key structural components of small plasma membrane invaginations called caveolae, which account for up to 40% of the adipocyte surface area, and play an important role in organising cell signalling and lipid trafficking. Most recently, genetic disruption of PCYT1A, encoding phosphate cytidylyltransferase 1, which plays a key role in de novo phosphatidylcholine biosynthesis, have also been demonstrated in patients with CGL[41].

Familial partial lipodystrophies (FPLD) are collectively far more common than CGL. They are usually not clinically manifest until puberty, when perturbation of pubertal adipose accretion unmasks the underlying abnormality. Women are more severely affected than men, likely due in large part to the naturally greater adiposity of women than men. At least seven genes with diverse roles in adipocyte biology have now been linked to partial lipodystrophy (**Table 1**). The first of these, LMNA, encodes a nearly ubiquitously expressed intermediate filament protein, Lamin A/C, which forms part of a structural network of proteins supporting the nuclear membrane[42]. Different LMNA mutations have also been linked to other disorders including muscular dystrophy, dilated cardiomyopathy, Charcot-Marie-Tooth neuropathy, premature aging syndromes, restrictive dermopathy and various overlap syndromes[43]. The mechanism(s) linking LMNA mutations to lipodystrophy are not fully understood, but roles have been proposed for structural defects in the nuclear envelope, and/or abnormal binding of the nuclear lamina to chromatin and transcription factors, thus altering gene expression.

The second gene to be affected in familial partial lipodystrophy is PPARG[44], encoding PPARγ, a nuclear hormone receptor most highly expressed in adipose tissue where it is essential for adipocyte differentiation, and which is the target of the thiazolidinedione class of antidiabetic agents. All known pathogenic mutations are heterozygous, and most have been shown to inhibit the function of co-expressed wildtype protein.

Although the remaining genes now known to cause partial lipodystrophy collectively account for only a tiny proportion of cases, their discovery has highlighted the critical importance of lipid droplet dynamics in human metabolic homeostasis, with defects in either CIDEC[45] or Perilipin[46], each of which resides on the surface of the lipid droplet and plays a regulatory role in triglyceride mobilisation, now implicated in human partial lipodystrophy.

Of note, several of the primary insulin signalling disorders also feature some degree of lipodystrophy, in keeping with an important role for insulin and IGF1 signalling in adipocyte differentiation. This is particularly pronounced in the case of AKT2 loss of function[26] and in SHORT syndrome[29, 31], but a generalised paucity of

adipose tissue is also commonly described in the more severe insulin receptoropathies. The potential importance of this for the IR subphenotype that is clinically expressed is discussed below.

Complex Monogenic Disorders Featuring Severe Insulin Resistance

A clinically heterogenous group of more complex monogenic conditions also exists that features severe IR disproportionate to whole body adiposity. These include the premature ageing syndromes Werner Syndrome[47, 48] and Bloom Syndrome[49], both caused by defects in DNA helicases, and a very recently described syndrome of primordial dwarfism caused my mutations in the NSMCE2 gene, which encodes a component of the Structural Maintenance of Chromosomes (SMC) 5/6 complex[50]. Each of these genes plays some role in permitting dividing cells to tolerate DNA damage, and in the maintenance of telomeres. Another recently described syndrome featuring loss of subcutaneous fat as well as severe IR is caused by heterozygous loss-of-function mutations in POLD1, encoding DNA polymerase delta, the dominant lagging strand DNA synthase in human cells[51]. This adds to the impression that some aspects of DNA damage repair or replication are critical to metabolic homeostasis. This notion is lent further credence by the observation that childhood irradiation as part of cancer therapy predisposes to later dyslipidaemia and IR, which may sometimes be severe[52]. Modelling of this phenomenon in leptin deficient, severely obese mice suggests that irradiation leads to adipocyte and preadipocyte death, effectively reducing adipose tissue expandability, leading to a worsened metabolic state because if, rather than despite, a reduction in adipose gain after irradiation[53].

A further tantalising hint of a specific relationship between a particular cellular function and whole body metabolic IR comes from the observation of severe IR in patients with genetic defects in different centrosomal components including ALMS1 [54], PCNT [55, 56], and POC1A[57]. Teasing out the pathogenic link between the defined molecular defect and severe systemic IR in these disorders promises to yield novel insights in future into the mechanistic basis of IR. Given the complex and pleiotropic nature of the genetic defects, however, these conditions are not suitable as a basis for the Mendelian randomisation approach.

Application of Mendelian Randomisation to Obesity-Associated Metabolic Disease

Many of the disorders described above, where the cellular function and immediate physiological consequences of the genetic defect can be defined with confidence, are appropriate instruments to use in a Mendelian randomisation-based attempt to address important questions about the causes and consequences of human IR. Several of these questions will be considered in turn, and the lessons to be drawn from monogenic disease will be assessed.

