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The role of Klotho in energy metabolism

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Abstract

A disproportionate expansion of white adipose tissue and abnormal recruitment of adipogenic precursor cells can not only lead to obesity but also impair glucose metabolism, which are both common causes of insulin resistance and diabetes mellitus. The development of novel and effective therapeutic strategies to slow the progression of obesity, diabetes mellitus and their associated complications will require improved understanding of adipogenesis and glucose metabolism. Klotho might have a role in adipocyte maturation and systemic glucose metabolism. Klotho increases adipocyte differentiation *in vitro*, and mice that lack Klotho activity are lean owing to reduced white adipose tissue accumulation; moreover, mice that lack the *Kl* gene (which encodes Klotho) are resistant to obesity induced by a high-fat diet. Knockout of *Kl* in leptin-deficient $Lep^{ob/ob}$ mice reduces obesity and increases insulin sensitivity, which lowers blood glucose levels. Energy metabolism might also be influenced by Klotho. However, further studies are needed to explore the possibility that Klotho could be a novel therapeutic target to reduce obesity and related complications, and to determine whether and how Klotho might influence the regulation and function of a related protein, β-Klotho, which is also involved in energy metabolism.

Introduction

Excess adiposity is associated with the development of insulin resistance and subsequent metabolic disorders, including type 2 diabetes mellitus (T2DM), dyslipidaemia, hypertension and coronary heart disease.[1–7] Importantly, obesity-associated disorders, including hypertension and T2DM, can partly be ameliorated by reducing body adipose content. However, inadequate amounts of adipose tissue, such as those seen in patients with lipodystrophy, can also induce the same range of metabolic complications that are observed in patients with obesity—including insulin resistance, T2DM, dyslipidaemia and hepatic steatosis.[8] An adequate but not excessive amount of adipose tissue in the body is, therefore, an essential prerequisite for maintaining physiological energy balance.

Despite the identification of different stages and events in adipogenesis and glucose metabolism,[9,10] the factors involved in the regulation of energy metabolism are not yet fully defined. A major obstacle for developing an effective therapy to reduce obesity and minimizing its associated complications is that crucial factors involved in energy metabolism have not yet been identified or adequately characterized in an *in vivo* system. Clarification of the mechanisms underlying energy metabolism is both biologically and clinically important because obesity has been identified as the second most common factor contributing to preventable death (after smoking tobacco).[9]

Competing interests The author declares no competing interests.

A major research focus in the field of energy metabolism over the past decade has been the functional characterization of the peroxisome proliferator-activated receptor (PPAR) family. The nuclear receptors PPAR-α, PPAR-δ and PPAR-γ act as lipid sensors and jointly regulate the expression of several genes that are essential for the regulation of energy metabolism, although only Klotho associations with PPAR-γ have been investigated in depth.[10] PPAR-γ has been identified as a key transcriptional regulator of nutrient and energy metabolism.[11,12] PPAR-γ also induces expression of Klotho,[13] a multifunctional protein[14–19] involved in a number of physio logical processes and implicated in a number of diseases (Box 1).[20–48]

This Review examines the role of Klotho in energy metabolism and considers its contribution to adipogenesis and obesity. The effects of Klotho on glucose control and phosphate metabolism are discussed, along with the potential role of this enzyme in diabetes mellitus. The role of the related protein β-Klotho in FGF-19 and FGF-21 signalling is briefly described in the context of glucose and adipocyte turnover. Other molecular and clinical aspects of β-Klotho function are not discussed further in this article, as they have been extensively reviewed elsewhere.[49–52]

