

# A causal role for hyperinsulinemia in obesity

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## Abstract

Insulin modulates the biochemical pathways controlling lipid uptake, lipolysis and lipogenesis at multiple levels. Elevated insulin levels are associated with obesity, and conversely, dietary and pharmacological manipulations that reduce insulin have occasionally been reported to cause weight loss. However, the causal role of insulin hypersecretion in the development of mammalian obesity remained controversial in the absence of direct loss-of-function experiments. Here, we discuss theoretical considerations around the causal role of excess insulin for obesity, as well as recent studies employing mice that are genetically incapable of the rapid and sustained hyperinsulinemia that normally accompanies a high-fat diet. We also discuss new evidence demonstrating that modest reductions in circulating insulin prevent weight gain, with sustained effects that can persist after insulin levels normalize. Importantly, evidence from long-term studies reveals that a modest reduction in circulating insulin is not associated with impaired glucose homeostasis, meaning that body weight and lipid homeostasis are actually more sensitive to small changes in circulating insulin than glucose homeostasis in these models. Collectively, the evidence from new studies on genetic loss-of-function models forces a re-evaluation of current paradigms related to obesity, insulin resistance and diabetes. The potential for translation of these findings to humans is briefly discussed.

## Key Words

- ▶ insulin
- ▶ type 2 diabetes
- ▶ insulin resistance

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## Phylogeny and complexity of insulin-like peptides and the insulin signaling network

Insulin/Insulin-like growth factor 1 (IGF1)-like signaling is evolutionarily conserved, and its many functions are shared across species. This pathway plays a critical role in coordinating nutrient availability with energy storage, body size and longevity in both invertebrates and vertebrates (Fontana *et al.* 2010, van Heemst 2010, Rideout *et al.* 2015). Seminal studies have shown that worms or flies with reduced insulin peptides or insulin

signaling are leaner and live longer (Kenyon *et al.* 1993, Clancy *et al.* 2001).

Invertebrates produce numerous insulin-like peptides: there are at least 38 genes encoding putative insulin-like peptides in *Caenorhabditis elegans* (Pierce *et al.* 2001), and eight known genes in *Drosophila melanogaster* (Brogiolo *et al.* 2001). In mammals, the superfamily of insulin-like genes includes insulin, two insulin-like

growth factors (IGF1 and IGF2), relaxins (three relaxin genes in humans) and four additional insulin-like peptides (insulin-like peptides 3–6; Nakae *et al.* 2001, Shabanpoor *et al.* 2009, Fernandez & Torres-Aleman 2012). These mammalian peptides appear to be derived from a shared ancestral gene and have structural similarities, although the known biological functions of the majority of these peptides appear largely distinct from those of insulin and IGF1 (Shabanpoor *et al.* 2009). However, a recent study showed that insulin-like peptide 5 produced by enteroendocrine cells acts as an orexigenic hormone with circulating levels that change in response to feeding status, which suggests that this peptide also plays a role in coordinating energy homeostasis (Grosse *et al.* 2014).

Although most mammals and humans have only a single 'insulin' gene, mice and rats have two insulin peptides that are produced from separate insulin genes (Soares *et al.* 1985, Shiao *et al.* 2008). *Ins2* is the ancestral insulin gene that is analogous to human *INS*, whereas rodent-specific *Ins1* appears to have originated from the transposition of a reverse-transcribed, partially processed *Ins2* mRNA (Soares *et al.* 1985). Fully processed *INS1* and *INS2* peptides differ at the level of two B-chain amino acids, with additional differences evident in their C-peptides (Wentworth *et al.* 1986). Despite sharing sequence homology upstream of the transcription initiation site (Wentworth *et al.* 1986, Melloul *et al.* 2002), *Ins1* and *Ins2* do have notable differences with respect to certain promoter elements, expression patterns, translational efficiency and imprinting status (Wentworth *et al.* 1986, Linde *et al.* 1989, Deltour *et al.* 1995, Hay & Docherty 2006, Meur *et al.* 2011, Mehran *et al.* 2012). *Ins2* can be detected earlier in murine development and has a broader tissue distribution (Deltour *et al.* 1993, Fan *et al.* 2009, Mehran *et al.* 2012). Murine *Ins1* expression and promoter activity are largely restricted to the pancreas (Mehran *et al.* 2012, Thorens *et al.* 2015), and it does not contribute as much to pancreatic insulin production as *Ins2*, except in the mouse embryo during the early stages of pancreatic development (Wentworth *et al.* 1986, Linde *et al.* 1989, Deltour *et al.* 1993, Bengtsson *et al.* 2005). Complete inactivation of both *Ins* genes in mice (i.e. *Ins1<sup>-/-</sup>:Ins2<sup>-/-</sup>*) leads to severe diabetes and death within several days of birth, or weeks after conditional insulin gene deletion (Duvillie *et al.* 1998, Szabat *et al.* 2016). However, genetic inactivation of either *Ins* gene alone has not been reported to be associated with significant metabolic deficiencies (Leroux *et al.* 2001, Mehran *et al.* 2012, Templeman *et al.* 2015, 2016, Dionne *et al.* 2016). Instead, there is a partial compensatory elevation of

