AGEING AND METABOLISM: DRUG DISCOVERY OPPORTUNITIES

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Abstract | There has recently been significant progress in our understanding of the mechanisms that regulate ageing, and it has been shown that changes in single genes can dramatically extend lifespan and increase resistance to many diseases. Furthermore, many of these genes belong to evolutionarily conserved pathways that also control energy metabolism. In this review, we describe the shared molecular machinery that regulates ageing and energy metabolism. Although drugs to slow ageing face severe regulatory hurdles, it is likely that an understanding of ageing pathways will help to identify novel drug targets to treat metabolic disorders and other age-related diseases.

TYPE 2 DIABETES
Also referred to as adult-onset or non-insulin-dependent diabetes, type 2 diabetes is primarily a disease of insulin insensitivity and is characterized by elevated insulin levels, early in the disease.

ENERGY HOMEOSTASIS
The tendency to maintain the stability of normal biological states during adjustments to environmental changes.

CALORIE RESTRICTION
Limiting of macronutrient sources of protein, carbohydrate and lipid while maintaining micronutrient sources.

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Ageing is an important risk factor for most of the common diseases, including TYPE 2 DIABETES, cardiovascular disease, cancer and neurodegeneration. A fundamental process associated with ageing is dysregulation of ENERGY HOMEOSTASIS. Manipulations that extend lifespan, such as restriction of caloric intake (CALORIE RESTRICTION), can prevent or delay these metabolic changes and confer resistance to many diseases in a range of organisms[1,2]. More recently, specific genetic pathways that modulate ageing in invertebrates and rodents have been identified[3]. Changes in single genes within these pathways can cause dramatic increases in lifespan and these long-lived organisms are less susceptible to age-related diseases. Of particular interest, many of the genes that regulate lifespan also play central roles in the regulation of energy homeostasis and are evolutionarily conserved.

Here we describe the intimate relationship between the regulation of ageing and energy homeostasis, both in terms of epidemiology and at the molecular level. On the basis of this perspective, we discuss how some of the existing therapies for metabolic diseases (for instance, the antidiabetic drug METFORMIN) might exert beneficial effects by tapping into the machinery that regulates ageing. In addition, we illustrate how emerging knowledge of the mechanisms that underlie ageing can provide new targets for drug discovery. By identifying pharmacologically tractable drug targets within ageing pathways, it will be possible to develop novel therapies for the treatment and prevention of numerous diseases (FIG. 1), with an emphasis on the metabolic diseases.

Ageing and metabolism: phenotypic links

Dysregulation of energy homeostasis is a pathology of ageing[4]. Older individuals experience a decrease in the ratio of lean mass to fat mass, especially in muscle, and a progressive redistribution of fat from subcutaneous to visceral regions[5,6]. This redistribution of fat contributes to a feed-forward cycle of increased VISCERAL FAT MASS, decreased INSULIN SENSITIVITY in many tissues and increased serum insulin. In the most severe cases, these pathological processes lead to type 2 diabetes and consequent end-organ damage.

Ageing and certain metabolic dysregulations are risk factors for a variety of diseases. The degree of obesity is inversely correlated with expected lifespan, with the risk increasing twofold in the morbidly obese compared with the non-overweight[5,6]. Younger individuals with increased visceral fat mass are more likely to suffer several diseases commonly associated with the elderly. They are at a higher risk of developing type 2 diabetes[7], coronary heart disease[8], hypertension[9], gallbladder disease[10] and neurodegenerative diseases, including Parkinson's disease and vascular dementia[11,12]. In addition, they are at a higher risk of mortality from the majority of cancers, including those of the colon,

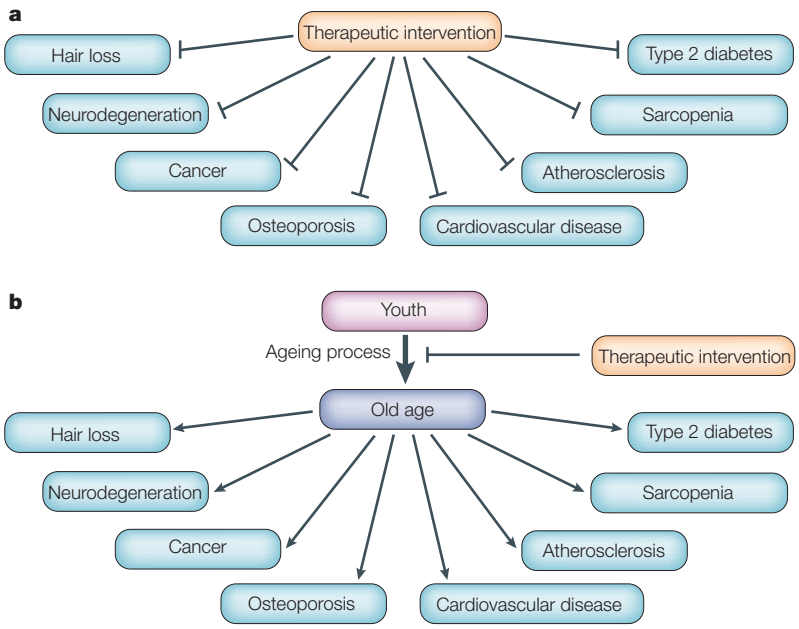


Figure 1 | **Ageing is a risk factor for a large number of impairments and diseases. a** | Drug discovery efforts traditionally target each of these diseases individually. **b** | A complementary approach is to develop therapies to treat and prevent these diseases by targeting their root cause — the mechanisms that regulate ageing.

prostate, pancreas, breast and ovary[13]. The type of obesity is important in determining disease predisposition; increased visceral fat mass seems to be a risk factor for type 2 diabetes and cardiovascular disease. By contrast, when the majority of fat is deposited subcutaneously (that is, under the skin) and visceral fat mass is low, obese individuals are relatively healthy[14,15]. Taken together, these findings forge a strong link between metabolic dysregulations involving visceral obesity and the diseases associated with old age. In the following sections, we review the evidence that ageing and metabolic disease share common molecular mechanisms.

Ageing and metabolism: metabolic links

Studies in rodents conducted during the past 70 years have shown that lifespan is extended by calorie restriction[1,2,16]. Similar effects have been observed in a wide range of organisms, including protozoans, yeast, nematodes and dogs[17–20]. Preliminary results from ongoing studies in monkeys indicate that a similar phenomenon occurs in primates[21], and a clinical trial recently initiated in non-obese humans is examining the short-term effects of calorie restriction on physiology, body composition and risk factors for age-related pathologies[22]. Calorie-restricted organisms not only live longer but also have increased resistance to disease and seem physically to age more slowly. For example, calorie restriction postpones many signs of ageing in rodents, including skin changes, obesity, decline in motor function, and loss of learning and memory[23–27]. In addition, calorie-restricted rodents are less prone to a large number of diseases of ageing, including nephropathy, immune dysfunction, heart disease, cancers and neurodegeneration[1,28–36].

