

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

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1 **Abstract**

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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.

## 1 **Origins of the concept of insulin resistance**

2 The discovery and clinical introduction of purified insulin in the early 1920s  
3 transformed diabetes mellitus from a rapidly fatal childhood malady into a  
4 manageable chronic disease. However as the effort to translate therapeutic proof of  
5 principle into cheap, widely available insulin treatment gathered momentum in  
6 ensuing years, it became apparent that a subgroup of people with diabetes did not  
7 exhibit the dramatic response of the emaciated, ketotic patients first treated. Most  
8 prominent in studying and articulating this was Harold Himsworth who, in the late  
9 1930s, discriminated between “sensitive diabetics” and “insensitive diabetics”  
10 (generally older, obese and hypertensive, commonly with arteriosclerosis, and  
11 exhibiting insidious disease onset with rare ketosis and insulin-induced  
12 hypoglycaemia)<sup>1</sup>. Physiological studies showed that “insulin insensitive” patients  
13 were slow to show a hypoglycaemic response to exogenous insulin<sup>1</sup>. Upon  
14 development of radioimmunoassays in 1960, formal proof was obtained that insulin  
15 resistant individuals do indeed have high or normal insulin levels in the face of  
16 hyperglycaemia<sup>2,3</sup>.

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

## 25 **Mechanisms of insulin action**

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

1 dimeric tyrosine kinase<sup>11</sup>, a member of what is now known as a large family of  
2 receptor tyrosine kinases of immense medical significance in endocrinology, cancer,  
3 and beyond.

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

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

1 potential for huge combinatorial signalling complexity. Finally, cellular studies are  
2 beginning to unpick the importance of different temporal sequences of signalling  
3 events after receptor stimulation, which, allied to different thresholds for activation of  
4 metabolic endpoints, introduces yet more tiers of signalling complexity<sup>15</sup>.

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

### 11 **Quantitative Definitions of Insulin Resistance**

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

1 surrogate indices of IR based on other plasma analytes<sup>18, 19</sup>, none of these has yet  
2 been widely clinically adopted.

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

### 13 **Mendelian Randomisation to Test Causality in Human Disease**

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

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

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

## 5 **An Analytic "Toolkit": Subtypes of Human Monogenic Insulin Resistance**

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

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

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

## 10 **Primary Lipodystrophies**

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

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



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

16 The second gene to be affected in familial partial lipodystrophy is PPARG<sup>44</sup>,  
17 encoding PPAR $\gamma$ , a nuclear hormone receptor most highly expressed in adipose  
18 tissue where it is essential for adipocyte differentiation, and which is the target of the  
19 thiazolidinedione class of antidiabetic agents. All known pathogenic mutations are  
20 heterozygous, and most have been shown to inhibit the function of co-expressed  
21 wildtype protein.

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

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

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

#### 4 **Complex Monogenic Disorders Featuring Severe Insulin Resistance**

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

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

## 1 **Application of Mendelian Randomisation to Obesity-Associated Metabolic** 2 **Disease**

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

### 9 **How does Obesity Give Rise to Insulin Resistance?**

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

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

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

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

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

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

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

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

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

#### 4 **Does Insulin Resistance Cause Fatty Liver and Dyslipidaemia?**

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

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

28 These human observations suggest that "partial" IR – that is, IR affecting only  
29 some parts of the insulin signalling network, may play an important role in the  
30 pathogenesis of major IR-related pathologies. This hypothesis was first widely  
31 promulgated in the 1980s<sup>4</sup>, and has been supported previously by observations

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

### 17 **Does Insulin Resistance Cause Polycystic Ovary Syndrome?**

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

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

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

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

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



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

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

## 21 **Does Insulin Resistance Cause Cancer?**

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

1 cancer in lipodystrophies<sup>95</sup>. However it has also been argued, with some  
2 experimental evidence, that insulin may directly drive tumour growth<sup>93, 96</sup>.