mRNA and protein of the corresponding non-deleted *Ins* gene, and increased beta-cell mass, which suggests a degree of redundancy between *Ins1* and *Ins2* (Leroux *et al.* 2001, Mehran *et al.* 2012, Templeman *et al.* 2015, 2016, Dionne *et al.* 2016). However, there is evidence to suggest that *Ins1* may have been subjected to positive selective mechanisms to maintain the two-*Ins* gene system in mice (Shiao *et al.* 2008), and it is unclear whether *INS1* and *INS2* or their peptide products have distinct functional roles. In unpublished studies, we have found that *Ins1* and *Ins2* peptides had similar biological activities using 3T3L1 pre-adipocyte differentiation as an assay. We and others have taken advantage of the unique properties of the two insulin genes to gain novel insight into the biology of this essential hormone.

Mammalian insulin/IGF1 signaling involves multiple receptors, and a network of signaling pathways can be triggered through activation of each receptor subtype, with the added complexity of interactions and crosstalk between the downstream pathways (White 2003, van Heemst 2010). The classical tyrosine kinase receptor ligands in mammals are insulin and IGF1, which bind with variable affinity to the primary mammalian insulin/IGF1 tyrosine kinase receptors, consisting of the insulin receptor (IR), which has two functionally distinct splice isoforms (IRa and IRb), and the IGF1 receptor (IGF1R; van Heemst 2010). Moreover, as these receptors are tetrameric proteins, functional hybrid heterodimers can form between insulin and IGF1 receptor subunits (e.g. IRa:IGF1R, IRb:IGF1R; van Heemst 2010). Receptor activation and subsequent phosphorylation of proximal effectors, such as the scaffolding proteins insulin receptor substrate (IRS) 1 or IRS2, initiate signal transduction via two major branches of insulin/IGF1 signaling: phosphatidylinositol 3-kinase (PI3K)/AKT and the mitogen-activated protein kinase (MAPK) pathways (Taniguchi *et al.* 2006). The precise mechanisms differentiating the downstream signaling of insulin receptors from IGF1 receptors have not been delineated (Nakae *et al.* 2001). However, insulin is widely considered to be the primary ligand of interest with respect to metabolic processes, despite the improbability of there being a complete divergence between the functions of insulin and IGF1 (Werner *et al.* 2008, Fu *et al.* 2013).

### Insulin as a key metabolic hormone

Insulin plays fundamental and conserved roles in maintaining energy homeostasis (Fontana *et al.* 2010, van Heemst 2010). Since the discovery of insulin in 1922

(Banting *et al.* 1922), the majority of research pertaining to this hormone has revolved around the two diseases of absolute and relative insulin insufficiency, known as type 1 diabetes and type 2 diabetes, respectively. Lately, there has been a resurgence of interest in conditions associated with excessive insulin production. Hyperinsulinemia, which for the purpose of this review will broadly refer to elevated basal and/or stimulated insulin secretion, is associated with obesity, and is nearly universal in the early stages of type 2 diabetes (Tabak *et al.* 2009). However, these correlative associations do not provide information as to whether excess insulin might be a causal contributor to obesity and its associated complications.