Research in the past 15 years has provided seminal insights into the molecular mechanisms of ageing. Previously, ageing was considered an unregulated process of decay. Surprisingly, genetic studies in lower organisms show that ageing is a regulated process and that dramatic increases or decreases in lifespan can be achieved by changes in single genes[37,38] (TABLE 1). Much of this research was pioneered in the nematode *Caenorhabditis elegans*, where it was found that reducing the activity of a single gene could lead to a doubling of lifespan[37,38]. Subsequently, lifespan-extending mutations were isolated in other multicellular organisms, including the fruitfly *Drosophila* and rodents.

Pertinent to ageing in humans, lifespan is controlled by orthologous genes in many organisms and these genes assemble into genetic pathways that are evolutionarily conserved from invertebrates to mammals (TABLE 1; FIG. 2). For example, *daf-2* encodes a protein in *C. elegans* that is orthologous to the receptors for insulin and insulin-like growth factor-1 (IGF-1), two key regulators of growth and metabolism in mammals[39]. Downregulation of *daf-2* not only doubles the lifespan of *C. elegans* but also delays the age-dependent decline in tissue integrity and muscle function, delays the age of onset of protein-aggregation disease, and increases resistance to high temperature, hypoxia and bacterial pathogenesis[38,40–45]. Consistent with the functional conservation of these pathways, insulin receptors and IGF-1 receptors also regulate lifespan in *Drosophila* and mice[46–48]. Furthermore, mouse mutants with reduced levels of pituitary growth hormone (GH), a key positive regulator of IGF-1, have extended lifespan[49–51].

Lifespan extension of *daf-2* mutants requires another conserved protein, the forkhead box O (FOXO) transcription factor DAF-16[38,52,53]. DAF-16 extends lifespan by a complex mechanism involving the transcriptional regulation of genes encoding metabolic regulators, stress-response proteins (for example, heat-shock proteins) and antimicrobial peptides[54–56]. DAF-16 co-regulates the expression of several genes with another transcription factor known to positively modulate lifespan, the heat-shock factor HSF-1[57,58]. Indeed, coordinate activation of both DAF-16 and HSF-1 is required for maximal extension of lifespan in worms. This is intriguing because various mammalian heat-shock proteins (that are regulated by heat-shock factors) function as chaperone proteins and prevent cell death in brain and heart ischaemia, as well as neurodegeneration associated with protein misfolding and aggregation[59,60].

The AMP-activated protein kinase (AMPK) complex is yet another conserved positive regulator of both energy homeostasis and lifespan, and it has been shown recently that overexpression of AMPK promotes lifespan extension in *C. elegans*[61]. A role for AMPK in ageing has also been found in *Drosophila* and yeast[62,63]. AMPK is activated by phosphorylation or by increases in the cellular AMP/ATP ratio and regulates energy homeostasis in mammals by modulating food intake and energy expenditure[64,65]. The antidiabetic compounds metformin and phenformin activate AMPK

METFORMIN
A member of the biguanide drug class, related to guanidine and the standard of care for type 2 diabetes. The related drugs phenformin and buformin have been withdrawn from the market.

VISCERAL FAT MASS
White fat tissue associated with the body cavities (particularly with organs in the abdominal cavity) that is anatomically and physiologically distinct from subcutaneous fat.

INSULIN SENSITIVITY
The capacity of cells to respond to insulin-stimulated glucose uptake following ingestion of carbohydrates.

Table 1 | **Genetic pathways that control lifespan**

Target	Lifespan-extending intervention	Organism	References
Insulin/IGF-1			
Insulin and IGF-1	Downregulate*	Worm	54,193–196
InsR and IGF-1R	Downregulate	Worm, fly, mouse	38,39,46–48
IRS	Downregulate	Worm, fly	197,198
PI3K	Downregulate	Worm	37,198,199
PTEN	Upregulate	Worm	200–205
PDK	Downregulate	Worm	206
AKT	Downregulate	Worm, yeast	207–209
FOXO	Upregulate	Worm, fly	38,52,53,78,210
GHR	Downregulate	Mouse	51
GHRHR	Downregulate	Mouse	50
Sir2			
Sir2 orthologues	Upregulate	Yeast, worm, fly	171–173
AMPK			
AMPK	Upregulate	Yeast, worm, fly	61–63
TSC	Upregulate	Fly	150
TOR	Downregulate	Worm, fly	149,150
S6K	Downregulate	Fly	150
Heat-shock proteins			
HSF	Upregulate	Worm	40, 57
HSP70	Upregulate	Worm, fly	211–213
Small HSPs	Upregulate	Worm, fly	57,214,215
Other			
Indy/NaCT	Downregulate	Worm, fly	185,186
p66shc	Downregulate	Mouse	216
JNK	Upregulate	Fly	213

*Also upregulated in *Caenorhabditis elegans* in the case of some insulins that inhibit their receptor. Targets listed are focused on pathways that appear to have an evolutionarily conserved function in the regulation of lifespan. Readers who are interested in a more comprehensive account of genes controlling lifespan are referred to recent reviews[3,156]. AMPK, AMP kinase; FOXO, forkhead box O; GHR, growth hormone receptor; GHRHR, growth hormone releasing hormone receptor; HSF, heat-shock factor; HSP70, heat-shock protein 70; IGF-1, insulin-like growth factor 1; IGFR, IGF receptor; IRS, insulin receptor substrate; PDK, phosphoinositide-dependent kinase; PI3K, phosphatidylinositol 3-kinase; PTEN, phosphatase and tensin homologue; S6K, S6 kinase; TOR, target of rapamycin.

by phosphorylation but without changes in the AMP/ATP ratio (likely through inhibition of mitochondrial respiration)[66–69]. Interestingly, phenformin treatment of non-diabetic rodents extends maximum lifespan by 20%[70,71]. Therefore, the metabolic benefits and lifespan-extending properties of phenformin seem to be linked to the activation of AMPK.