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

## 19 **Conclusions and Future Directions**

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

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

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## 11 **References**

- 12 1. Himsworth HP. Insulin Deficiency and Insulin Inefficiency. *Br Med J* 1940 **1** 719-722.
- 13 2. Yalow RS & Berson SA. Plasma insulin concentrations in nondiabetic and early diabetic  
14 subjects. Determinations by a new sensitive immuno-assay technic. *Diabetes* 1960 **9** 254-  
15 260.
- 16 3. Karam JH, Grodsky GM & Forsham PH. Excessive insulin response to glucose in obese  
17 subjects as measured by immunochemical assay. *Diabetes* 1963 **12** 197-204.
- 18 4. Reaven GM. Pathophysiology of insulin resistance in human disease. *Physiol Rev* 1995 **75**  
19 473-486.
- 20 5. Hanson RL, Imperatore G, Bennett PH & Knowler WC. Components of the "metabolic  
21 syndrome" and incidence of type 2 diabetes. *Diabetes* 2002 **51** 3120-3127.
- 22 6. Utzschneider KM & Kahn SE. Review: The role of insulin resistance in nonalcoholic fatty liver  
23 disease. *J Clin Endocrinol Metab* 2006 **91** 4753-4761.
- 24 7. Dunaif A, Segal KR, Futterweit W & Dobrjansky A. Profound peripheral insulin resistance,  
25 independent of obesity, in polycystic ovary syndrome. *Diabetes* 1989 **38** 1165-1174.
- 26 8. Pollak M. Insulin and insulin-like growth factor signalling in neoplasia. *Nat Rev Cancer* 2008 **8**  
27 915-928.
- 28 9. Freychet P, Roth J & Neville DM, Jr. Insulin receptors in the liver: specific binding of ( 125  
29 I)insulin to the plasma membrane and its relation to insulin bioactivity. *Proc Natl Acad Sci U*  
30 *SA* 1971 **68** 1833-1837.
- 31 10. Yip CC, Yeung CW & Moule ML. Photoaffinity labeling of insulin receptor of rat adipocyte  
32 plasma membrane. *J Biol Chem* 1978 **253** 1743-1745.
- 33 11. Ullrich A, Bell JR, Chen EY, Herrera R, Petruzzelli LM, Dull TJ, Gray A, Coussens L, Liao YC,  
34 Tsubokawa M, et al. Human insulin receptor and its relationship to the tyrosine kinase family  
35 of oncogenes. *Nature* 1985 **313** 756-761.
- 36 12. Taniguchi CM, Emanuelli B & Kahn CR. Critical nodes in signalling pathways: insights into  
37 insulin action. *Nat Rev Mol Cell Biol* 2006 **7** 85-96.
- 38 13. Cohen P. The twentieth century struggle to decipher insulin signalling. *Nat Rev Mol Cell Biol*  
39 2006 **7** 867-873.
- 40 14. Whiteman EL, Cho H & Birnbaum MJ. Role of Akt/protein kinase B in metabolism. *Trends*  
41 *Endocrinol Metab* 2002 **13** 444-451.

- 1 15. Humphrey SJ, James DE & Mann M. Protein Phosphorylation: A Major Switch Mechanism for  
2 Metabolic Regulation. *Trends Endocrinol Metab* 2015.
- 3 16. McGarry JD. What if Minkowski had been ageusic? An alternative angle on diabetes. *Science*  
4 1992 **258** 766-770.
- 5 17. Muniyappa R & Quon MJ. Assessing Insulin Sensitivity and Resistance in Humans. In  
6 *Endotext*. Eds LJ De Groot, P Beck-Peccoz, G Chrousos, K Dungan, A Grossman, JM  
7 Hershman, C Koch, R McLachlan, M New, R Rebar, F Singer, A Vinik & MO Weickert. South  
8 Dartmouth (MA), 2000.
- 9 18. Guerrero-Romero F, Simental-Mendia LE, Gonzalez-Ortiz M, Martinez-Abundis E, Ramos-  
10 Zavala MG, Hernandez-Gonzalez SO, Jacques-Camarena O & Rodriguez-Moran M. The  
11 product of triglycerides and glucose, a simple measure of insulin sensitivity. Comparison  
12 with the euglycemic-hyperinsulinemic clamp. *J Clin Endocrinol Metab* 2010 **95** 3347-3351.
- 13 19. Finucane FM, Luan J, Wareham NJ, Sharp SJ, O'Rahilly S, Balkau B, Flyvbjerg A, Walker M,  
14 Hojlund K, Nolan JJ, et al. Correlation of the leptin:adiponectin ratio with measures of insulin  
15 resistance in non-diabetic individuals. *Diabetologia* 2009 **52** 2345-2349.
- 16 20. Goldstein JL & Brown MS. The low-density lipoprotein pathway and its relation to  
17 atherosclerosis. *Annu Rev Biochem* 1977 **46** 897-930.
- 18 21. Yoshimasa Y, Seino S, Whittaker J, Kakehi T, Kosaki A, Kuzuya H, Imura H, Bell GI & Steiner  
19 DF. Insulin-resistant diabetes due to a point mutation that prevents insulin proreceptor  
20 processing. *Science* 1988 **240** 784-787.
- 21 22. Kadowaki T, Bevins CL, Cama A, Ojamaa K, Marcus-Samuels B, Kadowaki H, Beitz L, McKeon  
22 C & Taylor SI. Two mutant alleles of the insulin receptor gene in a patient with extreme  
23 insulin resistance. *Science* 1988 **240** 787-790.
- 24 23. Semple RK, Savage DB, Cochran EK, Gorden P & O'Rahilly S. Genetic syndromes of severe  
25 insulin resistance. *Endocr Rev* 2011 **32** 498-514.
- 26 24. Weiss RE. *Genetic diagnosis of endocrine disorders*. Boston, MA: Elsevier, 2015.
- 27 25. Taylor SI, Cama A, Accili D, Barbetti F, Quon MJ, de la Luz Sierra M, Suzuki Y, Koller E, Levy-  
28 Toledano R, Wertheimer E, et al. Mutations in the insulin receptor gene. *Endocr Rev* 1992 **13**  
29 566-595.
- 30 26. George S, Rochford JJ, Wolfrum C, Gray SL, Schinner S, Wilson JC, Soos MA, Murgatroyd PR,  
31 Williams RM, Acerini CL, et al. A family with severe insulin resistance and diabetes due to a  
32 mutation in AKT2. *Science* 2004 **304** 1325-1328.
- 33 27. Dash S, Sano H, Rochford JJ, Semple RK, Yeo G, Hyden CS, Soos MA, Clark J, Rodin A,  
34 Langenberg C, et al. A truncation mutation in TBC1D4 in a family with acanthosis nigricans  
35 and postprandial hyperinsulinemia. *Proc Natl Acad Sci U S A* 2009 **106** 9350-9355.
- 36 28. Moltke I, Grarup N, Jorgensen ME, Bjerregaard P, Treebak JT, Fumagalli M, Korneliusen TS,  
37 Andersen MA, Nielsen TS, Krarup NT, et al. A common Greenlandic TBC1D4 variant confers  
38 muscle insulin resistance and type 2 diabetes. *Nature* 2014 **512** 190-193.
- 39 29. Thauvin-Robinet C, Auclair M, Duplomb L, Caron-Debarle M, Avila M, St-Onge J, Le Merrer  
40 M, Le Luyer B, Heron D, Mathieu-Dramard M, et al. PIK3R1 mutations cause syndromic  
41 insulin resistance with lipoatrophy. *Am J Hum Genet* 2013 **93** 141-149.
- 42 30. Dymont DA, Smith AC, Alcantara D, Schwartzentruber JA, Basel-Vanagaite L, Curry CJ,  
43 Temple IK, Reardon W, Mansour S, Haq MR, et al. Mutations in PIK3R1 cause SHORT  
44 syndrome. *Am J Hum Genet* 2013 **93** 158-166.
- 45 31. Chudasama KK, Winnay J, Johansson S, Claudi T, Konig R, Haldorsen I, Johansson B, Woo JR,  
46 Aarskog D, Sagen JV, et al. SHORT syndrome with partial lipodystrophy due to impaired  
47 phosphatidylinositol 3 kinase signaling. *Am J Hum Genet* 2013 **93** 150-157.
- 48 32. Seip M & Trygstad O. Generalized Lipodystrophy. *Arch Dis Child* 1963 **38** 447-453.