Circulating insulin levels are rapidly adjusted in response to nutrients in the blood, including glucose, and undergo sustained basal alterations in response to chronic demand (Werner *et al.* 2008, Fu *et al.* 2013). The acute increase in circulating insulin induced by food intake promotes nutrient storage in its major peripheral target tissues, primarily by stimulating: (1) glucose uptake in adipose tissue and muscle; (2) glycolysis and glycogen synthesis in muscle and liver; (3) lipogenesis in adipose tissue and liver and (4) protein synthesis in muscle and liver. Conversely, insulin has repressive functions on glycogenolysis, lipolysis and protein breakdown, as well as gluconeogenesis and ketogenesis in the liver (Taniguchi *et al.* 2006, Cheng *et al.* 2010, Czech *et al.* 2013). Insulin may also exert local autocrine effects to promote beta-cell growth and survival, as well as influence its own production and secretion (Fig. 1; Luciani & Johnson 2005, Johnson *et al.* 2006, Beith *et al.* 2008, Leibiger *et al.* 2008, Mehran *et al.* 2012, Wang *et al.* 2013). In addition to its peripheral effects, insulin may affect energy balance and peripheral metabolism via central actions, such as through modulation of food intake; insulin signaling in the brain has been extensively reviewed elsewhere (e.g. Stockhorst *et al.* 2004, Schwartz & Porte 2005, Fernandez & Torres-Aleman 2012, Lee *et al.* 2016). Insulin clearly has essential roles in regulating energy balance and metabolic pathways, but there is still much to be learned about these and other critical functions of this hormone.

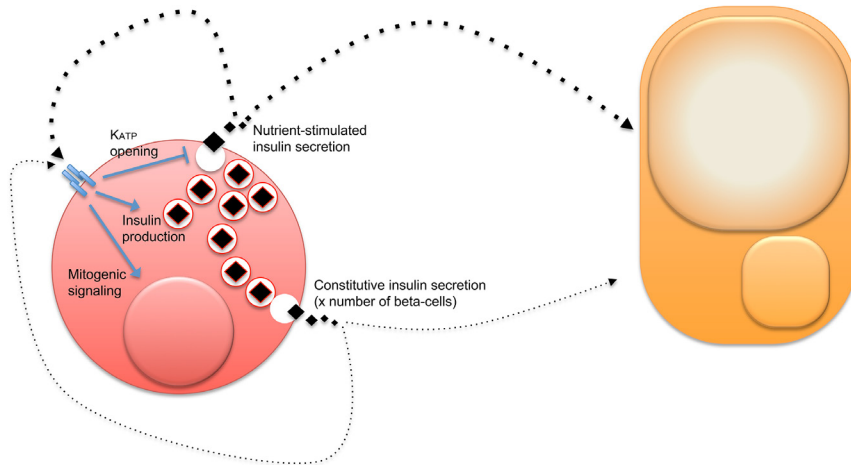
### Hyperinsulinemia in the etiology of insulin resistance and type 2 diabetes

Type 2 diabetes is defined as chronically elevated blood glucose that results from insufficient insulin production or impaired responsiveness to insulin (McGarry 1992, Prentki & Nolan 2006). Underlying factors that contribute

to both obesity and susceptibility to type 2 diabetes include genetic and epigenetic components, lifestyle and environmental conditions (McCarthy 2015). Chronic fuel surplus can lead to type 2 diabetes when there is a failure to compensate by adequately elevating insulin levels to meet demand (Prentki & Nolan 2006, Nolan *et al.* 2013, 2015). A decline in beta-cell function and/or mass is required for the onset of full-blown diabetes, and progression toward this state appears to be perpetuated by the metabolic disruptions associated with impaired tissue responsiveness to circulating insulin (Prentki & Nolan 2006, Nolan *et al.* 2011, 2015, Fu *et al.* 2013, Johnson & Olefsky 2013). The term 'insulin resistance' is widely used to specify an impairment of glucose disposal in response to insulin stimulation; however, this term is misleading when used broadly, because not all insulin-regulated processes are similarly affected in the insulin-resistant state that is associated with glucose intolerance (Saltiel & Kahn 2001, Biddinger & Kahn 2006). It has also been proposed that mild insulin resistance can be beneficial and protective, at least early in the disease progression (Nolan *et al.* 2015). Insulin resistance within tissues can arise at multiple levels of the insulin signaling pathway (Johnson & Olefsky 2013). Insulin resistance can also be caused by insulin receptor desensitization and removal from the plasma membrane (Knutson *et al.* 1983, Boothe *et al.* 2016).