The identification of genes that control lifespan in mice by regulating the function of adipose tissue provides further insight into the link between ageing and energy metabolism. C/EBPα and C/EBPβ are transcription factors that have a role in adipocyte differentiation and maturation[72]. Replacement of C/EBPα by C/EBPβ in transgenic mice (C/EBPβ/β mice) produces lean animals with adipocytes enriched in mitochondria, causing a shift towards energy dissipation rather than storage[73]. C/EBPβ/β mice live 22% longer than controls[74]. Similarly, mice lacking the insulin receptor only in adipose tissue (fat insulin receptor knockout (FIRKO) mice) maintain a lower proportion of body fat throughout life despite normal food intake, and FIRKO mice live 18% longer than wild-type mice[48,75]. Like the C/EBPβ/β mice, FIRKO mice have smaller adipocytes, which results in reduced fat storage[75]. As the regulation of lifespan by insulin signalling in adipose-like tissue has been demonstrated in *C. elegans* and *Drosophila*[76–78], the results obtained in mice suggest that this could also be conserved in humans.

These studies confirm that insulin signalling in adipocytes is important for ADIPOGENESIS and might also control secondary signals that regulate ageing in other tissues. These signals are likely to be secreted hormone-like molecules. In mammals, adipocytes secrete a large number of factors termed ADIPOKINES, including leptin, adiponectin, resistin, adipsin, monocyte chemoattractant protein (MCP1), plasminogen activator inhibitor 1 (PAI1), tumour-necrosis factor-α (TNFα) and interleukin-6 (IL-6), whose relative proportions are affected by insulin signalling[79,80]. Insulin can either inhibit signals that slow ageing and/or activate signals that promote ageing. It will be of interest to identify the complete set of adipocyte-derived secondary signals that control ageing and to determine whether these signals are also generated in tissues other than fat.

Insulin: good cop/bad cop

From the foregoing discussion it is clear that reduced insulin signalling extends lifespan and confers disease resistance in several model organisms. Given that insulin is crucial to the regulation of blood glucose levels, how do we reconcile the negative effect of insulin signalling on the ageing process with its beneficial effect in the regulation of glucose homeostasis? Insulin is secreted when food is consumed in order to dispose of excess blood glucose (FIG. 3). In type 2 diabetes inadequate insulin signalling in certain tissues results in decreased glucose uptake and increased hepatic glucose production, with consequent hyperglycaemia. The long-term consequences of diabetes include end-organ damage, such as retinopathy, neuropathy and nephropathy[81].

Insulin and other molecules involved in energy availability are well positioned to regulate both adipogenesis and the rate of ageing. Insulin is a potent stimulus of adipogenesis, and promotes the storage of energy as fat. Because mammals commonly encounter periods of food scarcity, the obesity that results from a sustained increase in insulin levels during times of food abundance is evolutionarily advantageous[82]. A program that extends lifespan in response to adverse environmental conditions (for example, starvation) is also evolutionarily advantageous, as this system allows the animal to survive until the return of conditions that favour reproduction and the survival of progeny[83]. Insulin-mediated glucose homeostasis therefore seems to be an evolutionary adaptation that tailors complementary strategies for survival to food availability.

ADIPOGENESIS
The development of fat precursor cells into mature white or brown fat tissue.

ADIPOKINES
Proteinaceous factors produced or released by fat cells.

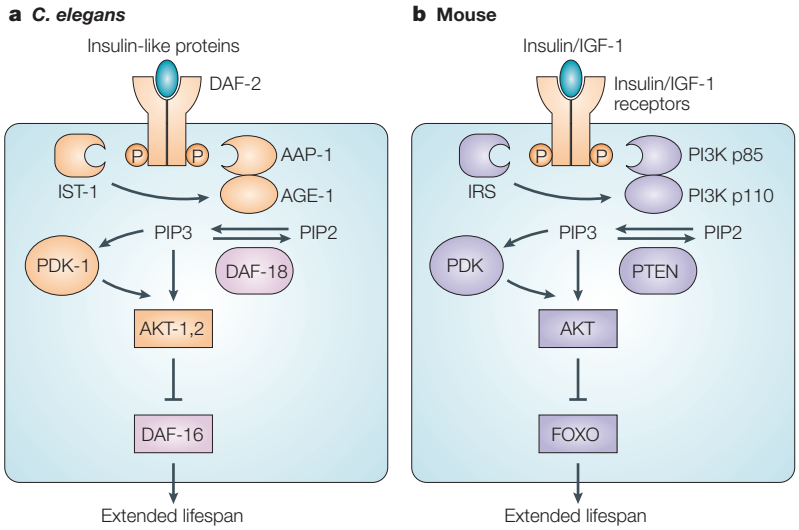


Figure 2 | **Conserved regulation of lifespan in *Caenorhabditis elegans* and mice by the insulin/IGF-1 pathway.** Notably, the pathway described for mouse is completely conserved in humans. Although worms have more than 35 different insulin-like proteins that bind to DAF-2, mammals have only insulin and insulin-like growth factor 1 (IGF-1). Stimulation of DAF-2 and insulin/IGF-1 receptors by their cognate ligands triggers a serine kinase cascade that culminates in phosphorylation and inactivation of the transcription factors DAF-16 and forkhead box O (FOXO) (in *C. elegans* and mouse, respectively). Reduced activity through this pathway activates DAF-16/FOXO, increasing the transcription of lifespan-promoting and disease-resistance genes. Positive regulators of lifespan in *C. elegans* are coloured in pink, whereas negative regulators are coloured in orange. IRS, insulin receptor substrate; PDK, phosphoinositide-dependent kinase; PI3K, phosphatidylinositol 3-kinase; PTEN, phosphatase and tensin homologue.

Cycles of food abundance and scarcity that were common to ancestral humans are not generally encountered today. In situations in which plentiful high-calorie food is combined with a sedentary existence, the pancreas increases insulin secretion above normal levels in order to dispose of sustained excess blood glucose which, over time, leads to the deposition of visceral fat. Increased visceral fat mass results in accelerated lipolysis, with consequent elevation in plasma free fatty acids, increases in muscle and liver fat content and, ultimately, activation of a serine-kinase cascade that impairs insulin signalling in these tissues[84–88].