- 1 33. Garg A, Wilson R, Barnes R, Arioglu E, Zaidi Z, Gurakan F, Kocak N, O'Rahilly S, Taylor SI, Patel  
2 SB, et al. A gene for congenital generalized lipodystrophy maps to human chromosome  
3 9q34. *J Clin Endocrinol Metab* 1999 **84** 3390-3394.
- 4 34. Agarwal AK, Arioglu E, De Almeida S, Akkoc N, Taylor SI, Bowcock AM, Barnes RI & Garg A.  
5 AGPAT2 is mutated in congenital generalized lipodystrophy linked to chromosome 9q34. *Nat*  
6 *Genet* 2002 **31** 21-23.
- 7 35. Magre J, Delepine M, Khallouf E, Gedde-Dahl T, Jr., Van Maldergem L, Sobel E, Papp J, Meier  
8 M, Megarbane A, Bachy A, et al. Identification of the gene altered in Berardinelli-Seip  
9 congenital lipodystrophy on chromosome 11q13. *Nat Genet* 2001 **28** 365-370.
- 10 36. Payne VA, Grimsey N, Tuthill A, Virtue S, Gray SL, Dalla Nora E, Semple RK, O'Rahilly S &  
11 Rochford JJ. The human lipodystrophy gene BSCL2/seipin may be essential for normal  
12 adipocyte differentiation. *Diabetes* 2008 **57** 2055-2060.
- 13 37. Szymanski KM, Binns D, Bartz R, Grishin NV, Li WP, Agarwal AK, Garg A, Anderson RG &  
14 Goodman JM. The lipodystrophy protein seipin is found at endoplasmic reticulum lipid  
15 droplet junctions and is important for droplet morphology. *Proc Natl Acad Sci U S A* 2007  
16 **104** 20890-20895.
- 17 38. Boutet E, El Mourabit H, Prot M, Nemani M, Khallouf E, Colard O, Maurice M, Durand-  
18 Schneider AM, Chretien Y, Gres S, et al. Seipin deficiency alters fatty acid Delta9  
19 desaturation and lipid droplet formation in Berardinelli-Seip congenital lipodystrophy.  
20 *Biochimie* 2009 **91** 796-803.
- 21 39. Kim CA, Delepine M, Boutet E, El Mourabit H, Le Lay S, Meier M, Nemani M, Bridel E, Leite  
22 CC, Bertola DR, et al. Association of a homozygous nonsense caveolin-1 mutation with  
23 Berardinelli-Seip congenital lipodystrophy. *J Clin Endocrinol Metab* 2008 **93** 1129-1134.
- 24 40. Hayashi YK, Matsuda C, Ogawa M, Goto K, Tominaga K, Mitsuhashi S, Park YE, Nonaka I,  
25 Hino-Fukuyo N, Haginoya K, et al. Human PTRF mutations cause secondary deficiency of  
26 caveolins resulting in muscular dystrophy with generalized lipodystrophy. *J Clin Invest* 2009  
27 **119** 2623-2633.
- 28 41. Payne F, Lim K, Grousse A, Brown RJ, Kory N, Robbins A, Xue Y, Sleigh A, Cochran E, Adams  
29 C, et al. Mutations disrupting the Kennedy phosphatidylcholine pathway in humans with  
30 congenital lipodystrophy and fatty liver disease. *Proc Natl Acad Sci U S A* 2014 **111** 8901-  
31 8906.
- 32 42. Shackleton S, Lloyd DJ, Jackson SN, Evans R, Niermeijer MF, Singh BM, Schmidt H, Brabant G,  
33 Kumar S, Durrington PN, et al. LMNA, encoding lamin A/C, is mutated in partial  
34 lipodystrophy. *Nat Genet* 2000 **24** 153-156.
- 35 43. Rankin J & Ellard S. The laminopathies: a clinical review. *Clin Genet* 2006 **70** 261-274.
- 36 44. Semple RK, Chatterjee VK & O'Rahilly S. PPAR gamma and human metabolic disease. *J Clin*  
37 *Invest* 2006 **116** 581-589.
- 38 45. Rubio-Cabezas O, Puri V, Murano I, Saudek V, Semple RK, Dash S, Hyden CS, Bottomley W,  
39 Vigouroux C, Magre J, et al. Partial lipodystrophy and insulin resistant diabetes in a patient  
40 with a homozygous nonsense mutation in CIDEC. *EMBO Mol Med* 2009 **1** 280-287.
- 41 46. Gandotra S, Le Dour C, Bottomley W, Cervera P, Giral P, Reznik Y, Charpentier G, Auclair M,  
42 Delepine M, Barroso I, et al. Perilipin deficiency and autosomal dominant partial  
43 lipodystrophy. *N Engl J Med* 2011 **364** 740-748.
- 44 47. Yamada K, Ikegami H, Yoneda H, Miki T & Ogihara T. All patients with Werner's syndrome are  
45 insulin resistant, but only those who also have impaired insulin secretion develop overt  
46 diabetes. *Diabetes Care* 1999 **22** 2094-2095.
- 47 48. Imura H, Nakao Y, Kuzuya H, Okamoto M, Okamoto M & Yamada K. Clinical, endocrine and  
48 metabolic aspects of the Werner syndrome compared with those of normal aging. *Adv Exp*  
49 *Med Biol* 1985 **190** 171-185.