Peripheral insulin resistance is closely related to elevated circulating insulin levels (Prentki & Nolan 2006, Shanik *et al.* 2008), and both of these features are linked to glucose intolerance (Nolan *et al.* 2011, Fu *et al.* 2013, Johnson & Olefsky 2013). Insulin resistance may be modulated by oscillatory dynamics of insulin secretion (Marsh *et al.* 1986, Shanik *et al.* 2008), and indeed, the loss of pulsatile insulin secretion is one of the earliest detectable defects in individuals who are at risk for type 2 diabetes (O'Rahilly *et al.* 1988, Satin *et al.* 2015). Currently, it is unclear whether hyperinsulinemia or altered insulin secretion dynamics play causal roles in the progression of type 2 diabetes. Theoretically, relative hyperinsulinemia could be maladaptive by enhancing weight gain, contributing to beta-cell exhaustion, or by exacerbating insulin receptor desensitization (i.e. insulin resistance).

Another interesting possibility is that insulin resistance at the level of the beta-cells could play a role in hyperinsulinemia, obesity, insulin resistance and type 2 diabetes (Fig. 1). Knockout mice lacking *Irs1* or *Akt* gene function exhibit basal hyperinsulinemia and, in some cases, increased beta-cell mass (Kido *et al.* 2000,

**Figure 1**

Insulin's effects are exerted through direct actions on target cells such as adipocytes, and also through autocrine feed-back on beta-cells. The number and function of beta-cells dictate insulin secretion levels and patterns that control adipogenesis and lipid homeostasis.

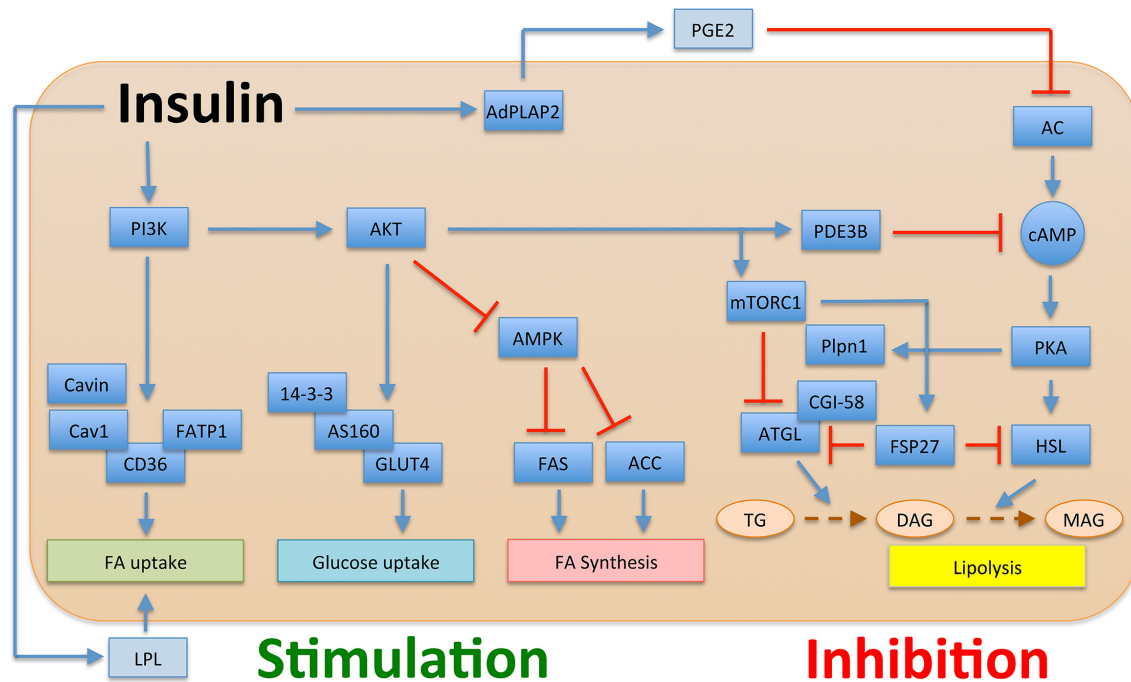
Bernal-Mizrachi *et al.* 2004), thereby mimicking the early stages in type 2 diabetes, but this concept should be tested directly with the insulin receptor and specific Cre deleter models. It has been shown that insulin acutely inhibits beta-cell electrical activity (Khan *et al.* 2001), meaning that local loss of insulin signaling might be expected to induce relative hyperinsulinemia. Indeed, insulin reduces its own secretion in healthy humans, but not in subjects that were obese (Cavallo-Perin *et al.* 1993). Human islets isolated from donors with type 2 diabetes were reported to have 50% reduced *INSR* expression (Gunton *et al.* 2005), but it is not known whether insulin receptors or insulin receptor signaling is affected in islets from hyperinsulinemic, obese, pre-diabetic individuals.