How does Obesity Give Rise to Insulin Resistance?

Cross sectional and longitudinal studies suggest strongly that obesity is the proximate cause of most pandemic IR, however the question then arises of the mechanistic link between these phenomena. Study of humans with single gene defects producing severe, early onset, hyperphagic obesity, for example due to genetic deficiency of either leptin or its receptor, confirm that IR is common in this group, however it is often not severe, and its severity is often somewhat less than that of the concomitant obesity. This implies that there are factors other than severity of obesity which influence the degree of obesity-related IR. Importantly, at the opposite end of the adipose spectrum, patients with congenital generalised lipodystrophy, who have negligible adipose tissue, develop extremely severe IR, dyslipidaemia and fatty liver, and thus monogenic lipodystrophy has been argued to be a potentially aetiologically informative model of the common metabolic syndrome[58].

These human observations lend strong support to the hypothesis that it is relative adipose failure that is critical to the pathogenesis of the obesity-related metabolic syndrome rather than the degree of obesity. According to this idea, the capacity of adipose tissue to continue expanding, while safely storing excess calories in the form of triglyceride, is finite, and when this capacity, which shows considerable inter-individual variation is exceeded, then "adipose failure", with knock-on effects on other, insulin-sensitive tissues, ensues[59-61]. Congenital generalised lipodystrophy represents an extreme example of this, where the adipose energy buffering capacity is negligable. In this setting patients remain lean, however

loss of the capacity of adipose tissue to buffer excess caloric intake by serving as a sump for free fatty acids and, to a much lesser extent, as a site of disposal of glucose is likely to result in remodelling of systemic free fatty acid flux, with increased uptake in other metabolically critically tissues such as liver, skeletal muscle and pancreatic beta cells, where harmful effects including loss of insulin sensitivity, secretory dysfunction and apoptosis may result[62]. This series of maladaptive changes are collectively unified by the term lipotoxicity.

In many monogenic partial lipodystrophies significant adipose depots remain, and indeed it is not uncommon for whole body adiposity to be elevated, as assessed by direct measurement and by surrogate indices such as plasma leptin levels. In these conditions it is adipose topography which appears to be critical to metabolic homeostasis. In all known genetic forms of partial lipodystrophy which feature severe IR femorogluteal depots are affected, while central fat and omental fat are often preserved or increased, meaning that Cushing's syndrome is commonly invoked as a differential diagnosis at first assessment. The impression that lower body subcutaneous fat is critical for metabolic health is bolstered by the observation that in acquired partial lipodystrophy, where subcutaneous fat is usually lost in a craniocaudal direction to the level of the umbilicus, there is only a low rate of systemic metabolic derangement[63]. Distributions of fat loss in partial lipodystrophies are illustrated in **Figure 3**.

Importantly, although some of the genes involved in lipodystrophy are expressed in cells other than adipocytes, raising concerns that they may exert phenotypic effects unrelated to lipodystrophy, thus invalidating them as instruments for Mendelian randomisation-based analysis, the closely similar metabolic phenotype seen in patients with acquired, autoimmune, lipodystrophy, and the fact that a least one of the genes implicated in genetic lipodystrophy, PLIN, is adipocyte-specific[46], argues against this.

Additional mechanisms beyond loss of energy buffering have been invoked to account for the link between increased adipose tissue and IR. One of these is perturbation of circulating levels of adipose-derived hormones, the so called adipokines. Evidence for a large number of these has now been put forward, but in

most cases evidence for a critical role in setting systemic insulin sensitivity is mixed, and most are best viewed as playing an accessory role only. Apart from leptin, best established of the *bona fide* adipokines, adiponectin, a large protein with homology to complement factor C1q, has attracted the most sustained and intense attention. It circulates at high levels in plasma as a complex mixture of oligomeric or multimeric forms. Unusually, increasing levels of adiposity and IR are associated with lower plasma adiponectin concentration, with a concomitant shift from higher molecular weight species to lower molecular weight forms. Much of the evidence that adiponectin is an insulin sensitiser come from murine studies. Genetic ablation of the adiponectin-encoding gene in mice produced IR in some, though not all reports, while administration of various different oligomeric adiponectin preparations has been shown in mice to improve insulin sensitivity[64, 65].