- 1 49. Diaz A, Vogiatzi MG, Sanz MM & German J. Evaluation of short stature, carbohydrate  
2 metabolism and other endocrinopathies in Bloom's syndrome. *Horm Res* 2006 **66** 111-117.
- 3 50. Payne F, Colnaghi R, Rocha N, Seth A, Harris J, Carpenter G, Bottomley WE, Wheeler E, Wong  
4 S, Saudek V, et al. Hypomorphism in human NSMCE2 linked to primordial dwarfism and  
5 insulin resistance. *J Clin Invest* 2014 **124** 4028-4038.
- 6 51. Weedon MN, Ellard S, Prindle MJ, Caswell R, Lango Allen H, Oram R, Godbole K, Yajnik CS,  
7 Sbraccia P, Novelli G, et al. An in-frame deletion at the polymerase active site of POLD1  
8 causes a multisystem disorder with lipodystrophy. *Nat Genet* 2013 **45** 947-950.
- 9 52. Mayson SE, Parker VE, Schutta MH, Semple RK & Rickels MR. Severe insulin resistance and  
10 hypertriglyceridemia after childhood total body irradiation. *Endocr Pract* 2013 **19** 51-58.
- 11 53. Ablamunits V, Weisberg SP, Lemieux JE, Combs TP & Klebanov S. Reduced adiposity in ob/ob  
12 mice following total body irradiation and bone marrow transplantation. *Obesity (Silver  
13 Spring)* 2007 **15** 1419-1429.
- 14 54. Marshall JD, Maffei P, Collin GB & Naggert JK. Alstrom syndrome: genetics and clinical  
15 overview. *Curr Genomics* 2011 **12** 225-235.
- 16 55. Huang-Doran I, Bicknell LS, Finucane FM, Rocha N, Porter KM, Tung YC, Szekeres F, Krook A,  
17 Nolan JJ, O'Driscoll M, et al. Genetic defects in human pericentrin are associated with severe  
18 insulin resistance and diabetes. *Diabetes* 2011 **60** 925-935.
- 19 56. Rauch A, Thiel CT, Schindler D, Wick U, Crow YJ, Ekici AB, van Essen AJ, Goecke TO, Al-Gazali  
20 L, Chrzanowska KH, et al. Mutations in the pericentrin (PCNT) gene cause primordial  
21 dwarfism. *Science* 2008 **319** 816-819.
- 22 57. Chen JH, Segni M, Payne F, Huang-Doran I, Sleigh A, Adams C, Consortium UK, Savage DB,  
23 O'Rahilly S, Semple RK, et al. Truncation of POC1A associated with short stature and extreme  
24 insulin resistance. *J Mol Endocrinol* 2015 **55** 147-158.
- 25 58. Gorden P, Lupsa BC, Chong AY & Lungu AO. Is there a human model for the 'metabolic  
26 syndrome' with a defined aetiology? *Diabetologia* 2010 **53** 1534-1536.
- 27 59. Virtue S & Vidal-Puig A. It's not how fat you are, it's what you do with it that counts. *PLoS  
28 Biol* 2008 **6** e237.
- 29 60. Shulman GI. Cellular mechanisms of insulin resistance. *J Clin Invest* 2000 **106** 171-176.
- 30 61. Danforth E, Jr. Failure of adipocyte differentiation causes type II diabetes mellitus? *Nat  
31 Genet* 2000 **26** 13.
- 32 62. Robbins AL & Savage DB. The genetics of lipid storage and human lipodystrophies. *Trends  
33 Mol Med* 2015 **21** 433-438.
- 34 63. Misra A, Peethambaram A & Garg A. Clinical features and metabolic and autoimmune  
35 derangements in acquired partial lipodystrophy: report of 35 cases and review of the  
36 literature. *Medicine (Baltimore)* 2004 **83** 18-34.
- 37 64. Cook JR & Semple RK. Hypoadiponectinemia--cause or consequence of human "insulin  
38 resistance"? *J Clin Endocrinol Metab* 2010 **95** 1544-1554.
- 39 65. Ye R & Scherer PE. Adiponectin, driver or passenger on the road to insulin sensitivity? *Mol  
40 Metab* 2013 **2** 133-141.
- 41 66. Semple RK, Soos MA, Luan J, Mitchell CS, Wilson JC, Gurnell M, Cochran EK, Gorden P,  
42 Chatterjee VK, Wareham NJ, et al. Elevated plasma adiponectin in humans with genetically  
43 defective insulin receptors. *J Clin Endocrinol Metab* 2006 **91** 3219-3223.
- 44 67. Semple RK, Halberg NH, Burling K, Soos MA, Schraw T, Luan J, Cochran EK, Dunger DB,  
45 Wareham NJ, Scherer PE, et al. Paradoxical elevation of high-molecular weight adiponectin  
46 in acquired extreme insulin resistance due to insulin receptor antibodies. *Diabetes* 2007 **56**  
47 1712-1717.
- 48 68. Semple RK, Cochran EK, Soos MA, Burling KA, Savage DB, Gorden P & O'Rahilly S. Plasma  
49 adiponectin as a marker of insulin receptor dysfunction: clinical utility in severe insulin  
50 resistance. *Diabetes Care* 2008 **31** 977-979.