Klotho proteins

The Klotho proteins (Klotho itself and the related enzyme β-Klotho) exert diverse effects on the physiological regulation of mineral ions (particularly calcium and phosphate) and energy metabolism by influencing the endocrine activities of fibroblast growth factors (FGFs), including FGF-19 and FGF-23.[17,19,51–54] The Klotho protein is approximately 130 kDa with a putative signal sequence at the N-terminus, a single transmembrane domain near the C-terminus and a short (10 amino acid) cytoplasmic domain.[55,56] The extracellular domain of Klotho consists of two internal repeats of approximately 550 amino acids each that share sequence similarity with β-glucosidase (Figure 1). This similarity gives Klotho a function analogous to that of β-glucosidase (albeit with weaker activity), although whether this activity occurs *in vivo* is not clear.[57] Both secreted and membrane-bound forms of Klotho can be detected in humans and other mammals. The secreted form is generated either by shedding the extracellular domain of the transmembrane protein or as a product of alternative splicing.[58] Klotho is expressed primarily in the kidney (distal convoluted tubules), parathyroid gland, brain (choroid plexus epithelium)[59] and adipose tissue.[60,61]

Distinguishing between the autocrine, paracrine, and endocrine actions of Klotho is often difficult because this enzyme seems to exert different functions in different cell types in a dependent or, possibly, independent manner. However, mouse genetic studies have led to the identification of a number of *in vivo* functions of Klotho, including roles in FGF signalling, calcium and phosphate ion transport, and energy metabolism (Box 2).[16,17,19,45,61–81]

Factors that induce the synthesis of the Klotho protein have not all been identified, although vitamin D seems to be an important regulator. In cells derived from the renal proximal, distal and collecting tubules, expression of Klotho (both membrane-bound and secreted splice variants) is regulated by 1,25-dihydroxyvitamin D.[82,83] Candidate vitamin D response elements (VDREs) were identified in the vicinity of mouse and human Klotho genes, and were found to be transcriptionally active sites, as determined by reporter gene assays.[83]

β-Klotho has 41% amino acid sequence similarity with Klotho and is primarily detected in the liver, pancreas, and adipose tissue.[84] β-Klotho regulates bile acid production, and mice lacking activity of this enzyme have a markedly increased synthesis and excretion of bile acids.[85] Binding of FGF-15 and FGF-19 to FGF receptor 4 (FGFR-4) is facilitated by β-Klotho, and suppresses the hepatic synthesis of CYP7A1, which in turn triggers a negative-

feedback loop to control bile acid metabolism by FGF-15. The overlapping phenotypes of *Fgfr4*$^{-/-}$,[86] viable *Fgf15*$^{-/-}$[87] and *Klb*$^{-/-}$[85] mice suggest that the products of these genes are involved in a common signalling cascade with molecular connections and interactions. In support of this hypothesis, FGF-19 can activate FGF signalling in FGFR-4-expressing hepatocytes to suppress the expression of CYP7A1, which is a rate-limiting enzyme in bile acid synthesis.[88]

β-Klotho is also involved in FGF-21 signalling.[89–91] For example, BaF3 cells (an immortalized murine bone-marrow-derived pro-B-cell line responsive to IL-3) are unresponsive to FGF-21 exposure, but co-expression of β-Klotho and FGFR1c in FGF-21-treated BaF3 cells leads to activation of downstream signalling events resulting in phosphorylation of FGFR substrate 2α (FRS2α), MAPK 2 and MAPK 3.[91] However, unlike *Fgf15*$^{-/-}$ mice, the phenotypes of *Fgf21*-knockout mice[92] differ from those of β-Klotho-knockout mice.[85] Importantly, the recombinant FGF-21 protein retains its biological activity in the *Klb*$^{-/-}$ mice, suggesting the existence of a β-Klotho-independent signalling pathway involving FGF-21.[93] However, whether Klotho and β-Klotho can influence each other's functions is not yet clear.