The metabolic role of adiponectin in humans is less clear. Until recently evidence for a causal role of low adiponectin in IR was largely correlative, based on studies of association of plasma adiponectin with metabolic disorders[64]. However genetic studies, several involving Mendelian randomisation, have also now been brought to bear on this issue. Plasma adiponectin is usually either preserved or frankly elevated, sometimes dramatically so, in the face of either genetic or acquired defects in insulin receptor function[66-68], and more limited evidence suggests that this is also true in SHORT syndrome, arguing that proximal defects in insulin signalling raise plasma adiponectin. This observation is not explained, but demonstrates that suppressed plasma adiponectin is not a consequences of reduced insulin signalling. Indeed, it may be viewed as an attempt to ameliorate insulin sensitivity in the face of a fixed signalling defect. A more direct test of adiponectin's potential to influence insulin sensitivity comes from true Mendelian randomisation, whereby association with IR of genetic variants at the locus of the adiponectin gene, ADIPOQ, that induce primary changes in plasma adiponectin, are assessed. This approach has been adopted by several groups with mixed results. Two studies found that genetically-determined lowering of plasma adiponectin did indeed associate with reduced insulin sensitivity[69, 70], while the largest study, with the greatest statistical power, found no such association[71]. One family has been reported in which a rare missense ADIPOQ variant suppressing plasma adiponectin was found to associate with IR[72]. More

systematic metabolic study of more families with rare ADIPOQ alleles identified in large scale exome sequencing studies will be of value to identify any that suppress plasma adiponectin, and to clarify the association further.

Does Insulin Resistance Cause Fatty Liver and Dyslipidaemia?

Understanding the relationship between plasma adiponectin levels and IR is important for the understanding of the pathogenesis of IR, and this is given extra weight by the potential exploitation of adiponectin for therapeutic benefit. Nevertheless low plasma adiponectin is a biochemical surrogate rather than a clinical disease. In contrast, the fatty liver disease spectrum, which is intimately linked to a pattern of dyslipidaemia characterised by low plasma HDL cholesterol and high plasma triglyceride, exacts an immense toll of human suffering. To this, too, Mendelian randomisation using rare disorders of insulin action may be applied. Before diabetes supervenes, primary IR due to genetic defects in the insulin receptor is a model of global, compensated IR with extreme hyperinsulinaemia. Strikingly, however, patients with genetic or acquired INSR defects appear to be entirely protected from "metabolic" dyslipidaemia[73]. Moreover, they do not exhibit increased liver fat, and rates of hepatic de novo lipogenesis, which has been proposed to be an important contributor to fatty liver and dyslipidaemia in human IR[74, 75], are similar to controls[73]. This argues that fatty liver disease and dyslipidaemia can be accounted for neither by generalised IR, nor by severe hyperinsulinaemia acting through non insulin receptor-dependent pathways.

Analysis of IR due to downstream signalling defects produces contrasting results: preliminary data suggests that in SHORT syndrome due to mutations in PIK3R1, lipid profiles are normal despite severe IR[31], while in contrast, 2 patients studied with severe IR due to defects in AKT2, three steps down the classical insulin signalling pathway from the insulin receptor and one from PI3K, did show exaggerated metabolic dyslipidaemia with severe fatty liver[73].

These human observations suggest that “partial” IR – that is, IR affecting only some parts of the insulin signalling network, may play an important role in the pathogenesis of major IR-related pathologies. This hypothesis was first widely promulgated in the 1980s[4], and has been supported previously by observations

made in genetically modified mice[76]. In the current context, “partial” IR may either be regarded as a cell autonomous phenomenon, whereby the pathway required for hyperinsulinaemia to drive liver fat accumulation and atherogenic patterns of VLDL secretion from liver cells depends on the insulin receptor and PIK3R1 but not AKT2, implying a signalling pathway proximal to AKT2 that drives hepatic *de novo* lipogenesis. However this is at odds with most of the considerable body of murine data, most of which suggest that AKT2 is critical in mediating the effect of insulin to drive liver fat accumulation[77, 78]. Other possibilities are that the critical elements of “partial” IR are differential effects of the same fixed defect in insulin signalling on different downstream pathways due to differing thresholds for activation[79] and/or differential effects of the same fixed defect on insulin sensitivity of different tissues[80]. Thus, it may be that the dominant effect in humans of the AKT2 mutation reported is in adipose tissue, where AKT2 is involved in suppressing adipocyte differentiation and the suppression of lipolysis, and that the resulting “adipose failure” swamps reduced ability of high insulin to drive de novo lipogenesis in the liver Possible models of partial IR are schematised in **Figure 4**.

Does Insulin Resistance Cause Polycystic Ovary Syndrome?