- 1 69. Mente A, Meyre D, Lanktree MB, Heydarpour M, Davis AD, Miller R, Gerstein H, Hegele RA,  
2 Yusuf S, Anand SS, et al. Causal relationship between adiponectin and metabolic traits: a  
3 Mendelian randomization study in a multiethnic population. *PLoS One* 2013 **8** e66808.
- 4 70. Gao H, Fall T, van Dam RM, Flyvbjerg A, Zethelius B, Ingelsson E & Hagg S. Evidence of a  
5 causal relationship between adiponectin levels and insulin sensitivity: a Mendelian  
6 randomization study. *Diabetes* 2013 **62** 1338-1344.
- 7 71. Yaghootkar H, Lamina C, Scott RA, Dastani Z, Hivert MF, Warren LL, Stancakova A, Buxbaum  
8 SG, Lyytikainen LP, Henneman P, et al. Mendelian randomization studies do not support a  
9 causal role for reduced circulating adiponectin levels in insulin resistance and type 2  
10 diabetes. *Diabetes* 2013 **62** 3589-3598.
- 11 72. Bueno AC, Sun K, Martins CS, Elias Junior J, Miranda W, Tao C, Foss-Freitas MC, Barbieri MA,  
12 Bettiol H, de Castro M, et al. A novel ADIPOQ mutation (p.M40K) impairs assembly of high-  
13 molecular-weight adiponectin and is associated with early-onset obesity and metabolic  
14 syndrome. *J Clin Endocrinol Metab* 2014 **99** E683-693.
- 15 73. Semple RK, Sleigh A, Murgatroyd PR, Adams CA, Bluck L, Jackson S, Vottero A, Kanabar D,  
16 Charlton-Menys V, Durrington P, et al. Postreceptor insulin resistance contributes to human  
17 dyslipidemia and hepatic steatosis. *J Clin Invest* 2009 **119** 315-322.
- 18 74. Lambert JE, Ramos-Roman MA, Browning JD & Parks EJ. Increased de novo lipogenesis is a  
19 distinct characteristic of individuals with nonalcoholic fatty liver disease. *Gastroenterology*  
20 2014 **146** 726-735.
- 21 75. Donnelly KL, Smith CI, Schwarzenberg SJ, Jessurun J, Boldt MD & Parks EJ. Sources of fatty  
22 acids stored in liver and secreted via lipoproteins in patients with nonalcoholic fatty liver  
23 disease. *J Clin Invest* 2005 **115** 1343-1351.
- 24 76. Brown MS & Goldstein JL. Selective versus total insulin resistance: a pathogenic paradox. *Cell*  
25 *Metab* 2008 **7** 95-96.
- 26 77. Wan M, Leavens KF, Saleh D, Easton RM, Guertin DA, Peterson TR, Kaestner KH, Sabatini DM  
27 & Birnbaum MJ. Postprandial hepatic lipid metabolism requires signaling through Akt2  
28 independent of the transcription factors FoxA2, FoxO1, and SREBP1c. *Cell Metab* 2011 **14**  
29 516-527.
- 30 78. Leavens KF, Easton RM, Shulman GI, Previs SF & Birnbaum MJ. Akt2 is required for hepatic  
31 lipid accumulation in models of insulin resistance. *Cell Metab* 2009 **10** 405-418.
- 32 79. Cook JR, Langlet F, Kido Y & Accili D. Pathogenesis of selective insulin resistance in isolated  
33 hepatocytes. *J Biol Chem* 2015 **290** 13972-13980.
- 34 80. Parker VE, Savage DB, O'Rahilly S & Semple RK. Mechanistic insights into insulin resistance in  
35 the genetic era. *Diabet Med* 2011 **28** 1476-1486.
- 36 81. Nandi A, Chen Z, Patel R & Poretsky L. Polycystic ovary syndrome. *Endocrinol Metab Clin*  
37 *North Am* 2014 **43** 123-147.
- 38 82. Goodarzi MO, Dumesic DA, Chazenbalk G & Azziz R. Polycystic ovary syndrome: etiology,  
39 pathogenesis and diagnosis. *Nat Rev Endocrinol* 2011 **7** 219-231.
- 40 83. Dunaif A. Insulin resistance and the polycystic ovary syndrome: mechanism and implications  
41 for pathogenesis. *Endocr Rev* 1997 **18** 774-800.
- 42 84. Poretsky L, Seto-Young D, Shrestha A, Dhillon S, Mirjany M, Liu HC, Yih MC & Rosenwaks Z.  
43 Phosphatidyl-inositol-3 kinase-independent insulin action pathway(s) in the human ovary. *J*  
44 *Clin Endocrinol Metab* 2001 **86** 3115-3119.
- 45 85. O'Reilly M, Gathercole L, Capper F, Arlt W & Tomlinson J. Effect of insulin on AKR1C3  
46 expression in female adipose tissue: in-vivo and in-vitro study of adipose androgen  
47 generation in polycystic ovary syndrome. *Lancet* 2015 **385** Suppl 1 S16.
- 48 86. Weber DR, Stanescu DE, Semple R, Holland C & Magge SN. Continuous subcutaneous IGF-1  
49 therapy via insulin pump in a patient with Donohue syndrome. *J Pediatr Endocrinol Metab*  
50 2014 **27** 1237-1241.