Once considered simply a fat-storage depot, adipose tissue is now also recognized to be an endocrine organ[79,80]. The sustained increase in insulin levels also shifts the balance of adipokines from those that promote insulin sensitivity in peripheral tissues (for example, adiponectin) to those that lower insulin sensitivity (for example, TNFα)[79,80]. As insulin sensitivity decreases in muscle and liver, the pancreas further increases insulin secretion to prevent an increase in blood glucose. A sustained increase in glucose input therefore triggers a cycle of increasing insulin levels, visceral fat mass and insulin resistance in peripheral tissues (FIG. 3)[89]. As visceral obesity develops, macrophages infiltrate the adipose tissue, which seems to shift further the balance of adipocyte-secreted factors towards those that cause insulin resistance in peripheral tissues[90,91]. Eventually, this feed-forward cycle leads to an altered metabolic state that involves very high levels of insulin (HYPERINSULINAEMIA), even under fasting conditions. This state triggers a constellation of related complications, collectively referred to as the METABOLIC SYNDROME[89]. Individuals with metabolic syndrome are at higher risk of developing type 2 diabetes and cardiovascular disease, and also have a shorter life expectancy[92]. As described above, age is also a risk factor for these diseases, and the elderly are more likely to experience insulin resistance and hyperinsulinaemia[4]. The increase in visceral fat mass observed in older subjects contributes to the insulin resistance that occurs in peripheral tissues, including muscle and liver[93–95]. The redistribution of fat towards visceral regions that can occur with age or obesity promotes an altered metabolic state that involves insulin resistance and hyperinsulinaemia. It should be noted that late in the course of type 2 diabetes, pancreatic β-cell apoptosis can lead to a lack of insulin, which requires the administration of exogenous insulin to normalize blood glucose.

Increased insulin signalling is responsible for many conditions associated with ageing and the metabolic syndrome. Insulin resistance is characterized by the loss of insulin-stimulated glucose uptake, leading to hyperglycaemia. However, other downstream pathways of insulin signalling, which are not related to the loss of normal glucose uptake, can lead to many of the negative consequences of type 2 diabetes. First, overstimulation of the insulin receptor can occur in tissues in which the cellular response to insulin is relatively preserved. As an example, insulin regulation of renal sodium-ion retention is not compromised in individuals with the metabolic syndrome; therefore, hyperinsulinaemia promotes fluid retention and puts them at risk of developing hypertension[96]. Second, hyperinsulinaemia can have detrimental effects in tissues with impaired glucose uptake in which other signalling pathways downstream of the insulin receptor are not impaired. For example, in type 2 diabetic patients with impaired insulin-stimulated glucose uptake in muscle, the activation of mitogen-activated protein kinase signalling by insulin is normal[97]. Hyperinsulinaemia might overstimulate cell division and increase the incidence of tumours. Accordingly, hyperinsulinaemia is a risk factor for breast cancer[98] and insulin therapy increases the risk of colorectal cancer among type 2 diabetics[99]. Last, insulin and IGF-1 signalling through their cognate receptors in vascular endothelial cells has been implicated in the pathogenesis of vascular retinopathy, suggesting that specific blockade of these receptors in the vasculature could ameliorate the negative sequelae of diabetic retinopathy[100].

It is noteworthy that many interventions that extend lifespan, including calorie restriction and knocking out the insulin receptor in fat cells, prevent the cycle that leads to visceral obesity, insulin resistance and hyperinsulinaemia. In humans, preventing this cycle seems to confer some protection from the diseases of ageing. In a study of healthy, non-obese individuals, those with the greatest insulin sensitivity

HYPERINSULINAEMIA
Blood insulin levels that exceed normal physiological fluctuations of fasting and feeding.

METABOLIC SYNDROME
A constellation of dysfunctions in glucose metabolism (such as insulin sensitivity and glucose tolerance), lipid metabolism and cardiac function; also referred to as syndrome X or insulin-resistance syndrome.

did not develop hypertension, coronary heart disease, stroke, cancer or type 2 diabetes after an average follow-up of more than 6 years, whereas those with the greatest insulin resistance developed at least one of those conditions[101]. In addition, insulin resistance and hyperinsulinaemia are uncommon in centenarians, whose insulin resistance is notably low compared with that of healthy younger adults (BOX 1)[102].

The foregoing suggests that a chronic excess of insulin is detrimental and that lowering insulin levels would extend lifespan as long as there is sufficient insulin to maintain glucose homeostasis. Interventions that block the cycle that leads to insulin resistance and hyperinsulinaemia provide an avenue to reap the lifespan-extending benefits of lower insulin signalling. In the next section we discuss how this might be done, and describe additional entry points for intervention in the machinery that regulates ageing and age-related disease.

