

A causal role for hyperinsulinemia in obesity

Nicole M Templeman¹, Søs Skovsø¹, Melissa M Page¹, Gareth E Lim¹ and James D Johnson^{1,2}

¹Department of Cellular and Physiological Sciences, Diabetes Research Group, Life Sciences Institute, University of British Columbia, Vancouver, British Columbia, Canada

²Institute for Personalized Therapeutic Nutrition, Vancouver, British Columbia, Canada

Correspondence should be addressed to J D Johnson

Email
James.d.johnson@ubc.ca

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

Journal of Endocrinology
(2017) **232**, R173–R183

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^{-/-}:Ins2^{-/-}*) 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.

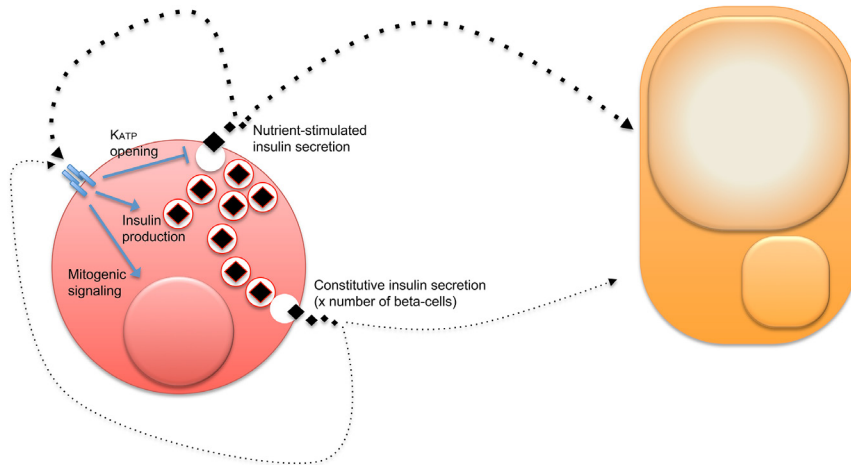
Hyperinsulinemia in the etiology of insulin resistance and type 2 diabetes

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.

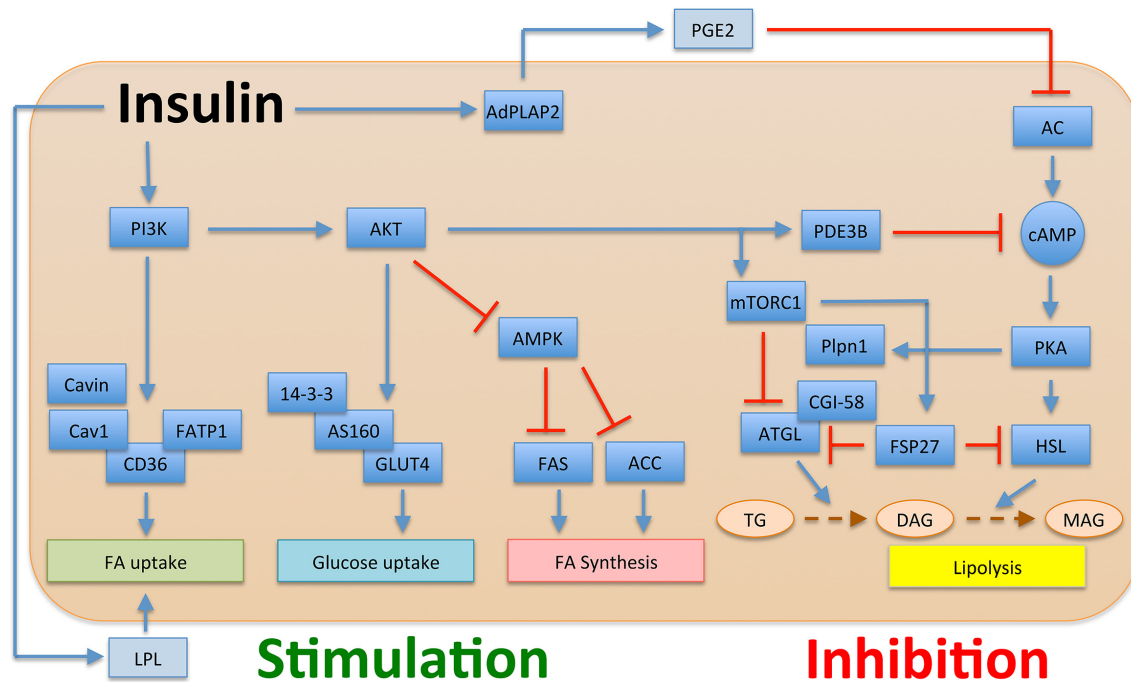
Bernal-Mizrachi *et al.* 2004), thereby mimicking the early stages in type 2 diabetes, but this concept should be tested directly with the insulin receptor and specific Cre deleter models. It has been shown that insulin acutely inhibits beta-cell electrical activity (Khan *et al.* 2001), meaning that 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) β and C/EBP δ leading to induction of the master adipogenic regulators C/EBP α and peroxisome proliferator-activated receptor (PPAR) γ ([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, Odeyeye *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*^{+/-}:*Ins2*^{-/-} mice) to their littermate controls that had both *Ins1* alleles (*Ins1*^{+/+}:*Ins2*^{-/-} mice) for one year, and we confirmed that the *Ins1*^{+/-}:*Ins2*^{-/-} mice exhibited the expected sustained reduction in circulating insulin, which was associated with reduced beta-cell mass. Remarkably, we found that male *Ins1*^{+/-}:*Ins2*^{-/-} 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*^{+/+}:*Ins2*^{-/-} 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*^{+/-}:*Ins2*^{-/-} 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*^{-/-}:*Ins2*^{+/-} experimental mice and their littermate *Ins1*^{-/-}:*Ins2*^{+/+} 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*^{-/-}:*Ins2*^{+/-} and *Ins1*^{-/-}:*Ins2*^{+/+} 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*^{-/-}:*Ins2*^{+/-} mice and *Ins1*^{-/-}:*Ins2*^{+/+} 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.