- 1 87. Brisigotti M, Fabbretti G, Pesce F, Gatti R, Cohen A, Parenti G & Callea F. Congenital bilateral  
2 juvenile granulosa cell tumor of the ovary in leprechaunism: a case report. *Pediatr Pathol*  
3 1993 **13** 549-558.
- 4 88. Stubbs SA, Webber LJ, Stark J, Rice S, Margara R, Lavery S, Trew GH, Hardy K & Franks S. Role  
5 of Insulin-like growth factors in initiation of follicle growth in normal and polycystic human  
6 ovaries. *J Clin Endocrinol Metab* 2013 **98** 3298-3305.
- 7 89. Poretsky L, Cataldo NA, Rosenwaks Z & Giudice LC. The insulin-related ovarian regulatory  
8 system in health and disease. *Endocr Rev* 1999 **20** 535-582.
- 9 90. Kaltsas GA, Androulakis II, Tziveriotis K, Papadogias D, Tsikini A, Makras P, Dimitriou K,  
10 Stathopoulou A & Piaditis G. Polycystic ovaries and the polycystic ovary syndrome  
11 phenotype in women with active acromegaly. *Clin Endocrinol (Oxf)* 2007 **67** 917-922.
- 12 91. Arioglu E, Andewelt A, Diabo C, Bell M, Taylor SI & Gorden P. Clinical course of the syndrome  
13 of autoantibodies to the insulin receptor (type B insulin resistance): a 28-year perspective.  
14 *Medicine (Baltimore)* 2002 **81** 87-100.
- 15 92. Idkowiak J, Lavery GG, Dhir V, Barrett TG, Stewart PM, Krone N & Arlt W. Premature  
16 adrenarche: novel lessons from early onset androgen excess. *Eur J Endocrinol* 2011 **165** 189-  
17 207.
- 18 93. Giovannucci E, Harlan DM, Archer MC, Bergenstal RM, Gapstur SM, Habel LA, Pollak M,  
19 Regensteiner JG & Yee D. Diabetes and cancer: a consensus report. *Diabetes Care* 2010 **33**  
20 1674-1685.
- 21 94. Nead KT, Sharp SJ, Thompson DJ, Painter JN, Savage DB, Semple RK, Barker A, Australian  
22 National Endometrial Cancer Study G, Perry JR, Attia J, et al. Evidence of a Causal Association  
23 Between Insulinemia and Endometrial Cancer: A Mendelian Randomization Analysis. *J Natl*  
24 *Cancer Inst* 2015 **107**.
- 25 95. Safar Zadeh E, Lungu AO, Cochran EK, Brown RJ, Ghany MG, Heller T, Kleiner DE & Gorden P.  
26 The liver diseases of lipodystrophy: the long-term effect of leptin treatment. *J Hepatol* 2013  
27 **59** 131-137.
- 28 96. Belfiore A & Malaguarnera R. Insulin receptor and cancer. *Endocr Relat Cancer* 2011 **18**  
29 R125-147.

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## Tables

**Table 1: Known genetic forms of partial lipodystrophy**

<b>Clinical Disorder</b>	<b>Gene</b>	<b>Inheritance</b>	<b>Distribution of Adipose Loss</b>	<b>Comment</b>
FPLD2	<i>LMNA</i>	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	<i>PPARG</i>	AD	Limbs	LD may be subtle, limited to limbs, and associated with centripetal obesity
FPLD4	<i>PLIN1</i>	AD	Limbs	
FPLD6	<i>AKT2</i>	AD	Limbs	Single family reported to date
FPLD5	<i>CIDEA</i>	AR	Limbs	Single patient reported
SHORT Syndrome	<i>PIK3R1</i>	AD or sporadic	S/C, variable	Not always seen. Not associated with dyslipidaemia
MDP Syndrome	<i>POLD1</i>	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 (p110 $\alpha$  or p110 $\beta$ ) and one of four regulatory subunits, three of which (p85 $\alpha$ , p55 $\alpha$ , p50 $\alpha$ ) are encoded by the *PIK3R1* gene mutated in SHORT syndrome. PIP<sub>2</sub> = Phosphatidylinositol-(4,5)-bisphosphate; PIP<sub>3</sub> = 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.