Obesity is often associated with peripheral insulin resistance, and with hyperinsulinemia. Indeed, it is difficult to conceptually or experimentally separate the phenomena of hyperinsulinemia and insulin resistance. The commonly accepted paradigm proposes that the hyperbolic relationship between impairment of insulin-stimulated glucose uptake and insulin secretion signifies that hyperinsulinemia is a compensatory response to prevent hyperglycemia when peripheral tissues are failing to take in excess glucose due to conditions resulting from obesity (Shanik *et al.* 2008). However, the chronology of obesity, hyperinsulinemia and insulin resistance is not always clear (Shanik *et al.* 2008). Characteristics of the obese state, such as toxic accumulation of lipids in non-adipose tissue and increased levels of proinflammatory cytokines, can induce or exacerbate insulin resistance, and the resultant elevated blood glucose has the potential to stimulate increased insulin secretion (Saltiel & Kahn 2001, Biddinger & Kahn 2006, Johnson & Olefsky 2013). On the other hand, clinical and experimental evidence

indicates that hyperinsulinemia can precede and promote both obesity (Genuth *et al.* 1971, Odeleye *et al.* 1997, Corkey 2012, Mehran *et al.* 2012) and insulin resistance or dysglycemia (Cusin *et al.* 1990, Le Stunff & Bougnères 1994, Dankner *et al.* 2009). Moreover, drugs that alleviate or prevent hyperinsulinemia can lead to weight loss (Alemzadeh *et al.* 1996, 1998, Lustig *et al.* 2005) and can enhance insulin-stimulated glucose uptake (Alemzadeh *et al.* 1996, Gray *et al.* 2010, Pedersen *et al.* 2015). Measured baseline insulin levels within healthy human and mouse populations are subject to wide variance (McAuley *et al.* 2001, Li *et al.* 2006, Berglund *et al.* 2008, Templeman *et al.* 2016), and diet intervention studies have indicated that obese individuals with the highest insulin secretion respond best to diets that reduce postprandial glycemia and insulinemia, whereas obese subjects with relatively lower insulin levels experience equivalent weight loss on low-fat diets (Pittas *et al.* 2005, Ebbeling *et al.* 2007). Collectively, these studies suggest that insulin has more of a causal role in perpetuating obesity and peripheral insulin resistance than is widely supposed.

### Direct mechanisms by which insulin may influence obesity

The expansion of white adipose tissue (WAT) that defines obesity can occur through both adipocyte hypertrophy (lipid filling) and hyperplasia (adipogenesis or increased differentiation of adipocyte precursor cells into adipocytes), but the mechanisms controlling these processes have not yet been fully delineated (Berry *et al.* 2014, Lim *et al.* 2015). Biochemical studies have shown that insulin plays key roles in regulating white adipocyte lipid

**Figure 2**

Insulin acts through multiple mechanisms to stimulate lipid uptake and storage and to inhibit lipid breakdown. Additional details have been expertly reviewed by [Czech et al. \(2013\)](#).

accumulation, by inhibiting lipolysis as well as promoting fatty acid uptake and triglyceride synthesis (lipogenesis) and also by stimulating the expression of genes involved in lipid uptake and storage ([Kersten 2001](#), [Czech et al. 2013](#)) ([Fig. 2](#)). Over a longer term, insulin signaling can drive adipogenesis, an orchestrated progression of events involving transient expression of the transcription factors CCAAT/enhancer-binding protein (C/EBP)  $\beta$  and C/EBP $\delta$  leading to induction of the master adipogenic regulators C/EBP $\alpha$  and peroxisome proliferator-activated receptor (PPAR)  $\gamma$  ([Cook & Cowan 2008](#), [Berry et al. 2014](#), [Lim et al. 2015](#)). Recently, it has also been shown that hyperinsulinemia promotes adipose tissue inflammation in mice, which contributes to disruption of metabolic processes such as *de novo* lipogenesis in WAT ([Pedersen et al. 2015](#)). Although the mechanisms guiding WAT growth are not fully understood, insulin appears to have the potential to influence several prominent steps. Indeed, deletion of insulin receptors from WAT protects mice from obesity, demonstrating a fundamental requirement for insulin in adipocyte differentiation and/or hyperplasia and/or hypertrophy ([Katic et al. 2007](#), [Boucher et al. 2012](#), [Softic et al. 2016](#)).