Funding

Related work in the JDJ laboratory has been funded by the Canadian Institutes for Health Research, the Canadian Diabetes Association (CDA), and the Juvenile Diabetes Research Foundation. N M T was supported by a Natural Science and Engineering Council of Canada scholarship and a Four-Year Fellowship from the University of British Columbia. M M P is supported by a CDA Fellowship. S S is supported by a Benzon Foundation Fellowship.

Acknowledgements

The authors thank colleagues including Timothy Kieffer (University of British Columbia), Ernesto Bernal-Mizrachi (University of Miami), Patrick MacDonald (University of Alberta) and many others for stimulating

discussions on the biology of insulin. They thank members of the Institute for Personalized Therapeutic Nutrition (University of British Columbia), including Sean McKelvey and Jonathan Little, for discussions on the nutritional implications of this research area.

References

- Alemzadeh R, Jacobs W & Pitukcheewanont P 1996 Antiobesity effect of diazoxide in obese Zucker rats. *Metabolism* **45** 334–341. (doi:10.1016/S0026-0495(96)90287-5)
- Alemzadeh R, Langley G, Upchurch L, Smith P & Slonim AE 1998 Beneficial effect of diazoxide in obese hyperinsulinemic adults. *Journal of Clinical Endocrinology and Metabolism* **83** 1911–1915. (doi:10.1210/jc.83.6.1911)
- Balkau B, Mhamdi L, Oppert JM, Nolan J, Golay A, Porcellati F, Laakso M, Ferrannini E & EGIR-RISC Study Group 2008 Physical activity and insulin sensitivity: the RISC study. *Diabetes* **57** 2613–2618. (doi:10.2337/db07-1605)
- Banting FG, Best CH, Collip JB, Campbell WR & Fletcher AA 1922 Pancreatic extracts in the treatment of diabetes mellitus. *Canadian Medical Association Journal* **12** 141–146.
- Beith JL, Alejandro EU & Johnson JD 2008 Insulin stimulates primary beta-cell proliferation via Raf-1 kinase. *Endocrinology* **149** 2251–2260. (doi:10.1210/en.2007-1557)
- Bengtsson M, Stahlberg A, Rorsman P & Kubista M 2005 Gene expression profiling in single cells from the pancreatic islets of Langerhans reveals lognormal distribution of mRNA levels. *Genome Research* **15** 1388–1392. (doi:10.1101/gr.3820805)
- Beranger GE, Karbiener M, Barquissau V, Pisani DF, Scheideler M, Langin D & Amri EZ 2013 In vitro brown and 'brite'/'beige' adipogenesis: human cellular models and molecular aspects. *Biochimica et Biophysica Acta* **1831** 905–914. (doi:10.1016/j.bbali.2012.11.001)
- Berglund ED, Li CY, Poffenberger G, Ayala JE, Fueger PT, Willis SE, Jewell MM, Powers AC & Wasserman DH 2008 Glucose metabolism in vivo in four commonly used inbred mouse strains. *Diabetes* **57** 1790–1799. (doi:10.2337/db07-1615)
- Bernal-Mizrachi E, Fatrai S, Johnson JD, Ohsugi M, Otani K, Han Z, Polonsky KS & Permutt MA 2004 Defective insulin secretion and increased susceptibility to experimental diabetes are induced by reduced Akt activity in pancreatic islet beta cells. *Journal of Clinical Investigation* **114** 928–936. (doi:10.1172/JCI200420016)
- Berry R, Jeffery E & Rodeheffer MS 2014 Weighing in on adipocyte precursors. *Cell Metabolism* **19** 8–20. (doi:10.1016/j.cmet.2013.10.003)
- Biddinger SB & Kahn CR 2006 From mice to men: insights into the insulin resistance syndromes. *Annual Review of Physiology* **68** 123–158. (doi:10.1146/annurev.physiol.68.040104.124723)
- Boothe T, Lim GE, Cen H, Skovso S, Piske M, Li SN, Nabi IR, Gilon P & Johnson JD 2016 Inter-domain tagging implicates caveolin-1 in insulin receptor trafficking and Erk signaling bias in pancreatic beta-cells. *Molecular Metabolism* **5** 366–378. (doi:10.1016/j.molmet.2016.01.009)
- Boucher J, Mori MA, Lee KY, Smyth G, Liew CW, Macotela Y, Rourk M, Bluher M, Russell SJ & Kahn CR 2012 Impaired thermogenesis and adipose tissue development in mice with fat-specific disruption of insulin and IGF-1 signalling. *Nature Communications* **3** 902.
- Brauner R, Serreau R, Souberbielle JC, Pouillot M, Grouazel S, Recasens C, Zerah M, Sainte-Rose C & Treluyer JM 2016 Diazoxide in children with obesity after hypothalamic-pituitary lesions: a randomized, placebo-controlled trial. *Journal of Clinical Endocrinology and Metabolism* **101** 4825–4833. (doi:10.1210/jc.2016-2126)
- Broggiolo W, Stocker H, Ikeya T, Rintelen F, Fernandez R & Hafen E 2001 An evolutionarily conserved function of the *Drosophila* insulin receptor and insulin-like peptides in growth control. *Current Biology* **11** 213–221. (doi:10.1016/S0960-9822(01)00068-9)
- Buyken AE, Goletzke J, Joslowski G, Felbick A, Cheng G, Herder C & Brand-Miller JC 2014 Association between carbohydrate quality