FGF-21 can regulate insulin-independent glucose transport in adipocytes.[49] In fact, when differentiated mouse 3T3-L1 adipocytes were treated with FGF-21, uptake of glucose was stimulated, in association with increased expression of GLUT1.[94] Furthermore, systemic administration of FGF-21 to diabetic animals led to a significant reduction in blood glucose and triglyceride levels, and *Fgf21*-transgenic mice, which overexpress FGF-21, were resistant to obesity induced by a high-fat diet.[87,95]

Adipogenesis regulation

Adipogenesis is the process by which preadipocytes differentiate into mature adipocytes (Figure 2). Over the past decade, a number of important factors have been identified that contribute to the initiation and progression of adipocyte maturation. Dysregulation of these factors results in altered production and distribution of adipose tissue. For example, heterozygous missense mutations in PPAR-γ (a master regulator of adipocyte differentiation) have been linked to the disease phenotype in familial partial lipodystrophy.[96,97]

Klotho can influence *in vitro* adipose cell maturation by promoting differentiation of preadipocyte cells into adipocyte cells.[61] Overexpression of Klotho in 3T3-L1 cells can upregulate adipogenic factors, including PPAR-γ, FABP4 and the CCAAT–enhancer binding proteins, which initiates the maturation process.[61] Klotho expression is induced by PPAR-γ,[13] and treatment with PPAR-γ agonists (such as thiazolidinediones) increases the synthesis of both Klotho mRNA and protein in renal epithelial cell lines; this induction of Klotho can be blocked by either PPAR-γ antagonists or by silencing of PPAR-γ using small interfering RNAs. Furthermore, a noncanonical PPAR-responsive element was detected within the 5'-flanking region of the human Klotho gene, *KL*. Importantly, this identified site is functionally active, as demonstrated by increased transcriptional activity of a reporter gene following PPAR-γ agonist (rosiglitazone) treatment.[13] This increased activity could be abolished by a PPAR-γ antagonist (GW9662).[13] C57BL6 mice treated with thiazolidinediones demonstrated increased renal expression of Klotho,[13] and adenovirus-mediated over-expression of PPAR-γ also upregulates Klotho expression in the kidney.[13] Similarly, Klotho expression was reduced in the kidneys of Otsuka Long-Evans Tokushima fatty (OLETF) rats, an animal model of metabolic syndrome. However, treatment with troglitazone (an insulin sensitizer and PPAR-γ agonist) induced renal Klotho expression in these rats.[98] These *in vitro* and *in vivo* results suggest that PPAR-γ increases Klotho

expression,[13,16,99] but that Klotho can also induce PPAR-γ synthesis during adipocyte maturation.[61]

Although these experimental observations are suggestive of a potential role of Klotho in adipocyte maturation, the regulatory steps of the adipocyte differentiation process that might be affected by Klotho remain to be identified, and whether Klotho can direct the commitment of multipotent mesenchymal stem cells (MSCs) to an adipogenic lineage still needs to be determined experimentally. Interestingly, stem cell transplantation can alter Klotho levels.[100–102] Mice lacking Klotho activity (both $Kl^{-/-}$ and $Kl^{kl/k}$) have less detectable adipose tissue content either in the abdominal cavity or under the skin than wild-type mice.[74,75,103]

Obesity regulation

Although Klotho can induce the expression of adipogenic factors, exactly how either the soluble or the membrane-bound Klotho protein activates their transcription is not clear. Genetically abolishing Klotho function in mice by knockout or knockdown of *Kl* resulted in the generation of lean mice with decreased white adipose tissue accumulation, including a reduced subcutaneous adipose tissue layer compared with that in wild-type mice.[75,103–105] Despite the obvious reduction in white adipose tissue mass in the *Kl*-knockdown ($Kl^{kl/kl}$) mice, which retain a very low level of Klotho, no such reduction could be detected in brown adipose tissue mass.[73] Moreover, $Kl^{kl/kl}$ mice had reduced glycogen content in the liver and decreased insulin content in the pancreas compared with their wild-type littermates.[73] Similarly, $Kl^{kl/kl}$ mice had decreased lipid content in brown adipose tissues (which can provide heat insulation), along with reduced *Ucp1* expression, which was associated with low body temperature.[73] In summary, the reduced adipose tissue phenotypes of *Kl*-mutant $Kl^{kl/kl}$ and $Kl^{-/-}$ mice[103,105,106] and the *in vitro* adipocyte-promoting ability of Klotho[61] imply that this protein might contribute to either adipocyte maturation or intracellular lipid accumulation. Intracellular lipid accumulation, particularly in skeletal muscle and liver, is usually associated with insulin resistance. Elimination of Klotho function from $Lep^{ob/ob}$ mice suppressed hepatic intracellular lipid accumulation in $Kl^{-/-}Lep^{ob/ob}$ double-knockout mice,[62] which raises the possibility that Klotho has a role in intracellular lipid accumulation. Whether Klotho could increase lipid storage in addition to promoting lipid synthesis remains to be elucidated.