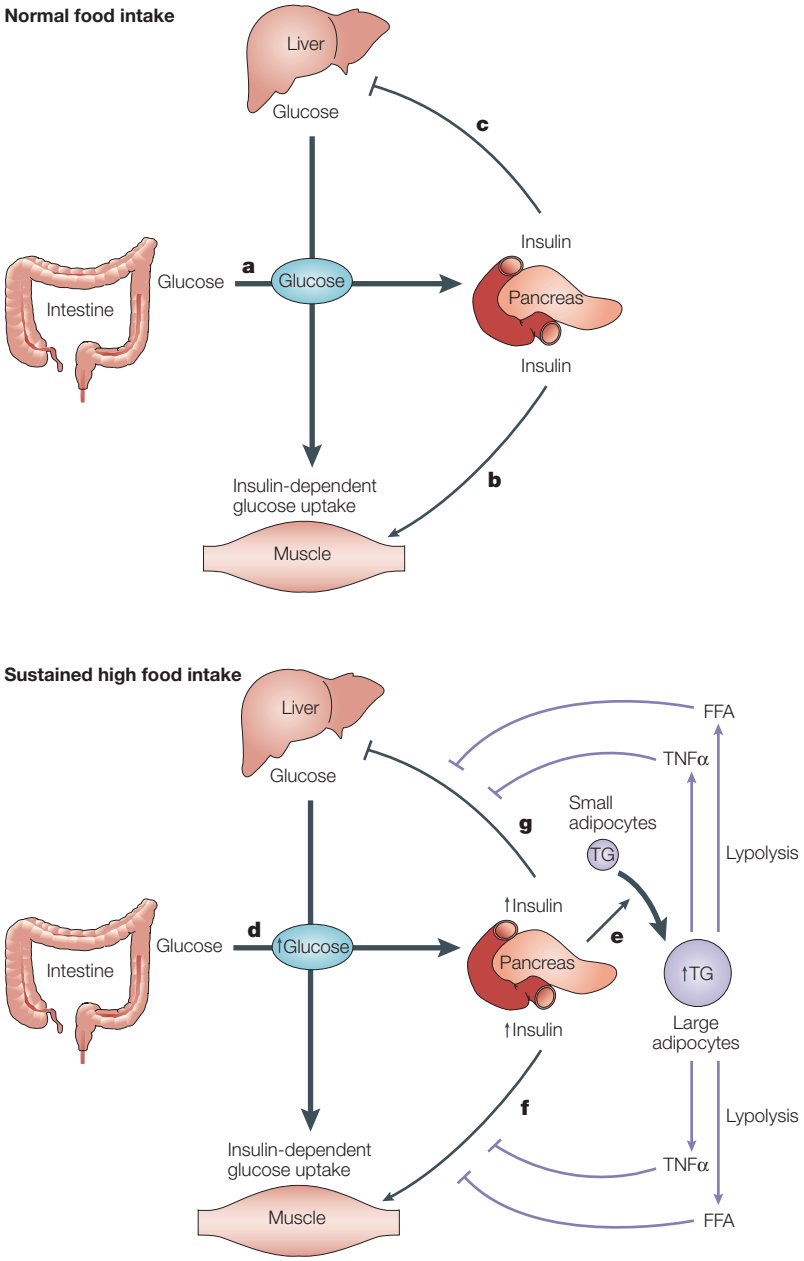


Figure 3 | **Tissue cross-talk in the regulation of blood-glucose levels.** When the level of food intake is normal, glucose derived from food intake (**a**) and from endogenous sources stimulates secretion of insulin by the pancreas. Insulin lowers blood-glucose levels by promoting glucose uptake in skeletal muscle (**b**) and inhibiting hepatic gluconeogenesis (**c**). During sustained high intake of food, a sustained increase in glucose intake (**d**) increases insulin secretion from the pancreas above normal levels (**e**), which promotes accumulation of triglycerides in adipocytes and, over time, results in visceral obesity. Under these conditions, visceral adipose tissue secretes a variety of factors (for simplicity only free fatty acids (FFA) and tumour-necrosis factor-α (TNFα) are illustrated) that inhibit insulin-mediated glucose uptake into skeletal muscle (**f**) and inhibition of hepatic gluconeogenesis (**g**). To compensate for this attenuated response to insulin and to prevent an increase in blood glucose, the pancreas increases insulin secretion. Eventually this feed-forward cycle leads to an altered metabolic state involving visceral obesity, insulin resistance and hyperinsulinaemia.

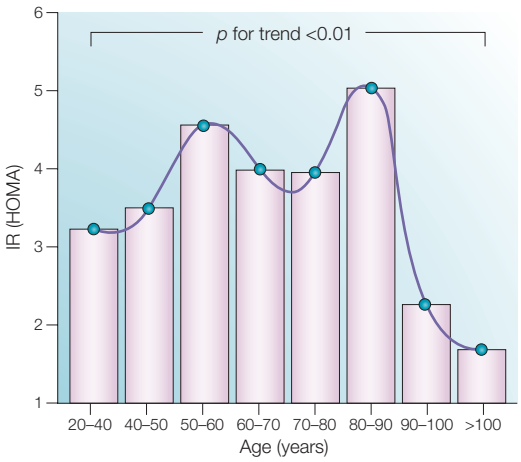
Drug discovery outlook

How does one take advantage of our emerging knowledge of ageing for drug discovery and development? Given the intimate relationship between ageing and the regulation of energy homeostasis, we suggest that pathways shown to function in ageing will provide targets for the treatment of metabolic diseases. We first discuss how some existing therapies for obesity and type 2 diabetes might already exert their beneficial effects by inadvertently tapping into these ageing pathways. We will then focus on how new discoveries in the field of ageing can result in novel therapies for the treatment of metabolic diseases. We suggest that drugs targeting the mechanisms of ageing are inherently more valuable than drugs ameliorating only the disease by treating the symptoms. For instance, a diabetes drug that normalizes blood glucose *and* improves other clinical outcomes is preferable to one that only affects glucose homeostasis.

Reducing food intake. The front-line treatment for obesity and type 2 diabetes remains improved diet and increased exercise, with the goal of reducing body weight. The metabolic benefits of intentional weight loss through diet or stomach-reduction surgery are well known. These interventions decrease visceral fat mass, lower insulin levels, curb type 2 diabetes progression and reduce type 2 diabetes-related mortality and all-cause mortality[103–108]. It is tempting to speculate that some of these benefits are conferred by the attendant calorie restriction, because they cannot be accounted for solely by the lost body weight. For example, the increased insulin sensitivity and decreased insulin levels achieved following stomach-reduction surgery are much greater than predicted by the actual weight lost[109]. Likewise, calorie restriction for only a week increases insulin sensitivity in type 2 diabetics, even though only a small loss of weight occurs in this period[110].

Although intentional weight loss is beneficial, it is unlikely that a voluntary drastic reduction of caloric intake will ever be widely embraced, because lifestyle modifications involving reduced food intake have very high failure rates[111]. Therefore, pharmacological regulation of nutrient intake is expected to be more successful in treating obesity and diabetes. There are two potential ways to achieve reduced caloric intake: suppression of feeding behaviour or blockade of nutrient absorption.

Box 1 | **Centenarians**



The study of centenarians is beginning to yield clues about the genetic variations that confer extreme longevity in human populations. Centenarians seem to be protected from the cycle that leads to insulin resistance and hyperinsulinaemia, and their insulin resistance is as low, if not lower, than that of healthy younger adults (see box figure; reproduced with permission from REF. 102). The graph shows age-related insulin resistance (IR) in healthy individuals. IR was calculated using the homeostasis model assessment (HOMA). Centenarians also show a lower incidence and significant delay in the onset of age-related diseases, including cardiovascular disease, type 2 diabetes, cancer and Alzheimer's disease[217]. Recent studies point to individual genetic loci that favour long lifespan[218]. One study found an association of a polymorphism in the gene encoding the microsomal transfer protein (MTP), which controls the rate-limiting step in lipoprotein assembly[219]. Another study found that Ashkenazi Jewish centenarians and their offspring have larger high-density and low-density lipoprotein particles than a control group; a search among candidate genes known to control lipoprotein particle size identified a variant of the cholesteryl ester transfer protein (CETP) associated with very long lifespan in that population[220,221]. In addition, the apolipoprotein E ε-4 allele, which increases the risk of Alzheimer's disease, is very rare among centenarians[222]. Taken together, these studies suggest that proper regulation of lipoprotein metabolism can confer the ability to reach extreme old age. Future studies could provide insights on the mechanisms by which centenarians seem to be protected from impairments in the response to insulin, hyperinsulinaemia and the detrimental effects on the ageing process.