Like type 2 diabetes, polycystic ovary syndrome has a high prevalence, estimated to be of the order of 10% in Western populations, and confers a high burden of morbidity, due in particular to subfertility and to psychological distress related to the cosmetic effects of clinical hyperandrogenism. However like the label "type 2 diabetes", the label "PCOS" does not imply aetiopathogenesis. Instead it simply describes the syndrome, and is the unifying designation for the common clinical expression of a range of primary underlying defects rather than being a single entity. Indeed, although PCOS has been the focus of an extensive literature encompassing clinical, model organism and cellular studies, debate continues about key aspects of its pathogenesis.

IR and the metabolic syndrome are very well described associations of PCOS, with a prevalence in the region of 50-70%, depending on definitions used and population studied[81, 82]. However there has been uncertainty as to whether IR in PCOS has unique characteristics. Moreover primary androgen excess of a variety of

causes may reduce insulin sensitivity in adipose and other tissues, so an argument has sometimes been made that the IR in PCOS is caused by ovarian dysfunction rather than *vice versa*. Where IR is accepted as a cause of some forms of PCOS, partial IR, as in the liver, has been invoked to explain PCOS, with the ovary argued to be an non insulin-resistant "bystander" tissue, responding to high levels of circulating insulin set by selective defects in the metabolic actions of insulin in other tissues[83].

In the face of complex and sometimes competing hypotheses, observations in humans with single gene severe IR are simple: Ovulatory dysfunction and hyperandrogenism, which may be very severe, are seen in nearly all known monogenic forms of severe IR. They are seen in exaggerated form in the context of genetic or acquired insulin receptoropathy, of downstream insulin signalling defects in AKT2 or PIK3R1, of partial or generalised lipodystrophy and pleiotropic syndromes, all of which commonly exhibit severe clinical and biochemical hyperandrogenism, oligo- or amenorrhoea and classical polycystic ovaries. In other words, while dyslipidaemia and fatty liver are seen only in some types of IR, the ovarian phenotype is nearly universal. There is no evidence that it differs between global IR at the level of the receptor, and selective IR affecting PI3K/AKT signalling, generally regarded as the more metabolic arm of the insulin signalling pathway, in keeping with prior evidence that PI3K does not mediate the effects of insulin on human granulosa cells in culture[84]. Furthermore, although perturbed androgen metabolism has been suggested to play an important role in PCOS in some forms of insulin resistance[85], the severe PCOS seen in generalised lipodystrophy argues that adipose androgen is not an obligate link between severe IR and PCOS.

In Donohue syndrome cystic ovarian enlargement may be seen in infancy, and this can be massive, even when no functional insulin receptors are expressed[86, 87]. While this ovarian pathology is not identical to that of polycystic ovary syndrome, it is plausible to suggest that it shares the same underlying mechanism, namely synergic stimulation of follicles by extremely elevated insulin and the gonadotrophins which are elevated in the early months of life before secondary suppression. If this parallel holds true, then this suggests that the adverse effects of insulin on the ovary need not be exerted through the insulin receptor itself. These observations suggest that

the ovarian component of the IR syndrome, quite unlike fatty liver and dyslipidaemia, do not require intact INSR function, although they do require severe hyperinsulinaemia. These findings may be reconciled by hypothesizing that very high levels of insulin enhance signalling through the IGF receptor, in keeping with a large body of evidence for an important role for IGFs in follicular maturation[88, 89]. PCOS is reported to be common in premenopausal women with acromegaly[90], however it is not usually as severe as that seen in the context of severe IR. This discrepancy may be accounted for by endocrine or paracrine enhancement of IGF1 bioavailability or receptor expression in IR in addition to an IGF-like action of very high levels of circulating insulin.

Observations in defined monogenic forms of severe insulin resistance may not necessarily be extrapolatable to "common" PCOS. On the other hand, the extremely high penetrance of a PCOS phenotype in the face of a diverse array of genetic forms of severe IR, and the reversible PCOS seen in acquired states of insulin receptor dysfunction[91], which are not mimicked by insulin deficient states, show that IR with compensatory hyperinsulinaemia is sufficient to cause a severe PCOS-like state without needing to invoke additional mechanisms or the importance of a specific subtype of insulin resistance. The ability of primary hyperandrogenaemia due to genetic abnormalities in steroid metabolism also to phenocopy prevalent PCOS[92] reaffirm, however, that there is more than one pathway to the PCOS phenotype.

Does Insulin Resistance Cause Cancer?