- and inflammatory markers: systematic review of observational and interventional studies. *American Journal of Clinical Nutrition* **99** 813–833. (doi:10.3945/ajcn.113.074252)
- Carpentier A, Mittelman SD, Lamarche B, Bergman RN, Giacca A & Lewis GF 1999 Acute enhancement of insulin secretion by FFA in humans is lost with prolonged FFA elevation. *American Journal of Physiology* **276** E1055–E1066.
- Cavallo-Perin P, Bruno A, Scaglione L, Gruden G, Cassader M & Pagano G 1993 Feedback inhibition of insulin and glucagon secretion by insulin is altered in abdominal obesity with normal or impaired glucose tolerance. *Acta Diabetologica* **30** 154–158. (doi:10.1007/BF00572860)
- Cheng Z, Tseng Y & White MF 2010 Insulin signaling meets mitochondria in metabolism. *Trends in Endocrinology and Metabolism* **21** 589–598. (doi:10.1016/j.tem.2010.06.005)
- Chondronikola M, Volpi E, Borsheim E, Porter C, Saraf MK, Annamalai P, Yfanti C, Chao T, Wong D, Shinoda K, et al. 2016 Brown adipose tissue activation is linked to distinct systemic effects on lipid metabolism in humans. *Cell Metabolism* **23** 1200–1206. (doi:10.1016/j.cmet.2016.04.029)
- Clancy DJ, Gems D, Harshman LG, Oldham S, Stocker H, Hafen E, Leever SJ & Partridge L 2001 Extension of life-span by loss of CHICO, a Drosophila insulin receptor substrate protein. *Science* **292** 104–106. (doi:10.1126/science.1057991)
- Cook A & Cowan C 2008 Adipose. In *StemBook*. Ed. The Stem Cell Research Community. Boston, MA, USA: Massachusetts General Hospital/Harvard Stem Cell Institute. (doi:10.3824/stembook.1.40.1)
- Corkey BE 2012 Banting lecture 2011: hyperinsulinemia: cause or consequence? *Diabetes* **61** 4–13. (doi:10.2337/db11-1483)
- Cusin I, Terretz J, Rohner-Jeanrenaud F & Jeanrenaud B 1990 Metabolic consequences of hyperinsulinaemia imposed on normal rats on glucose handling by white adipose tissue, muscles and liver. *Biochemical Journal* **267** 99–103.
- Czech MP, Tencerova M, Pedersen DJ & Aouadi M 2013 Insulin signalling mechanisms for triacylglycerol storage. *Diabetologia* **56** 949–964. (doi:10.1007/s00125-013-2869-1)
- Dankner R, Chetrit A, Shanik MH, Raz I & Roth J 2009 Basal-state hyperinsulinemia in healthy normoglycemic adults is predictive of type 2 diabetes over a 24-year follow-up: a preliminary report. *Diabetes Care* **32** 1464–1466. (doi:10.2337/dc09-0153)
- Deltour L, Leduque P, Blume N, Madsen O, Dubois P, Jami J & Bucchini D 1993 Differential expression of the two nonallelic proinsulin genes in the developing mouse embryo. *PNAS* **90** 527–531. (doi:10.1073/pnas.90.2.527)
- Deltour L, Montagutelli X, Guenet JL, Jami J & Paldi A 1995 Tissue- and developmental stage-specific imprinting of the mouse proinsulin gene, Ins2. *Developmental Biology* **168** 686–688. (doi:10.1006/dbio.1995.1114)
- Dionne DA, Skovso S, Templeman NM, Clee SM & Johnson JD 2016 Caloric restriction paradoxically increases adiposity in mice with genetically reduced insulin. *Endocrinology* **157** 2724–2734. (doi:10.1210/en.2016-1102)
- Duvillie B, Bucchini D, Tang T, Jami J & Paldi A 1998 Imprinting at the mouse Ins2 locus: evidence for cis- and trans-allelic interactions. *Genomics* **47** 52–57. (doi:10.1006/geno.1997.5070)
- Ebbeling CB, Leidig MM, Feldman HA, Lovesky MM & Ludwig DS 2007 Effects of a low-glycemic load vs low-fat diet in obese young adults: a randomized trial. *JAMA* **297** 2092–2102. (doi:10.1001/jama.297.19.2092)
- Fan Y, Rudert WA, Grupillo M, He J, Sisino G & Trucco M 2009 Thymus-specific deletion of insulin induces autoimmune diabetes. *EMBO Journal* **28** 2812–2824. (doi:10.1038/emboj.2009.212)
- Fernandez AM & Torres-Aleman I 2012 The many faces of insulin-like peptide signalling in the brain. *Nature Reviews Neuroscience* **13** 225–239. (doi:10.1038/nrn3209)
- Fontana L, Partridge L & Longo VD 2010 Extending healthy life span – from yeast to humans. *Science* **328** 321–326. (doi:10.1126/science.1172539)