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

7

#### 8 **Figure 4: Models for Role of Partial Insulin Resistance in Disease**

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

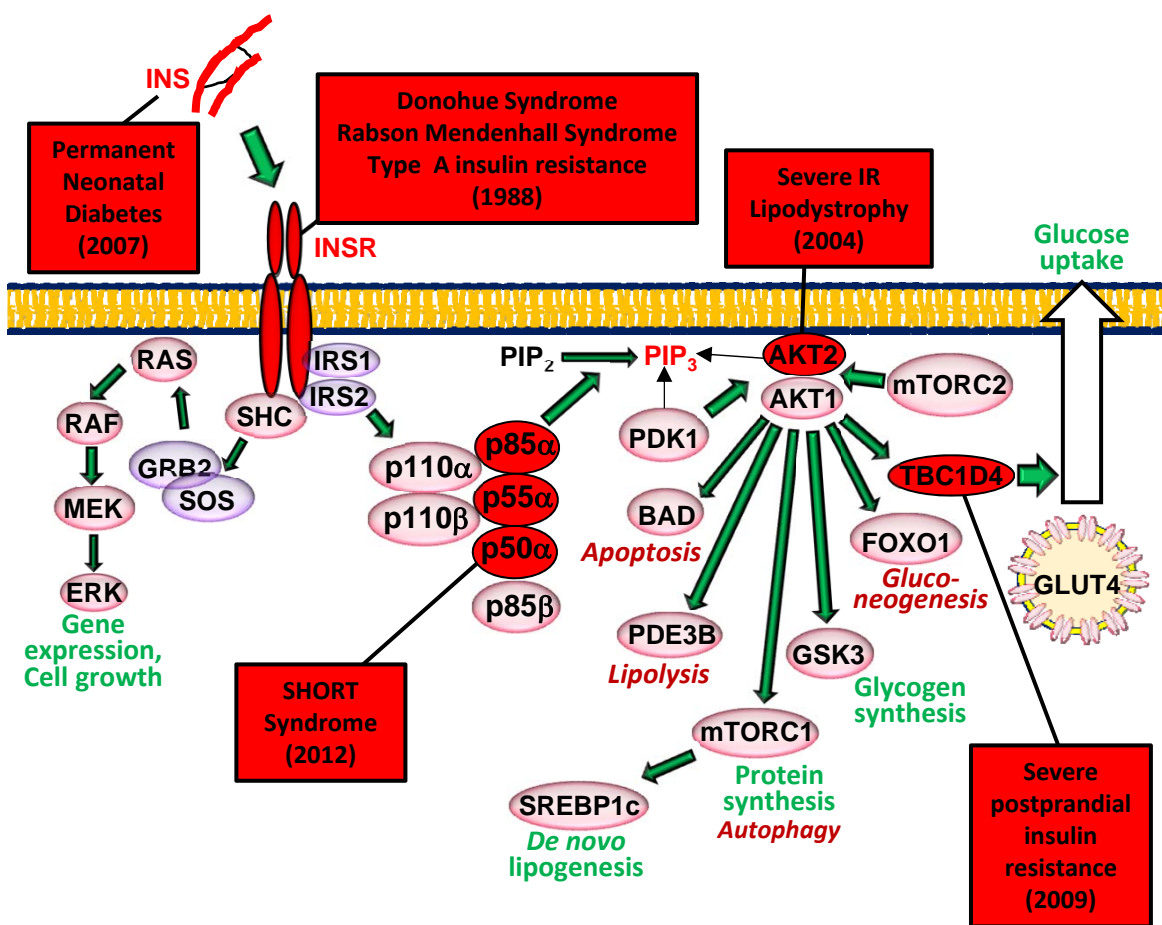


Figure 1: Insulin Signalling Pathway with Known Human Monogenic Disorders

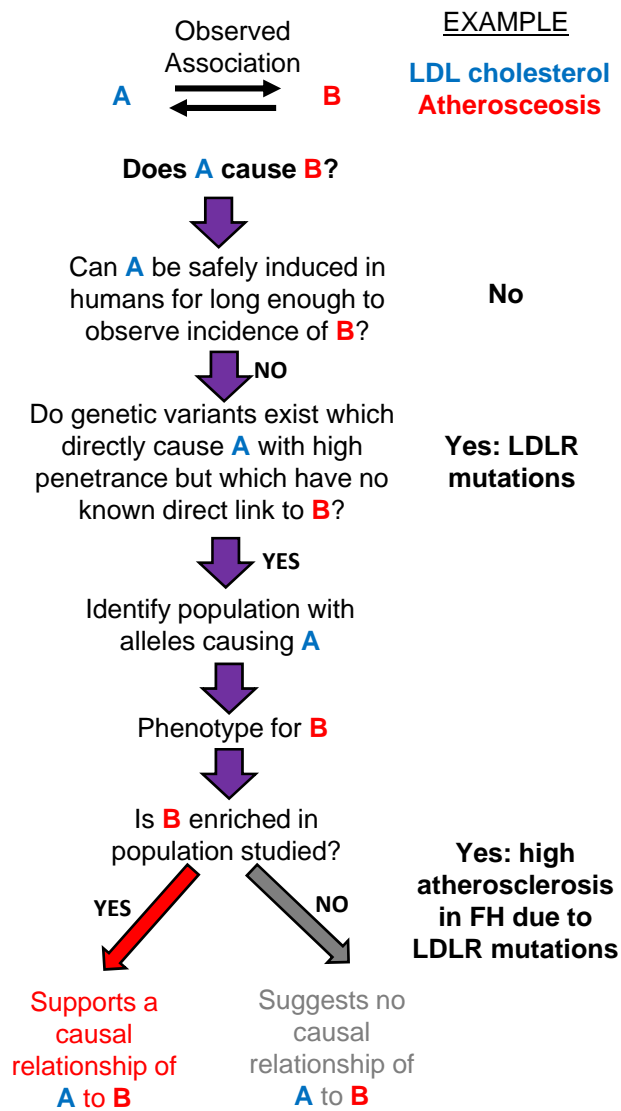