Increasing attention has been paid in recent years to the association in population-based studies between IR and some cancers, including those of the breast, colon, prostate and endometrium[93]. For some cancers there are plausible endocrine or metabolic explanations for a causal link between IR and the tumour that do not involve direct effects of insulin on the tumour itself. For example, hyperinsulinemia induces the oestrogen replete but oligomenorrhoeic state commonly seen in PCOS, indirectly increasing risk of endometrial cancer[94]. Furthermore it is well established that the fatty liver associated with lipodystrophy is not benign, but rather features a high risk of cirrhosis and ultimately hepatocellular

cancer in lipodystrophies[95]. However it has also been argued, with some experimental evidence, that insulin may directly drive tumour growth[93, 96].

Cancer pathogenesis is complex and multifactorial, and hyperinsulinaemia is likely to be only one facilitatory element in a complex sequence of events. Nevertheless it is to be predicted that in severe monogenic forms of IR the cancer risk may be further increased from the risk attributed to IR in the general population. Indeed, several non malignant elements of the severe IR syndrome, namely the dermal and epidermal hyperplasia of acanthosis nigricans, the organomegaly of infantile receptoropathy, and the pseudoacromegaloid soft tissue overgrowth common to many forms of IR, attest to the tissue defective growth-promoting effects of hyperinsulinaemia. In the most severe hyperinsulinaemia seen in Donohue syndrome, ovarian tumours may arise in infancy[86, 87], while colonic polyposis is also sometimes seen in either recessive or dominant insulin receptoropathies. Thus, while syndromes of severe insulin resistance, except those featuring an underlying defect in DNA damage repair such as Werner and Bloom's syndromes, are not penetrant cancer predisposition syndromes, the mitogenic consequences of sustained severe hyperinsulinaemia are apparent, and this adds weight to the notion of a role for hyperinsulinaemia per se in increasing prevalent cancer risk.

Conclusions and Future Directions

IR is not a disease in itself, but rather an endocrine derangement associated with several pandemic diseases and tissue pathologies. Study of rare single gene forms of insulin resistance has yielded valuable insights into several aspects of the pathogenesis of these diseases. Current findings are consistent with the "adipose failure" model linking obesity to prevalent metabolic disease, and suggest that compensatory hyperinsulinaemia may play a critical role in the pathogenesis of PCOS as well as fatty liver and metabolic dyslipidaemia. Specifically, differences among different subtypes of insulin signalling defect demonstrate that insulin resistance with respect to glucose lowering may be uncoupled from other components of the prevalent metabolic syndrome including fatty liver and dyslipidaemia, depending on the precise nature of the underlying signalling defect. In contrast the "trophic" features of insulin resistance including acanthosis nigricans,

soft tissue overgrowth and PCOS are seen in essentially all insulin resistant hyperinsulinaemic states. Teasing out the mechanistic basis of these findings in humans offers the possibility to identify novel strategies to isolate and treat several different components of the complex of obesity and insulin resistance-related diseases.

Acknowledgements

Dr Semple is supported by a Senior Research Fellowship from the Wellcome Trust [Grant WT098498].

Disclosure Statement

Dr Semple has received speaker fees from Novo Nordisk and Sandoz.

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Tables

Table 1: Known genetic forms of partial lipodystrophy

Clinical Disorder	Gene	Inheritance	Distribution of Adipose Loss	Comment
FPLD2	*LMNA*	AD	S/C sparing head, neck and labia majora	Commonest cause of FPLD. May be mistaken for Cushing's syndrome due to preserved head and neck fat
FPLD3	*PPARG*	AD	Limbs	LD may be subtle, limited to limbs, and associated with centripetal obesity
FPLD4	*PLIN1*	AD	Limbs	
FPLD6	*AKT2*	AD	Limbs	Single family reported to date
FPLD5	*CIDEC*	AR	Limbs	Single patient reported
SHORT Syndrome	*PIK3R1*	AD or sporadic	S/C, variable	Not always seen. Not associated with dyslipidaemia
MDP Syndrome	*POLD1*	AD or sporadic	S/C	Also loss of limb muscle, scleroderma-like features, deafness and male hypogonadism

FPLD = Familial Partial Lipodystrophy; SHORT = Short stature, Hyperextensibility, Ocular depression, Hernias, Rieger Anomaly; MDP = Mandibular hypoplasia, Deafness, Progeroid features; AD = autosomal dominant; AR = autosomal recessive; S/C = subcutaneous