- Fontes G, Zarrouki B, Hagman DK, Latour MG, Semache M, Roskens V, Moore PC, Prentki M, Rhodes CJ, Jetton TL, et al. 2010 Glucolipotoxicity age-dependently impairs beta cell function in rats despite a marked increase in beta cell mass. *Diabetologia* **53** 2369–2379. (doi:10.1007/s00125-010-1850-5)
- Fu Z, Gilbert ER & Liu D 2013 Regulation of insulin synthesis and secretion and pancreatic Beta-cell dysfunction in diabetes. *Current Diabetes Reviews* **9** 25–53. (doi:10.2174/157339913804143225)
- Fuente-Martin E, Argente-Arizon P, Ros P, Argente J & Chowen JA 2013 Sex differences in adipose tissue: it is not only a question of quantity and distribution. *Adipocyte* **2** 128–134. (doi:10.4161/adip.24075)
- Genuth SM, Przybylski RJ & Rosenberg DM 1971 Insulin resistance in genetically obese, hyperglycemic mice. *Endocrinology* **88** 1230–1238. (doi:10.1210/endo-88-5-1230)
- Gray SL, Donald C, Jetha A, Covey SD & Kieffer TJ 2010 Hyperinsulinemia precedes insulin resistance in mice lacking pancreatic β -cell leptin signaling. *Endocrinology* **151** 4178–4186. (doi:10.1210/en.2010-0102)
- Grosse J, Heffron H, Burling K, Akhter Hossain M, Habib AM, Rogers GJ, Richards P, Larder R, Rimmington D, Adriaenssens AA, et al. 2014 Insulin-like peptide 5 is an orexigenic gastrointestinal hormone. *PNAS* **111** 11133–11138. (doi:10.1073/pnas.1411413111)
- Guerra C, Navarro P, Valverde AM, Arribas M, Bruning J, Kozak LP, Kahn CR & Benito M 2001 Brown adipose tissue-specific insulin receptor knockout shows diabetic phenotype without insulin resistance. *Journal of Clinical Investigation* **108** 1205–1213. (doi:10.1172/JCI13103)
- Gunton JE, Kulkarni RN, Yim S, Okada T, Hawthorne WJ, Tseng YH, Roberson RS, Ricordi C, O'Connell PJ, Gonzalez FJ, et al. 2005 Loss of ARNT/HIF1 β mediates altered gene expression and pancreatic islet dysfunction in human type 2 diabetes. *Cell* **122** 337–349. (doi:10.1016/j.cell.2005.05.027)
- Hatori M, Vollmers C, Zarrinpar A, DiTacchio L, Bushong EA, Gill S, Leblanc M, Chaix A, Joens M, Fitzpatrick JA, et al. 2012 Time-restricted feeding without reducing caloric intake prevents metabolic diseases in mice fed a high-fat diet. *Cell Metabolism* **15** 848–860. (doi:10.1016/j.cmet.2012.04.019)
- Hay CW & Docherty K 2006 Comparative analysis of insulin gene promoters: implications for diabetes research. *Diabetes* **55** 3201–3213. (doi:10.2337/db06-0788)
- Holloszy JO 2005 Exercise-induced increase in muscle insulin sensitivity. *Journal of Applied Physiology* **99** 338–343. (doi:10.1152/jappphysiol.00123.2005)
- Jeffery E, Wing A, Holtrup B, Sebo Z, Kaplan JL, Saavedra-Pena R, Church CD, Colman L, Berry R & Rodeheffer MS 2016 The adipose tissue microenvironment regulates depot-specific adipogenesis in obesity. *Cell Metabolism* **24** 142–150. (doi:10.1016/j.cmet.2016.05.012)
- Jeffrey KD, Alejandro EU, Luciani DS, Kalynyak TB, Hu X, Li H, Lin Y, Townsend RR, Polonsky KS & Johnson JD 2008 Carboxypeptidase E mediates palmitate-induced beta-cell ER stress and apoptosis. *PNAS* **105** 8452–8457. (doi:10.1073/pnas.0711232105)
- Johnson AM & Olefsky JM 2013 The origins and drivers of insulin resistance. *Cell* **152** 673–684. (doi:10.1016/j.cell.2013.01.041)
- Johnson JD, Bernal-Mizrachi E, Alejandro EU, Han Z, Kalynyak TB, Li H, Beith JL, Gross J, Warnock GL, Townsend RR, et al. 2006 Insulin protects islets from apoptosis via Pdx1 and specific changes in the human islet proteome. *PNAS* **103** 19575–19580. (doi:10.1073/pnas.0604208103)
- Katic M, Kennedy AR, Leykin I, Norris A, McGettrick A, Gesta S, Russell SJ, Blüher M, Maratos-Flier E & Kahn CR 2007 Mitochondrial gene expression and increased oxidative metabolism: role in increased lifespan of fat-specific insulin receptor knock-out mice. *Aging Cell* **6** 827–839. (doi:10.1111/j.1474-9726.2007.00346.x)
- Kenyon C, Chang J, Gensch E, Rudner A & Tabtiang R 1993 A C. elegans mutant that lives twice as long as wild type. *Nature* **366** 461–464. (doi:10.1038/366461a0)