There are multiple types of adipose tissue with distinct functions, spatial distribution and contributions to obesity, and it is not clear whether insulin affects all

types of adipocytes similarly. WAT is the primary type of adipose tissue that serves as an energy reserve, and WAT also secretes an array of factors with autocrine, paracrine and endocrine functions ([Berry et al. 2014](#)). Subcutaneous and visceral depots of WAT have distinct characteristics, differing in cellular composition, innervation, metabolic characteristics and secretory profile; in general, visceral WAT is more implicated in perpetuating metabolic dysfunction, compared to subcutaneous WAT ([Lee et al. 2013](#), [Lim et al. 2015](#)). Interestingly, insulin treatment may have selective trophic effects on subcutaneous WAT when compared with visceral WAT in a rat model of late-stage type 2 diabetes ([Skovso et al. 2015](#)). The mechanisms regulating the specific expansion of distinct WAT depots are not well understood, although physiological factors such as age and sex have an influence on adipose tissue distribution ([Fuente-Martin et al. 2013](#), [Lee et al. 2013](#), [Jeffery et al. 2016](#), [Kwok et al. 2016](#)). For instance, central adiposity tends to increase with aging, and many of the pathologic conditions or diseases associated with obesity, including glucose intolerance and type 2 diabetes, insulin resistance, a proinflammatory state, coronary heart disease, hypertension, atherosclerosis and some cancers, are also associated with aging ([Ebbeling et al. 2007](#)). It seems that certain stages of life, such as the perinatal period and adolescence, are particularly



influential for determining the metabolic and adipocyte characteristics and thus shaping future susceptibility to obesity (Lawlor & Chaturvedi 2006, Buyken *et al.* 2014, Templeman *et al.* 2015).

Brown adipose tissue (BAT) and the brown-like adipocytes that can be found in some WAT depots are distinguished from WAT by a marked capacity to uncouple the electron transport chain-generated proton gradient from ATP synthesis, and thus expend energy through thermogenesis (Beranger *et al.* 2013). Although increasing the activity of BAT or expansion of brown-like adipocytes has been proposed as a treatment for obesity, the overall contribution of BAT and brown-like adipocytes to whole body metabolism is still being debated (Chondronikola *et al.* 2016, Labbe *et al.* 2016). BAT-specific insulin receptor knockout in mice results in the loss of fat-burning BAT tissue, illustrating an important role for insulin in this tissue (Guerra *et al.* 2001). Overall, there is ample evidence supporting effects of insulin signaling in adipose tissue that could influence the onset and development of obesity.

### Prevention of obesity in mice genetically incapable of sustained hyperinsulinemia

Although clinical and experimental evidence indicated that hyperinsulinemia could precede and promote obesity (Genuth *et al.* 1971, Odeleye *et al.* 1997, Corkey 2012), what was previously missing from the field was a genetic loss-of-function experiment in a mammalian model system that could directly establish the causal requirement for hyperinsulinemia in diet-induced obesity. To address this unmet need, we designed a series of studies in which we modulated the insulin gene dosage, and, to varying extents, levels of circulating insulin in mice (Mehran *et al.* 2012, Templeman *et al.* 2015, 2016). The fact that mice have two insulin genes allowed us to compare effects over a range of insulin reductions, and to avoid the compensation that occurs between the two insulin genes.

In the first such study, we used mice that were null for the *Ins2* gene to focus attention only on insulin coming from the pancreas (Mehran *et al.* 2012). Thus, on the *Ins2*-null background, we compared *Ins1* heterozygous mice (*Ins1<sup>+/-</sup>:Ins2<sup>-/-</sup>* mice) to their littermate controls that had both *Ins1* alleles (*Ins1<sup>+/+</sup>:Ins2<sup>-/-</sup>* mice) for one year, and we confirmed that the *Ins1<sup>+/-</sup>:Ins2<sup>-/-</sup>* mice exhibited the expected sustained reduction in circulating insulin, which was associated with reduced beta-cell mass. Remarkably, we found that male *Ins1<sup>+/-</sup>:Ins2<sup>-/-</sup>* mice were