Figure Legends

Figure 1: Simplified Schematic of Insulin Signalling Pathway with Known Human Monogenic Disorders. Genes that have been implicated in Mendelian disorders of insulin signalling are shown in red with the corresponding disease and year of discovery in linked boxes. Cellular process stimulated by insulin are shown in green, while those inhibited by insulin are shown in red italics. INS = insulin; INSR = the insulin receptor; IRS1/2 = Insulin receptor substrate 1/2; Phosphatidylinositol-3-Kinase is illustrated as a heterodimer of of one of two catalytic subunits (p110α or p110β) and one of four regulatory subunits, three of which (p85α, p55α, p50α) are encoded by the *PIK3R1* gene mutated in SHORT syndrome. PIP_2 = Phosphatidylinositol-(4,5)-bisphosphate; PIP_3 = Phosphatidylinositol-(3,4,5)-trisphosphate; PDK1 = 3-phosphoinositide dependent protein kinase-1; AKT = Protein kinase B; GSK3 = Glycogen synthase kinase 3; FOXO1 = Forkhead box protein O1 (FOXO1); mTORC1/2 = Mammalian target of rapamycin complex 1/2; TBC1D4 = gene encoding AKT substrate of 160KDa; BAD = ;PDE3B = ;SREBP1c = ; SHC = Src-homology-2-containing protein; GRB2 = Growth factor receptor-bound protein 2; SOS = Son of Sevenless; MEK = MAPK/ERK Kinase ;ERK = Extracellular signal-regulated kinases.

Figure 2: Principle of Mendelian Randomisation. Algorithm for the use of Mendelian disorders to test possible causality underlying biological association, illustrated by the example of LDL cholesterol and atherosclerosis.

Figure 3: Distribution of Adipose Loss in Lipodystrophies. Areas of deficient adipose tissue are illusrtaed in red, with preserved adipose tissue represented in green. CGL = congenital generalised lipodytrophy; FPLD = familial partial lipodystrophy; APLD = acquired partial lipodystrophy; WAT = white adipose tissue; s/c = subcutaneous; BM = bone marrow; IR = insulin resisance; DL = dyslipidaemia; NAFLD = non alcoholic fatty liver disease.

Figure 4: Models for Role of Partial Insulin Resistance in Disease Pathogenesis. Hypoglycaemic and lipogenic actions of insulin are used to illustrate each model. **A.** Cell autonomous selective signalling defect model, with disproportionate attenuation of insulin action to lower glucose, leading to compensatory hyperinsulinaemia, and exposure of intact insulin signalling pathway, to higher levels of insulin action. **B.** Differential dose response model. This is a variation of the above theme whereby a fixed reduction in insulin action across all arms of the signalling pathway has a greater effect on arms of the pathway showing a shallow dose response curve to insulin than those showing a steep dose response curve. **C.** Differential tissue resistance to insulin model. Where an organ with the dominant role in insulin-mediated glucose disposal is more insulin resistant than other tissues, it effectively sets the level of systemic insulin, potentially exposing other more responsive tissues to abnormally high insulin action. These models are non mutually exclusive and may all play some role *in vivo*.