- Kersten S 2001 Mechanisms of nutritional and hormonal regulation of lipogenesis. *EMBO Reports* **2** 282–286. (doi:10.1093/embo-reports/kve071)
- Khan FA, Goforth PB, Zhang M & Satin LS 2001 Insulin activates ATP-sensitive K(+) channels in pancreatic beta-cells through a phosphatidylinositol 3-kinase-dependent pathway. *Diabetes* **50** 2192–2198. (doi:10.2337/diabetes.50.10.2192)
- Kido Y, Burks DJ, Withers D, Bruning JC, Kahn CR, White MF & Accili D 2000 Tissue-specific insulin resistance in mice with mutations in the insulin receptor, IRS-1, and IRS-2. *Journal of Clinical Investigation* **105** 199–205. (doi:10.1172/JCI7917)
- Knutson VP, Ronnett GV & Lane MD 1983 Rapid, reversible internalization of cell surface insulin receptors. Correlation with insulin-induced down-regulation. *Journal of Biological Chemistry* **258** 12139–12142.
- Kwok KH, Lam KS & Xu A 2016 Heterogeneity of white adipose tissue: molecular basis and clinical implications. *Experimental and Molecular Medicine* **48** e215. (doi:10.1038/emm.2016.5)
- Labbe SM, Caron A, Chechi K, Laplante M, Lecomte R & Richard D 2016 Metabolic activity of brown, 'beige,' and white adipose tissues in response to chronic adrenergic stimulation in male mice. *American Journal of Physiology: Endocrinology and Metabolism* **311** E260–E268. (10.1152/ajpendo.00545.2015)
- Lawlor DA & Chaturvedi N 2006 Treatment and prevention of obesity – are there critical periods for intervention? *International Journal of Epidemiology* **35** 3–9. (doi:10.1093/ije/dyi309)
- Le Stunff C & Bougnères P 1994 Early changes in postprandial insulin secretion, not in insulin sensitivity, characterize juvenile obesity. *Diabetes* **43** 696–702.
- Lee MJ, Wu Y & Fried SK 2013 Adipose tissue heterogeneity: implication of depot differences in adipose tissue for obesity complications. *Molecular Aspects of Medicine* **34** 1–11. (doi:10.1016/j.mam.2012.10.001)
- Lee SH, Zabolotny JM, Huang H, Lee H & Kim YB 2016 Insulin in the nervous system and the mind: functions in metabolism, memory, and mood. *Molecular Metabolism* **5** 589–601. (doi:10.1016/j.molmet.2016.06.011)
- Leibiger IB, Leibiger B & Berggren PO 2008 Insulin signaling in the pancreatic beta-cell. *Annual Review of Nutrition* **28** 233–251. (doi:10.1146/annurev.nutr.28.061807.155530)
- Leroux L, Desbois P, Lamotte L, Duville B, Cordonnier N, Jackerott M, Jami J, Bucchini D & Joshi RL 2001 Compensatory responses in mice carrying a null mutation for Ins1 or Ins2. *Diabetes* **50** (Supplement 1) S150–S153. (doi:10.2337/diabetes.50.2007.S150)
- Li C, Ford ES, McGuire LC, Mokdad AH, Little RR & Reaven GM 2006 Trends in hyperinsulinemia among nondiabetic adults in the U.S. *Diabetes Care* **29** 2396–2402. (doi:10.2337/dc06-0289)
- Lim GE, Albrecht T, Piske M, Sarai K, Lee JT, Ramshaw HS, Sinha S, Guthridge MA, Acker-Palmer A, Lopez AF, et al. 2015 14-3-zeta coordinates adipogenesis of visceral fat. *Nature Communications* **6** 7671. (doi:10.1038/ncomms8671)
- Linde S, Nielsen JH, Hansen B & Welinder BS 1989 Reversed-phase high-performance liquid chromatographic analyses of insulin biosynthesis in isolated rat and mouse islets. *Journal of Chromatography* **462** 243–254. (doi:10.1016/S0021-9673(00)91351-7)
- Longo VD & Panda S 2016 Fasting, circadian rhythms, and time-restricted feeding in healthy lifespan. *Cell Metabolism* **23** 1048–1059. (doi:10.1016/j.cmet.2016.06.001)
- Luciani DS & Johnson JD 2005 Acute effects of insulin on beta-cells from transplantable human islets. *Molecular and Cellular Endocrinology* **241** 88–98. (doi:10.1016/j.mce.2005.06.006)
- Lunetta M, Di Mauro M, Le Moli R & Burrafato S 1996 Long-term octreotide treatment reduced hyperinsulinemia, excess body weight and skin lesions in severe obesity with acanthosis nigricans. *Journal of Endocrinological Investigation* **19** 699–703. (doi:10.1007/BF03349042)