completely protected from diet-induced obesity, as well as related sequelae including fatty liver, over this time frame. We observed an increase in energy expenditure in mice with less insulin, associated with browning of WAT, but no differences in food intake. Interestingly, control female *Ins1<sup>+/+</sup>:Ins2<sup>-/-</sup>* mice did not develop significant hyperinsulinemia in a comparison between these high-fat and moderate-fat diets, so we were unable to test our hypothesis in females. It should be noted that male and female mice with only a single *Ins1* allele (on an *Ins2*-null background) had relatively normal glucose homeostasis when housed in our conventional facility (Mehran *et al.* 2012), but diabetes is now found in over half of the male *Ins1<sup>+/+</sup>:Ins2<sup>-/-</sup>* mice after they were rederived into new 'modified barrier' and 'specific pathogen-free' facilities. The reason for this increased diabetes susceptibility in male mice with a single remaining *Ins1* allele is not clear, but could be related to gut microbiota, environmental stresses or genetic drift within this strain. Notwithstanding, the results of this first study demonstrated for the first time in any mammal that hyperinsulinemia is required for weight gain.

In a second study, we studied female *Ins1<sup>-/-</sup>:Ins2<sup>+/-</sup>* experimental mice and their littermate *Ins1<sup>-/-</sup>:Ins2<sup>+/+</sup>* controls for one year. In this experiment, reduced *Ins2* gene dosage led to a transient decrease in circulating insulin that was significant at 8 and 28 weeks, but had returned to hyperinsulinemic levels at one year (Templeman *et al.* 2015). This transient reduction of diet-induced hyperinsulinemia provided sustained protection from obesity, although in this less-severe model of insulin reduction, we did not observe evidence of significant browning of WAT (Templeman *et al.* 2015). A pilot study provided evidence that partial normalization of *INS2* peptide levels, via mini-osmotic pump, was sufficient to partially reverse the protective anti-obesity effects of reducing *Ins2* gene dosage.

Our third study focused on males with *Ins1<sup>-/-</sup>:Ins2<sup>+/-</sup>* and *Ins1<sup>-/-</sup>:Ins2<sup>+/+</sup>* genotypes. In this case, circulating insulin levels were hypervariable compared with their female littermates, and were not significantly suppressed by reducing insulin gene dosage. The wide variation in circulating insulin in these male mice is similar to human populations and other mouse studies (Li *et al.* 2006, Berglund *et al.* 2008). Although this meant we could not test the hypothesis by comparing the body weight means between *Ins1<sup>-/-</sup>:Ins2<sup>+/-</sup>* mice and *Ins1<sup>-/-</sup>:Ins2<sup>+/+</sup>* controls, we still observed a significant positive correlation between body mass and fasting insulin levels in one-year-old male mice (Templeman *et al.* 2016).

These three studies, alongside data from mice with insulin receptor knock-out in adipose tissue (Katic *et al.* 2007, Boucher *et al.* 2012), strongly support the concept that excess insulin contributes to diet-induced obesity. Moreover, across multiple models, we found that obesity and lipid homeostasis are more sensitive to small changes in circulating insulin levels than glucose homeostasis. Collectively, these experiments also fit well with clinical and pre-clinical studies demonstrating that therapy with long-acting insulin analogues leads to weight gain, and with small trials showing weight loss with drugs that reduce insulin secretion (Alemzadeh *et al.* 1998, Lustig *et al.* 2005, ORIGIN Trial Investigators *et al.* 2012, Skovso *et al.* 2015). For example, a recent investigation reported that chronic insulin infusion via mini-osmotic pump leads to WAT expansion in mice (Rajan *et al.* 2016). Clearly, more work needs to be done, but the idea that insulin plays an important role in lipid homeostasis *in vivo* appears to be broadly supported.

### Nutritional regulation of insulin secretion

Circulating insulin levels are affected by nutrients and numerous circulating factors, such as certain amino acids, fatty acids, sex hormones, melatonin, leptin, growth hormone, glucose-dependent insulinotropic polypeptide and glucagon-like peptide 1 (Melloul *et al.* 2002, Fu *et al.* 2013). Insulin levels can be tempered through stimulation or repression of its secretion, and insulin production is also subject to modulation, at the level of transcription, mRNA stability, translation and processing (Melloul *et al.* 2002, Fu *et al.* 2013). Basal insulin levels are also modulated chronically through changes in the number of beta-cells and their differentiation status (Szabat *et al.* 2012). One of the unexpected insights from our studies on insulin-mutant mice is that although differences in gene dosage (and therefore insulin synthesis) usually translate into measurable changes in insulin secretion in the long-term (Mehran *et al.* 2012, Templeman *et al.* 2015), this is not always the case (Templeman *et al.* 2016). Both stimulated and basal insulin secretion are under multifactorial control and can be modulated through numerous means.