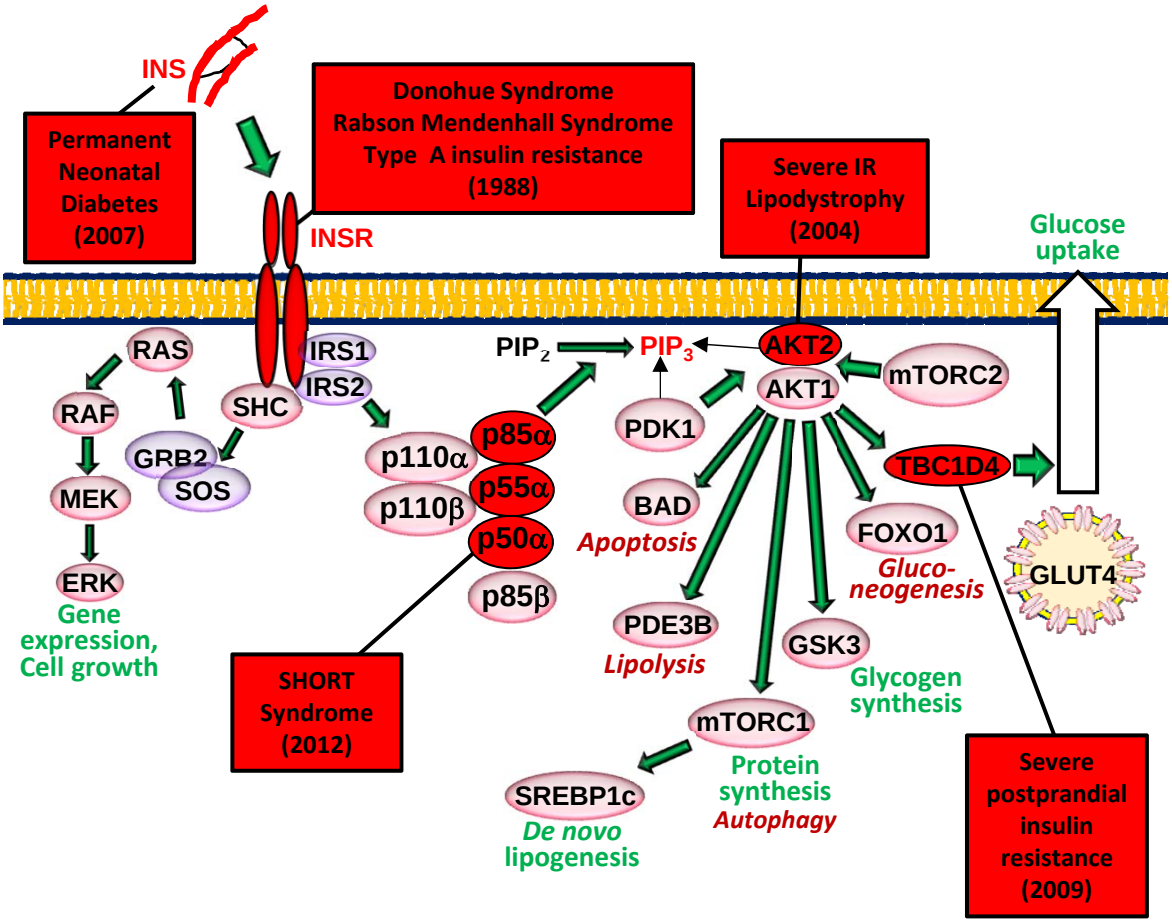


Figure 1: Insulin Signalling Pathway with Known Human Monogenic Disorders

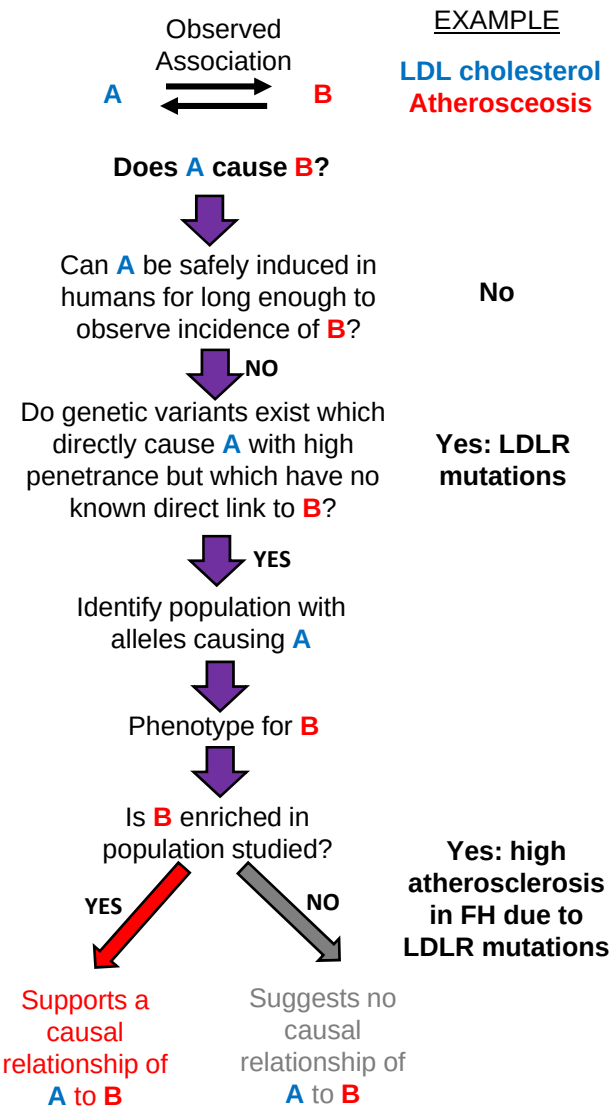
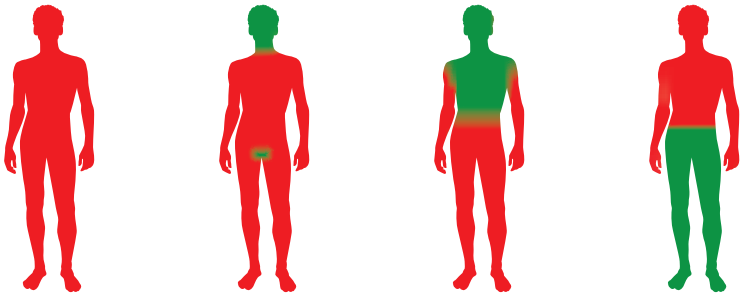