- Lustig RH, Greenway F, Velasquez-Mieyer P, Heimbürger D, Schumacher D, Smith D, Smith W, Soler N, Warsi G, Berg W, et al. 2005 A multicenter, randomized, double-blind, placebo-controlled, dose-finding trial of a long-acting formulation of octreotide in promoting weight loss in obese adults with insulin hypersecretion. *International Journal of Obesity* **30** 331–341. (doi:10.1038/sj.ijo.0803074)
- Marsh BD, Marsh DJ & Bergman RN 1986 Oscillations enhance the efficiency and stability of glucose disposal. *American Journal of Physiology* **250** E576–E582.
- McAuley KA, Williams SM, Mann JI, Walker RJ, Lewis-Barned NJ, Temple LA & Duncan AW 2001 Diagnosing insulin resistance in the general population. *Diabetes Care* **24** 460–464.
- McCarthy MI 2015 Genomic medicine at the heart of diabetes management. *Diabetologia* **58** 1725–1729. (doi:10.1007/s00125-015-3588-6)
- McCormack SE, Shaham O, McCarthy MA, Deik AA, Wang TJ, Gerszten RE, Clish CB, Mootha VK, Grinspoon SK & Fleischman A 2013 Circulating branched-chain amino acid concentrations are associated with obesity and future insulin resistance in children and adolescents. *Pediatric Obesity* **8** 52–61. (doi:10.1111/j.2047-6310.2012.00087.x)
- McGarry JD 1992 What if Minkowski had been ageusic? An alternative angle on diabetes. *Science* **258** 766–770. (doi:10.1126/science.1439783)
- Mehran AE, Templeman NM, Brigidi GS, Lim GE, Chu KY, Hu X, Botzelli JD, Asadi A, Hoffman BG, Kieffer TJ, et al. 2012 Hyperinsulinemia drives diet-induced obesity independently of brain insulin production. *Cell Metabolism* **16** 723–737. (doi:10.1016/j.cmet.2012.10.019)
- Melloul D, Marshak S & Cerasi E 2002 Regulation of insulin gene transcription. *Diabetologia* **45** 309–326. (doi:10.1007/s00125-001-0728-y)
- Meur G, Qian Q, da Silva Xavier G, Pullen TJ, Tsuboi T, McKinnon C, Fletcher L, Tavares JM, Hughes S, Johnson P, et al. 2011 Nucleocytoplasmic shuttling of FoxO1 directly regulates mouse Ins2 but not Ins1 gene expression in pancreatic beta cells (MIN6). *Journal of Biological Chemistry* **286** 13647–13656. (doi:10.1074/jbc.M110.204248)
- Nair KS & Short KR 2005 Hormonal and signaling role of branched-chain amino acids. *Journal of Nutrition* **135** 1547S–1552S.
- Nakae J, Kido Y & Accili D 2001 Distinct and overlapping functions of insulin and IGF-I receptors. *Endocrine Reviews* **22** 818–835. (doi:10.1210/edrv.22.6.0452)
- Nelson RK, Horowitz JF, Holleman RG, Swartz AM, Strath SJ, Kriska AM & Richardson CR 2013 Daily physical activity predicts degree of insulin resistance: a cross-sectional observational study using the 2003–2004 National Health and Nutrition Examination Survey. *International Journal of Behavioral Nutrition and Physical Activity* **10** 10. (doi:10.1186/1479-5868-10-10)
- Nolan CJ, Damm P & Prentki M 2011 Type 2 diabetes across generations: from pathophysiology to prevention and management. *Lancet* **378** 169–181. (doi:10.1016/S0140-6736(11)60614-4)
- Nolan CJ, Ruderman NB & Prentki M 2013 Intensive insulin for type 2 diabetes: the risk of causing harm. *Lancet Diabetes and Endocrinology* **1** 9–10. (doi:10.1016/S2213-8587(13)70027-5)
- Nolan CJ, Ruderman NB, Kahn SE, Pedersen O & Prentki M 2015 Insulin resistance as a physiological defense against metabolic stress: implications for the management of subsets of type 2 diabetes. *Diabetes* **64** 673–686. (doi:10.2337/db14-0694)
- O'Rahilly S, Turner RC & Matthews DR 1988 Impaired pulsatile secretion of insulin in relatives of patients with non-insulin-dependent diabetes. *New England Journal of Medicine* **318** 1225–1230. (10.1056/nejm198805123181902)
- Odeleye OE, de Courten M, Pettitt DJ & Ravussin E 1997 Fasting hyperinsulinemia is a predictor of increased body weight gain and obesity in Pima Indian children. *Diabetes* **46** 1341–1345. (doi:10.2337/diab.46.8.1341)
- ORIGIN Trial Investigators, Gerstein HC, Bosch J, Dagenais GR, Diaz R, Jung H, Maggioni AP, Pogue J, Probstfeld J, Ramachandran A, et al. 2012 Basal insulin and cardiovascular and other outcomes in dysglycemia. *New England Journal of Medicine* **367** 319–328. (doi:10.1056/NEJMoa1203858)