There are two approved classes of drugs that block nutrient absorption. First, inhibitors of α-glucosidase, such as acarbose (Precose; Bayer) and miglitol (Glyset; Pfizer), inhibit the hydrolysis of complex carbohydrates in the small intestine and reduce peak blood glucose after feeding, with a reduction in insulin levels[112]. This type of food limitation might have effects similar to calorie restriction; treatment with acarbose has been shown to prevent or delay the onset of type 2 diabetes, high blood pressure and cardiovascular complications among individuals with impaired glucose tolerance[113,114]. The other class of drugs that blocks nutrient absorption is the inhibitors of gastrointestinal lipases, such as orlistat (Xenical; Roche), which blocks the uptake of fats from the intestine after feeding, resulting in anti-obesity effects[115].

With respect to appetite suppressants, currently marketed drugs work primarily by increasing the availability of neurotransmitters that reduce feeding in the central nervous system (CNS)[116]. Sibutramine (Meridia; Abbott Labs) blocks reuptake of serotonin and adrenaline, and is approved for weight loss and weight maintenance in conjunction with a reduced-calorie diet[117]. However, these appetite suppressants have limited efficacy and intensive drug discovery efforts are aimed at the feeding circuits in the CNS. Peptides derived from the gut, stomach and adipose tissue play a major role in regulating feeding behaviours, mainly by acting on neuronal populations in the HYPOTHALAMUS that regulate appetite[118]. Agonists of the melanocortin 4 receptor and antagonists of the ghrelin receptor and melanin-concentrating hormone receptor are some avenues currently being explored[119–121]. The cannabinoid receptor antagonist rimonabant (Acomplia; Sanofi-Aventis) currently looks to be the most promising anti-obesity candidate[122].

Regulating the function of adipocytes. Strategies that focus on reducing visceral fat are likely to have better clinical outcomes than drugs that only reduce subcutaneous fat. Surgical removal of visceral fat prevents type 2 diabetes and increases insulin sensitivity in old rats; the resulting insulin sensitivity is similar to that observed in old calorie-restricted and young rats[123]. In humans, removal of visceral fat in conjunction with stomach-reduction surgery increases insulin sensitivity and lowers insulin levels to a much larger extent than stomach-reduction surgery alone[124]. By contrast, liposuction of subcutaneous fat in obese women does not increase insulin sensitivity or lower insulin levels[125]. Pharmacological interventions could target visceral obesity by mimicking the changes observed in the FIRKO and C/EBPβ/β mice, in particular the increase in mitochondria-enriched small adipocytes. Human adipose tissue contains mesenchymal stem cells[126], which could be stimulated to differentiate into small adipocytes. One possible strategy involves activating the peroxisome proliferator-activated receptor-δ (PPARδ) transcription factor, which functions in adipocytes to induce a variety of genes required for catabolism of fat and energy dissipation[127]. Both GlaxoSmithKline and Mitsubishi Pharmaceuticals have PPARδ agonists in Phase II clinical trials (GW-501506 and netoglitazone, respectively).

As described previously, insulin signalling in adipocytes regulates secondary signals that affect insulin sensitivity in other tissues. An important therapeutic goal is to shift the balance of circulating adipokines towards those that promote insulin sensitivity. Some of these adipokines are also inflammatory mediators and a chronic sub-acute inflammatory state is commonly associated with the metabolic syndrome[128] and old age[129]. Inflammatory markers are correlated with the development of diseases of ageing, such as type 2 diabetes[130,131], atherosclerosis[132], vascular dementia and Alzheimer's disease[133]. Accordingly, high doses of aspirin reverse insulin resistance in obese rodents and type 2 diabetics, mediated by inhibition of IKK (inhibitor of nuclear factor-κB kinase β)[134,135]. Other opportunities for pharmacological intervention involve reducing the levels of individual cytokines, such a TNFα[136]. There are currently three marketed TNFα inhibitors: adalimumab (Humira; Abbott Labs), infliximab (Remicade; Centocor) and etanercept

HYPOTHALAMUS
A region of the diencephalon lying below the thalamus that controls feeding, thirst, body temperature, sleep and emotion, as well as pituitary and autonomic functions.

(Enbrel; Amgen/Wyeth). In addition, a recent study suggests that inhibition of TNFα by pentoxifylline might improve insulin sensitivity[137].

Activating AMPK. Physical exercise activates AMPK in muscle, liver and adipose tissue[138]. AMPK is partially required for exercise-induced glucose uptake[139] and is an important component of the adaptive response to exercise training in mice[140–142]. Therefore, it is possible that AMPK activation is responsible for some of the beneficial effects of exercise in extending lifespan[143]. Because AMPK is a key regulator of energy homeostasis, activation of AMPK has been proposed as a strategy for the treatment of the metabolic syndrome[144]. Indeed, the most commonly used drug for the management of type 2 diabetes, metformin, activates AMPK possibly via inhibition of Complex I of the respiratory chain[66–69]. Given that AMPK functions to extend lifespan in many organisms and that metformin reduces fasting insulin levels and visceral fat mass in humans[145,146], we speculate that metformin will extend lifespan and delay the onset of age-related diseases. Consistent with this view, there is some evidence that metformin treatment results in better clinical outcomes than other drugs that lower blood glucose levels by a similar degree. In one study that compared metformin with insulin or the sulphonylureas (which promote insulin secretion), metformin was superior with respect to end-organ damage, myocardial infarction and all-cause mortality[147]. Metformin outperformed therapies that increase blood insulin levels. Efforts to design improved activators of AMPK represent a fruitful approach to the next generation of antidiabetes drugs.

Other opportunities for pharmacological intervention in the AMPK pathway involve targeting downstream effectors of AMPK[64] (FIG. 4). In mammals, AMPK inhibits signalling by the kinase target of rapamycin (TOR)[148] and reduction of TOR in *C. elegans* and *Drosophila* extends lifespan by 30%[149,150]. Interestingly, inhibition of TOR by rapamycin and its analogues has been used for cancer treatment[151]. A key target of TOR phosphorylation is S6 kinase (S6K), which in *Drosophila* functions to extend lifespan[150]. Mice lacking S6K1 are protected from insulin resistance and obesity induced by old age or a high-fat/high-calorie diet[152]. Other key targets inhibited by AMPK include acetyl co-enzyme A carboxylase 2 (ACC-2) and 3-hydroxy-3-methylglutaryl-co-enzyme A-reductase (HMG-CoA reductase). AMPK promotes FATTY-ACID OXIDATION via phosphorylation and, therefore, inhibition of ACC-2[144]. Mice lacking ACC-2 are lean, continuously burn fat and are resistant to obesity induced by a high-fat/high-calorie diet[153,154]. AMPK also phosphorylates HMG-CoA reductase, which reduces its activity and inhibits cholesterol biosynthesis[64,65]. HMG-CoA reductase is the target of statins, a class of drugs that reduce plasma low-density lipoprotein cholesterol and lower the risk of coronary heart disease[155]. In *Drosophila*, statin treatment slows the neurodegeneration of AMPK loss-of-function mutants[62].