Leptin-deficient $Lep^{ob/ob}$ mice are overweight because of increased white adipose tissue deposition; these mice start to gain body weight from 3 weeks of age and are almost three times heavier than their wild-type counterparts by 9 weeks of age. Nonetheless, eliminating Klotho activity in $Lep^{ob/ob}$ mice that are then crossed with $Kl^{-/-}$ mice significantly reduces body weight in the resulting $Kl^{-/-}Lep^{ob/ob}$ double-knockout mice. Similarly, retro peritoneal, mesenteric and epididymal adipose tissue accumulations are significantly lower in the $Kl^{-/-}Lep^{ob/ob}$ double-knockout mice than in the $Lep^{ob/ob}$ mice, suggesting a potential role for Klotho in the excessive adipose tissue accumulation characteristic of $Lep^{ob/ob}$ mice.[62] In addition, a high-fat (60%) diet fed to $Kl^{-/-}$ mice did not lead to any gain in body weight compared with a standard-fat (20%) diet.[62] The results of this dietary manipulation study suggest that $Kl^{-/-}$ mice are resistant to obesity induced by a high-fat diet.[31] With our current level of understanding, it is not possible to explain the human relevance of such experimental observations. Moreover, the reader should bear in mind that animal models of diet-induced obesity are similar to, but might not always exactly mimic, human obesity. The main difference between $Lep^{ob/ob}$ mice and human metabolic disorders is that the $Lep^{ob/ob}$ mouse is a monogenic experimental model of obesity, while human metabolic disorders usually have multiple contributing factors, including genetic background, environment and

dietary habits. Moreover, the homozygous *Lep* mutation causes early-onset morbid obesity with diabetes mellitus in mice, whereas in humans such onset could happen later in life.

Importantly, reducing obesity via the genetic elimination of Klotho in $Lep^{ob/ob}$ mice subsequently crossed with $Kl^{-/-}$ mice also reduced blood glucose levels in the resulting $Kl^{-/-}Lep^{ob/ob}$ mice, suggesting that Klotho might influence glucose metabolism as well as adipogenesis (Figure 3).[62]

Glucose metabolism

The *in vivo* manipulation of Klotho function affects glucose metabolism;[62,72,73] $Kl^{kl/kl}$ mice have reduced pancreatic insulin content but still develop hypoglycaemia owing to increased insulin sensitivity (Figure 3).[72] After insulin injections, blood glucose levels were markedly reduced in the $Kl^{kl/kl}$ mice relative to those in wild-type controls.[62,72,73]

Transgenic mice that overexpress *Kl* (EFmKL46 and EFmKL48) have biochemical features of insulin resistance.[64] Low expression of Klotho in the pancreas of wild-type mice has been reported,[103] and whether such expression influences insulin production has not been shown. Furthermore, the hepatic expression of phosphoenolpyruvate carboxykinase, an enzyme that increases gluconeogenesis, was raised in the $Kl^{kl/kl}$ mice.[73] Non-alcoholic fatty liver disease is considered to be a hepatic manifestation of the metabolic syndrome, and is closely associated with obesity, insulin resistance, T2DM and dyslipidaemia.[107–109] Consistent with human studies,[107–109] studies in animal models of obesity and T2DM showed features of hepatic fatty changes in those animals that are similar to those seen in $Lep^{ob/ob}$ mice (Figure 4).[62,110] By contrast, fatty changes were not seen in livers of $Kl^{-/-}Lep^{ob/ob}$ mice without Klotho activity. The double-mutant mice had lower fat accumulation in the liver compared with the $Lep^{ob/ob}$ mice, which was reflected by reduced blood glucose levels in the double-mutant mice.[62]