- Paolisso G, Gambardella A, Amato L, Tortoriello R, D'Amore A, Varricchio M & D'Onofrio F 1995 Opposite effects of short- and long-term fatty acid infusion on insulin secretion in healthy subjects. *Diabetologia* **38** 1295–1299. (doi:10.1007/BF00401761)
- Pedersen DJ, Guilherme A, Danai LV, Heyda L, Matevossian A, Cohen J, Nicoloso SM, Straubhaar J, Noh HL, Jung D, et al. 2015 A major role of insulin in promoting obesity-associated adipose tissue inflammation. *Molecular Metabolism* **4** 507–518. (doi:10.1016/j.molmet.2015.04.003)
- Pierce SB, Costa M, Wisotzkey R, Devadhar S, Homburger SA, Buchman AR, Ferguson KC, Heller J, Platt DM, Pasquinelli AA, et al. 2001 Regulation of DAF-2 receptor signaling by human insulin and ins-1, a member of the unusually large and diverse *C. elegans* insulin gene family. *Genes and Development* **15** 672–686. (doi:10.1101/gad.867301)
- Pittas AG, Das SK, Hajduk CL, Golden J, Saltzman E, Stark PC, Greenberg AS & Roberts SB 2005 A low-glycemic load diet facilitates greater weight loss in overweight adults with high insulin secretion but not in overweight adults with low insulin secretion in the CALERIE trial. *Diabetes Care* **28** 2913–2941.
- Prentki M & Nolan CJ 2006 Islet beta cell failure in type 2 diabetes. *Journal of Clinical Investigation* **116** 1802–1812. (doi:10.1172/JCI29103)
- Rajan S, Shankar K, Beg M, Varshney S, Gupta A, Srivastava A, Kumar D, Mishra RK, Hussain Z, Gayen JR, et al. 2016 Chronic hyperinsulinemia reduces insulin sensitivity and metabolic functions of brown adipocyte. *Journal of Endocrinology* **230** 275–290. (doi:10.1530/JOE-16-0099)
- Rideout EJ, Narsaiya MS & Grewal SS 2015 The sex determination gene transformer regulates male-female differences in *Drosophila* body size. *PLoS Genetics* **11** e1005683. (doi:10.1371/journal.pgen.1005683)
- Robertson RP 2007 Estimation of beta-cell mass by metabolic tests: necessary, but how sufficient? *Diabetes* **56** 2420–2424. (doi:10.2337/db07-0742)
- Saltiel AR & Kahn CR 2001 Insulin signalling and the regulation of glucose and lipid metabolism. *Nature* **414** 799–806. (doi:10.1038/414799a)
- Satin LS, Butler PC, Ha J & Sherman AS 2015 Pulsatile insulin secretion, impaired glucose tolerance and type 2 diabetes. *Molecular Aspects of Medicine* **42** 61–77. (doi:10.1016/j.mam.2015.01.003)
- Schugar RC & Crawford PA 2012 Low-carbohydrate ketogenic diets, glucose homeostasis, and nonalcoholic fatty liver disease. *Current Opinion in Clinical Nutrition and Metabolic Care* **15** 374–380. (doi:10.1097/MCO.0b013e3283547157)
- Schwartz MW & Porte D Jr 2005 Diabetes, obesity, and the brain. *Science* **307** 375–379. (doi:10.1126/science.1104344)
- Shabanpoor F, Separovic F & Wade JD 2009 The human insulin superfamily of polypeptide hormones. *Vitamins and Hormones* **80** 1–31. (doi:10.1016/s0083-6729(08)00601-8)
- Shanik MH, Xu Y, Skrha J, Dankner R, Zick Y & Roth J 2008 Insulin resistance and hyperinsulinemia: is hyperinsulinemia the cart or the horse? *Diabetes Care* **31** (Supplement 2) S262–S268. (doi:10.2337/dc08-s264)
- Shiao MS, Liao BY, Long M & Yu HT 2008 Adaptive evolution of the insulin two-gene system in mouse. *Genetics* **178** 1683–1691. (doi:10.1534/genetics.108.087023)
- Skovso S, Damgaard J, Fels JJ, Olsen GS, Wolf XA, Rolin B & Holst JJ 2015 Effects of insulin therapy on weight gain and fat distribution in the HF/HS-STZ rat model of type 2 diabetes. *International Journal of Obesity* **39** 1531–1538.
- Soares MB, Schon E, Henderson A, Karathanasis SK, Cate R, Zeitlin S, Chirgwin J & Efstratiadis A 1985 RNA-mediated gene duplication: the rat preproinsulin I gene is a functional retroposon. *Molecular and Cellular Biology* **5** 2090–2103. (doi:10.1128/MCB.5.8.2090)
- Softic S, Boucher J, Solheim MH, Fujisaka S, Haering MF, Homan EP, Winnay J, Perez-Atayde AR & Kahn CR 2016 Lipodystrophy due to adipose tissue specific insulin receptor knockout results in progressive NAFLD. *Diabetes* **65** 2187–2200. (doi:10.2337/db16-0213)