Figure 2: Principle of Mendelian Randomisation



**Form of LD**

CGL

FPLD2

Other FPLD

APLD

**Genes**

***AGPAT2, BSLC2***  
*PTRF, CAV1*

***LMNA***

***PPARG, PLIN1***  
*AKT2, CIDEA*

---

**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

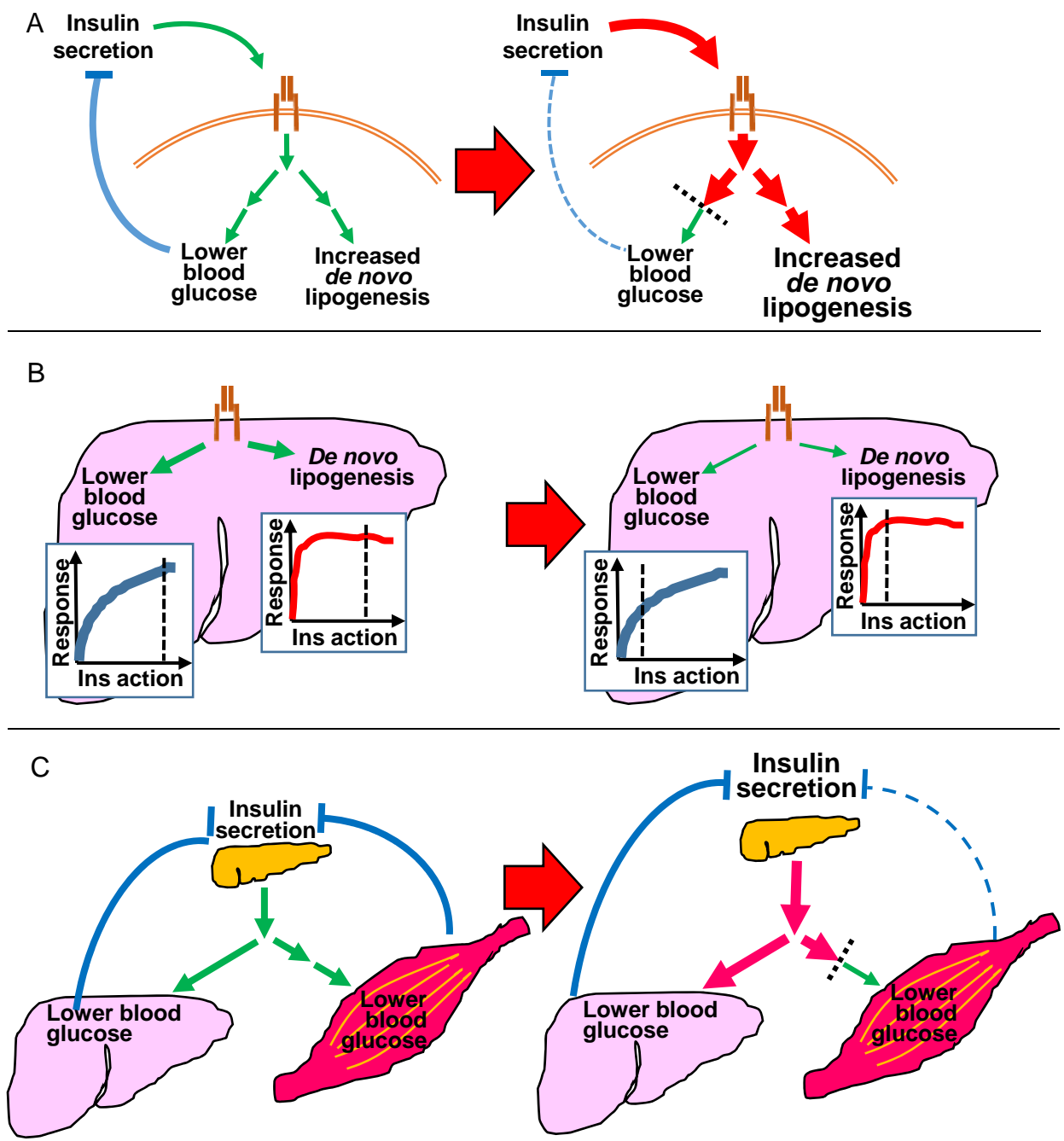


Figure 4: Models for Role of Partial Insulin Resistance in Disease Pathogenesis