FATTY-ACID OXIDATION
The burning of stored fat or fats obtained through the diet. Also known as β-oxidation, it efficiently produces NADH, $FADH_2$ and acetyl co-enzyme A.

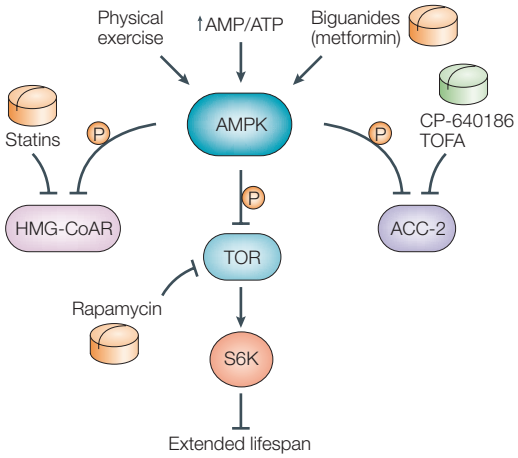


Figure 4 | **Potential pharmacological entry points into the AMPK pathway.** Physiologically, exercise and low energy stores (increased AMP/ATP ratio) activate AMP kinase (AMPK) activity. AMPK, in turn, phosphorylates and inactivates HMG-CoA reductase (HMG-CoAR), target of rapamycin (TOR) and ACC-2. Established drugs (yellow pill) such as the biguanide metformin activate AMPK, whereas statins and rapamycin inhibit HMG-CoAR and TOR, respectively. Experimental compounds (green pill) CP-640186 and TOFA are non-selective inhibitors of ACC, a key enzyme in fatty-acid biosynthesis[223,224]. ACC, acetyl co-enzyme A carboxylase.

Lowering the GH/IGF-1 axis. Consistent with the role of insulin/IGF-1 signalling in *C. elegans* and *Drosophila*, reduced IGF-1 signalling extends lifespan in rodents. Female mice that lack one functional copy of the IGF-1 receptor are long lived, have a normal size and show normal fertility and feeding behaviour[47]. GH stimulates production of IGF-1 and dwarf rodents with defects in GH production or signalling are long lived, have lower insulin levels and are resistant to many diseases of ageing, including cancer, osteoarthritis and cognitive decline[49–51,156,157]. By contrast, transgenic mice that express high levels of GH are short lived and show various signs of accelerated ageing[158,159]. A relatively small number of humans with untreated congenital GH or IGF-1 deficiencies seem to be long lived[160]. Unlike other interventions that extend lifespan in rodents, GH deficiency causes moderate obesity; however, most of the excess fat mass accumulates subcutaneously[161]. Opportunities for pharmacological intervention in this pathway include therapeutics that lower IGF-1 levels or its bioavailability, and antagonists of the GH and IGF-1 receptors, as well as compounds that decrease hypothalamic stimulation of GH secretion in the pituitary gland (FIG. 5). Octreotide (Sandostatin; Novartis) is a marketed somatostatin receptor agonist that decreases GH release and is used for the treatment of ACROMEGALY. In addition, octreotide might have utility in treating hypothalamic obesity[162,163], certain cancers[164] and diabetic end-organ damage[165]. Pegvisomant (Somavert; Pfizer), a GH receptor antagonist that lowers IGF-1 levels, was initially developed to treat acromegaly[166] but also might have activity

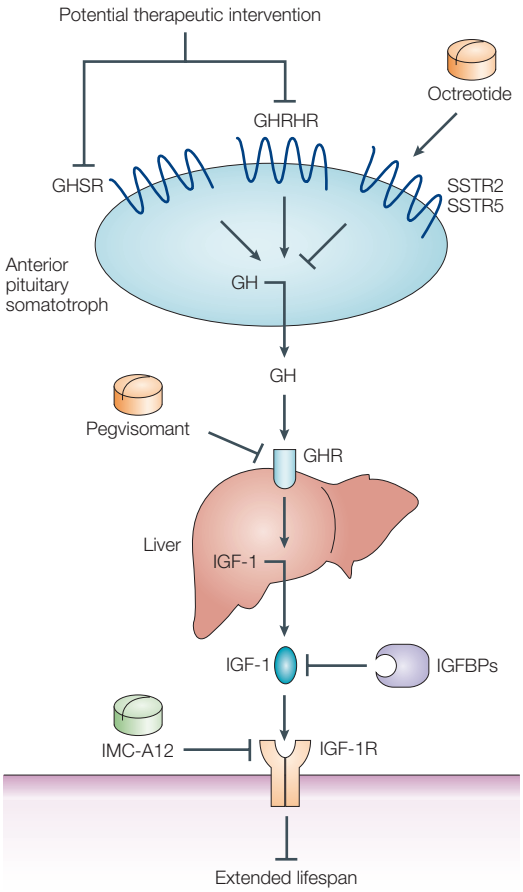


Figure 5 | **Potential therapeutic entry points into the GH–IGF-1 axis.** The release of growth hormone (GH) from the pituitary is stimulated by the growth hormone secretagogue receptor (GHSR) and growth hormone releasing hormone receptor (GHRHR), and inhibited by somatostatin receptors 2 and 5 (SSTR). Although GHRH activity is controlled predominantly by GH secretagogue ('ghrelin') at the level of the hypothalamus, GHSRs are localized on anterior pituitary somatotrophs and positively modulate GH release. Growth-hormone binding to its receptor (GHR) promotes insulin-like growth factor 1 (IGF-1) secretion, primarily by the liver. Approved drugs that modulate this pathway are shown as yellow pills (investigational drugs shown in green). Pegvisomant is a modified GH mutant that acts as a competitive inhibitor of GHR[225]. Monoclonal antibodies that block IGF-1 action are currently at the development stage. Other opportunities involve interventions that antagonize GHRH and GHS receptors, or that increase the level/activity of the IGF-1-binding proteins (IGFBPs), which normally lower IGF-1 bioavailability.

against malignancy[167]. Last, several biopharmaceutical companies, including ImClone, are developing IGF-1 receptor antagonists for the treatment of cancer.

In light of these studies in rodents, the current use of GH as an anti-ageing therapy in humans has been questioned[168]. Long-term studies of a sufficient scale to evaluate clinically relevant outcomes, such as improvements in ability to carry out activities of daily living or a lower rate of fractures, would be required to justify the use of GH as an anti-ageing therapy. Although short-term treatment with GH has beneficial effects in increasing lean body mass, long-term administration has been shown to increase the risk of type 2 diabetes and, possibly, cancer[169].