- Staa J, Ubhayasekera SJ, Sargsyan E, Chowdhury A, Kristinsson H, Manell H, Bergquist J, Forslund A & Bergsten P 2016 Initial hyperinsulinemia and subsequent beta-cell dysfunction is associated with elevated palmitate levels. *Pediatric Research* **80** 267–274. (doi:10.1038/pr.2016.80)
- Stockhorst U, de Fries D, Steingrueber HJ & Scherbaum WA 2004 Insulin and the CNS: effects on food intake, memory, and endocrine parameters and the role of intranasal insulin administration in humans. *Physiology and Behavior* **83** 47–54. (doi:10.1016/S0031-9384(04)00348-8)
- Szabat M, Lynn FC, Hoffman BG, Kieffer TJ, Allan DW & Johnson JD 2012 Maintenance of beta-cell maturity and plasticity in the adult pancreas: developmental biology concepts in adult physiology. *Diabetes* **61** 1365–1371. (doi:10.2337/db11-1361)
- Szabat M, Page MM, Panzhinskiy E, Skovso S, Mojibian M, Fernandez-Tajes J, Bruin JE, Bround MJ, Lee JT, Xu EE, et al. 2016 Reduced insulin production relieves endoplasmic reticulum stress and induces beta cell proliferation. *Cell Metabolism* **23** 179–193. (doi:10.1016/j.cmet.2015.10.016)
- Tabak AG, Jokela M, Akbaraly TN, Brunner EJ, Kivimaki M & Witte DR 2009 Trajectories of glycaemia, insulin sensitivity, and insulin secretion before diagnosis of type 2 diabetes: an analysis from the Whitehall II study. *Lancet* **373** 2215–2221. (doi:10.1016/S0140-6736(09)60619-X)
- Taniguchi CM, Emanuelli B & Kahn CR 2006 Critical nodes in signalling pathways: insights into insulin action. *Nature Reviews Molecular Cell Biology* **7** 85–96. (doi:10.1038/nrm1837)
- Templeman NM, Clee SM & Johnson JD 2015 Suppression of hyperinsulinaemia in growing female mice provides long-term protection against obesity. *Diabetologia* **58** 2392–2402. (doi:10.1007/s00125-015-3676-7)
- Templeman NM, Mehran AE & Johnson JD 2016 Hyper-variability in circulating insulin, high fat feeding outcomes, and effects of reducing Ins2 dosage in male Ins1-null mice in a specific pathogen-free facility. *PLoS ONE* **11** e0153280. (doi:10.1371/journal.pone.0153280)
- Thorens B, Tarussio D, Maestro MA, Rovira M, Heikkila E & Ferrer J 2015 Ins1(Cre) knock-in mice for beta cell-specific gene recombination. *Diabetologia* **58** 558–565. (doi:10.1007/s00125-014-3468-5)
- van Heemst D 2010 Insulin, IGF-1 and longevity. *Aging and Disease* **1** 147–157.
- van Loon LJ, Kruijshoop M, Menheere PP, Wagenmakers AJ, Saris WH & Keizer HA 2003 Amino acid ingestion strongly enhances insulin secretion in patients with long-term type 2 diabetes. *Diabetes Care* **26** 625–630. (doi:10.2337/diacare.26.3.625)
- Wang TJ, Larson MG, Vasan RS, Cheng S, Rhee EP, McCabe E, Lewis GD, Fox CS, Jacques PF, Fernandez C, et al. 2011 Metabolite profiles and the risk of developing diabetes. *Nature Medicine* **17** 448–453. (doi:10.1038/nm.2307)
- Wang M, Li J, Lim GE & Johnson JD 2013 Is dynamic autocrine insulin signaling possible? A mathematical model predicts picomolar concentrations of extracellular monomeric insulin within human pancreatic islets. *PLoS ONE* **8** e64860. (doi:10.1371/journal.pone.0064860)
- Wentworth BM, Schaefer IM, Villa-Komaroff L & Chirgwin JM 1986 Characterization of the two nonallelic genes encoding mouse preproinsulin. *Journal of Molecular Evolution* **23** 305–312. (doi:10.1007/BF02100639)
- Werner H, Weinstein D & Bentov I 2008 Similarities and differences between insulin and IGF-I: structures, receptors, and signalling pathways. *Archives of Physiology and Biochemistry* **114** 17–22. (doi:10.1080/13813450801900694)
- White MF 2003 Insulin signaling in health and disease. *Science* **302** 1710–1711. (doi:10.1126/science.1092952)

Received in final form 16 December 2016

Accepted 3 January 2017

Accepted Preprint published online 4 January 2017