Figure 2: Principle of Mendelian Randomisation



Form of LD	CGL	FPLD2	Other FPLD	APLD
Genes	***AGPAT2, BSLC2*** *PTRF, CAV1*	***LMNA***	***PPARG***, *PLIN1 AKT2, CIDEC*	---
Fat preserved	Sometimes mechanical WAT, BM WAT	visceral WAT head/neck WAT labial WAT	visceral WAT some s/c truncal WAT	infra-umbilical WAT visceral WAT
Metabolic Profile	**Severe IR** **Severe DL** Severe NAFLD	**Severe IR** **Severe DL** Severe NAFLD	**Severe IR** **Severe DL** Severe NAFLD	**Moderate or no IR** **Little DL** Little or no NAFLD

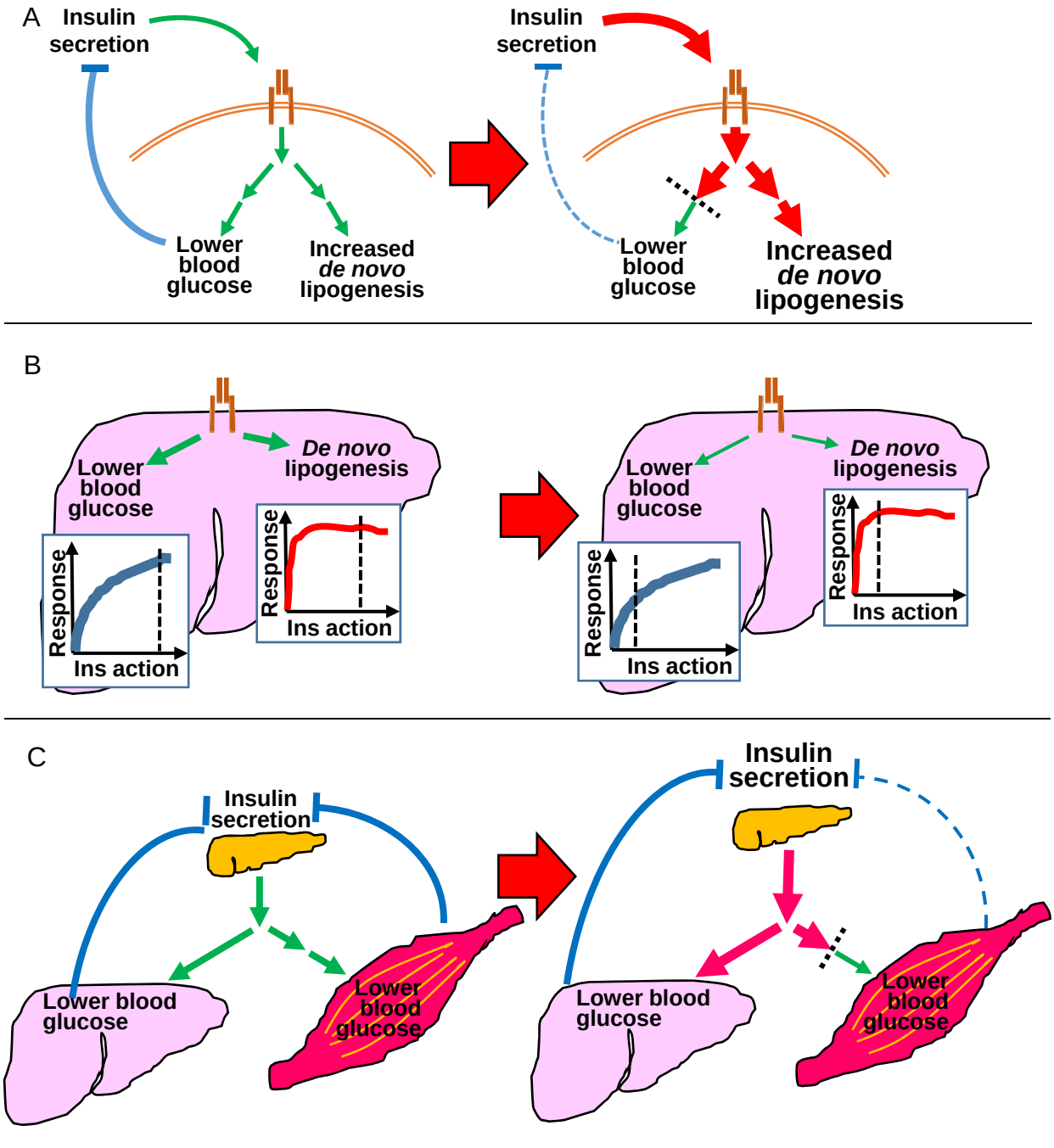


Figure 4: Models for Role of Partial Insulin Resistance in Disease Pathogenesis