Modulating Sir2-class enzymes. Sir2-class enzymes are NAD-dependent protein deacetylases that increase lifespan in yeast, *C. elegans* and *Drosophila*[170–173]. NAD is cleaved in the reaction to form nicotinamide, which is a feedback inhibitor of the enzyme[174]. Deacetylation is dependent on the concentration of NAD and, therefore, links lifespan to cellular energy levels and metabolism[174]. Increased *Sir2* gene copy number serves to extend lifespan and Sir2 enzymes seem to have a role in many lifespan pathways. For instance, overexpression of *sir2.1* in *C. elegans* extends lifespan via the FOXO transcription factor DAF-16[172], which also mediates the lifespan extension of insulin/IGF-1-pathway mutants. In *Drosophila*, *Sir2* mediates lifespan extension in response to calorie restriction[173]. Accordingly, pharmacological activation of Sir2-class enzymes could result in lifespan extension and disease resistance. This could be achieved by compounds that increase the concentration of NAD in the cell, perhaps via induction of enzymes involved in NAD biosynthesis, such as nicotinamide-phosphoribosyltransferase and nicotinamide mononucleotide-adenylyltransferase[175]. Recently, Sir2 activation in yeast was achieved with iso-nicotinamide, which prevents inhibition of Sir2 by nicotinamide[176].

Although lifespan regulation by any of the seven mammalian Sir2 homologues (SIRT1–7) has not been reported, recent studies indicate that some of these genes might contribute to the regulation of energy homeostasis. SIRT1, which is thought to be the orthologue of yeast Sir2 and *C. elegans* sir2.1, deacetylates FOXO transcription factors and alters their ability to modulate transcription of selected genes[177–180]. In mouse adipocytes, SIRT3 increases mitochondrial respiration and promotes thermogenesis via activation of the PPARγ co-activator (PGC-1α)[181]. Because starvation increases the levels of both SIRT1 and SIRT3 in adipose tissue[181,182], we speculate that SIRT1 and SIRT3 might function to extend mammalian lifespan by regulating adipocyte function.

Counter to the hypothesis that SIRT1 activation might be involved in disease resistance and lifespan extension, recent studies suggest that inhibition of SIRT1 could result in beneficial effects on energy metabolism. SIRT1 attenuates adipogenesis and promotes the release of free fatty acids via repression of the nuclear receptor PPARγ[183] in cultured mouse adipocytes, which might contribute to insulin resistance and hyperglycaemia. Hepatic SIRT1 protein levels increase after fasting to promote glucose production by stimulating transcription of genes that regulate gluconeogenesis via PGC-1α[184]. SIRT1-knockout mice have normal blood glucose and lower blood insulin, and they also seem to have higher insulin sensitivity in the fed state. Lower insulin levels are possibly due to SIRT1 promoting insulin secretion from pancreatic β-cells (L. Bordone and L. Guarente, personal communication).

ACROMEGALY
Enlargement of the hands, feet, head and face due to overproduction of growth hormone by the anterior pituitary gland.

Therefore, pharmacological inhibition of SIRT1 might reduce glucose and insulin levels, and improve insulin sensitivity in type 2 diabetics. A series of potent, specific, orally bioavailable and cell-permeable SIRT1 inhibitors has recently been identified (A. Napper, J. Solomon, L.J. Huber, P.S.D. and R.C., unpublished observations). These compounds could serve as tools for pharmacological investigations examining the role of SIRT1 enzymatic activity on metabolic profiles.

Inhibiting Indy/NaCT. Reducing the function of the *indy* ('I'm not dead yet') gene extends lifespan in *Drosophila* and *C. elegans*[185,186]. *Indy* and NaCT, its mammalian orthologue, are plasma-membrane dicarboxylate and tricarboxylate transporters that are expressed in tissues that regulate energy use and storage[187,188]. Citrate, a primary substrate of these transporters, is an energy-rich metabolite that is a precursor and positive regulator of fatty-acid and cholesterol synthesis. NaCT promotes utilization of blood citrate for fat synthesis in the liver[189] and treatment of bipolar patients with lithium, a potent NaCT activator[190], causes weight gain and enhances fat and cholesterol synthesis[191]. Selective blockers of citrate transport by NaCT could promote resistance to obesity, normalize lipid profiles and therefore extend lifespan.

Future prospects

The prospect of slowing the ageing process through pharmacological intervention addresses the number one risk factor for a large number of diseases — age[192]. An important challenge is to devise a strategy for clinical development and marketing of such therapeutics. Because of the links that exist between ageing and metabolic dysfunction, we speculate that drugs capable of regulating the ageing process are likely to be introduced initially as *treatments* for metabolic diseases (for example, type 2 diabetes) using established clinical endpoints such as haemoglobin-A1C levels. After establishing the utility of these therapeutics in a primary indication, the next logical step would be to demonstrate efficacy in delaying or *preventing* complications associated with that primary indication, such as cardiac disease, nephropathy, neuropathy and retinopathy in the case of type 2 diabetes. Subsequently, the use of these same drugs could be explored in trials of disease prevention in non-diabetics. For example, lowering visceral fat and insulin levels could positively affect the onset or progression of neurodegenerative diseases. Drugs that affect ageing pathways, such as metformin, which are currently used to treat type 2 diabetes, might be beneficial in treating or preventing such diseases. Finally, it might be desirable to address directly the *rate of ageing* in a clinical trial. Although studying lifespan in a controlled clinical setting is highly impractical and costly, it might be possible to develop biomarkers that indirectly measure ageing. On the basis of the foregoing discussion, visceral fat and insulin levels represent two promising candidate biomarkers. It will admittedly be challenging to establish such biomarkers in a manner that would satisfy clinicians and regulatory authorities, and would require extensive preclinical and clinical validation. A precedent exists in this regard, however, with the statins. This class of drugs gained approval, by the US Food and Drug Administration on the basis of measurements of a biomarker: plasma low-density lipoprotein cholesterol. Within a decade of approval, statins were proven to reduce the risk for cardiovascular disease[155].

Drugs that target the machinery of ageing offer the promise that metabolic, degenerative and other diseases could be treated or prevented. Although it could take decades to complete clinical trials assessing whether such therapies extend lifespan, the study of the mechanisms of ageing presently provides new avenues and perspectives for target identification and drug discovery.

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Competing interests statement

The authors declare competing financial interests: see Web version for details.

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