Although the carbohydrate glucose is clearly a dominant insulin secretagogue in adult beta-cells, evidence indicates that other sugars and non-carbohydrate substrates can also elicit significant insulin secretion under some conditions. For example, free fatty acids can acutely stimulate insulin secretion in some individuals, either alone or in the context of elevated glucose levels

(Paolisso *et al.* 1995, Carpentier *et al.* 1999, Jeffrey *et al.* 2008, Staaf *et al.* 2016). Prolonged exposure to high levels of lipids can also increase the number of beta-cells and induce fasting hyperinsulinemia in some rodent models (Fontes *et al.* 2010). Exposure to free fatty acids at very high concentrations, or for long periods of time, can eventually induce beta-cell death and dysfunction in human islets and in animal models (Jeffrey *et al.* 2008). Clearly, a better understanding of the effects of lipids on human beta-cells from a large array of donors is urgently needed.

Evidence similarly points to a role for some amino acids in acute and chronic insulin secretion (Nair & Short 2005). For example, arginine is a well-known insulin secretagogue used to 'maximally' stimulate insulin secretion in a clinical setting (Robertson 2007). Elevated circulating levels of branched-chain amino acids are associated with over-nutrition, obesity and type 2 diabetes (McCormack *et al.* 2013). Metabolomic profiling of 2422 Framingham Offspring individuals identified elevated levels of five branched-chain and aromatic amino acids (isoleucine, leucine, valine, tyrosine and phenylalanine) that were significantly associated with subsequent diabetes (Wang *et al.* 2011). Notably, branched-chain amino acids such as leucine can be powerful insulin secretagogues (van Loon *et al.* 2003). To date, no study has simultaneously compared the responsiveness to glucose, fatty acids and amino acids, but our anecdotal experience is that there is considerable variability in the relative responses to these macronutrient groups between individuals. Genetic profiling studies could be used to understand the mechanistic underpinnings of this variation. Answering this question could usher in a new era of nutrigenomics to minimize obesity and diabetes risk. If fats and proteins can stimulate robust insulin secretion in some individuals, it would mean that a one-size-fits-all prescription of simply avoiding excess carbohydrates would not be sufficient for these people. Maintaining insulin levels relatively in the healthy range might be more complex and individualized than once thought. It should be stressed that the healthy insulin range obviously has a minimum for each individual, below which diabetes occurs.

### Conclusions and future directions

Long-term studies using ever more powerful animal models, as well as carefully defined human trials, are required to further test the hypothesis that hyperinsulinemia is a driving force in obesity, insulin

resistance and type 2 diabetes and to determine its net contribution, which is likely to be context dependent. Although our published studies have demonstrated that preventing hyperinsulinemia can prevent obesity in euglycemic conditions, we have not yet shown that obesity can be treated by reducing insulin secretion in mice that have already gained weight or demonstrated that hyperinsulinemia has a similar role in a more typical scenario that includes dysglycemia. Clinical evidence to support reducing insulin as a therapy for obesity has been presented (Lunetta *et al.* 1996, Alemzadeh *et al.* 1998, Lustig *et al.* 2005), but conflicting data on specific patient populations have also been published (Brauner *et al.* 2016). Therefore, a focus on hyperinsulinemia as a target for obesity has not yet been widely adopted. It is likely that hyperinsulinemia contributes to the 'environmental' part of the obesity risk equation, as common variation in the *INS* gene has not been genetically associated with BMI or circulating triglycerides (<http://www.type2diabetesgenetics.org>). Nevertheless, insulin could be reduced in many individuals by adopting low-carbohydrate diets, time-controlled feeding or high-intensity exercise (Holloszy 2005, Balkau *et al.* 2008, Hatori *et al.* 2012, Schugar & Crawford 2012, Nelson *et al.* 2013, Longo & Panda 2016). Work in this area has the potential to change the paradigms of obesity and diabetes pathophysiology and to provide people with personalized nutritional advice for weight loss and disease prevention based around the maintenance of insulin within a healthy range. Lifestyle changes aimed at preventing excess circulating insulin could be a key to living a healthy long life.

#### Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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