Despite the intermediate body-weight phenotype of $Kl^{-/-}Lep^{ob/ob}$ mice that lacked Klotho activity, hepatic steatosis was completely eliminated.[62] Hepatic steatosis is an integral feature of the metabolic syndrome that leads to hepatic insulin resistance; thus, elimination of hepatic steatosis from $Kl^{-/-}Lep^{ob/ob}$ mice through suppression of Klotho activity is an important finding that requires further molecular clarification and understanding. Whether the reversal of hepatic steatosis in $Kl^{-/-}Lep^{ob/ob}$ mice lacking Klotho activity is a local effect or a systemic consequence is currently unclear. Importantly, $Kl^{-/-}$ mice are resistant to the development of hepatic steatosis induced by a high-fat diet.[62]

Hepatic glucose synthesis also has a key role in maintaining systemic glucose metabolism. Analyses of glucose tolerance and insulin tolerance show that, compared with $Lep^{ob/ob}$ mice, double-mutant $Kl^{-/-}Lep^{ob/ob}$ mice have greater insulin sensitivity and better glucose tolerance, which clearly suggests that Klotho has a role in the hyperglycaemia observed in $Lep^{ob/ob}$ mice.[62] Whether reducing Klotho activity in humans would have beneficial effects similar to those observed in $Kl^{-/-}Lep^{ob/ob}$ mice is unclear and warrants further study. A report from the Centers for Disease Control showed that in 2008, approximately 68% of adults with diabetes mellitus were aged 40–64 years at diagnosis, whereas only 17% were diagnosed at age ≥65 years.[111] A study of 804 community-dwelling adults showed that serum levels of Klotho decline gradually in individuals aged ≥65 years.[112] Whether the reduction in Klotho levels observed in this age group is associated with the markedly reduced incidence of new cases of diabetes mellitus is an important area of future research with clinical and therapeutic importance.

The soluble Klotho protein does not have any effect on IGF-1 production and/or insulin signalling in HEK293, L6 and HepG2 cells,[60] and Klotho levels did not correlate with the

development of insulin resistance in animal models of metabolic diseases (Wistar rats fed a high-fat diet and obese Zucker rats).[60] The mechanisms under lying the potential effects of soluble or membrane-bound Klotho on glucose metabolism require additional experimental clarification. An association between *KL* genotypes, determined by single nucleotide polymorphism analysis, and fasting levels of either high glucose or low insulin was reported in hospitalized elderly (age >65 years, mean age 79.04 ± 7.14) female patients.[113]

Phosphate metabolism

Another area that has received very little attention is the association between electrolyte homeostasis (especially of phosphate) and energy metabolism. FGF-23 is a crucial regulator of systemic phosphate metabolism, and requires Klotho to exert its effects.[15,19,69,114–120] Serum phosphate levels are reduced through increased urinary excretion, which is induced by FGF-23. The presence of Klotho increases the binding affinity of FGF-23 for its receptors,[70,71,121,122] which leads to phosphorylation of FGFR substrate 2, MAPK1 and MAPK2, and activation of downstream signalling events.[70,71,121–123] The occurrence of phosphate toxicity in $Kl^{-/-}$ or $Kl^{kl/kl}$ mice demonstrates the importance of Klotho in phosphate metabolism *in vivo*.[74,103,124,125] Importantly, mice that lack *Phex* (which encodes a phosphate-regulating protein), which is homolo gous to the endopeptidase-encoding genes located on the X chromosome, have increased urinary phosphate wasting and severe hypophosphataemia owing to the increased activity of FGF-23.[74,126,127] However, the genetic inactivation of *Kl* in these *Phex*-mutated mice changed their phenotype to hyperphosphataemia, even though the $Kl^{-/-}$*Phex* double-mutant mice have extremely high serum levels of FGF-23, clearly showing an essential requirement of Klotho for *in vivo* FGF-23 function.[17,74] In addition, a point mutation in human *KL* resulting in a His193Arg amino acid change in a 13-year-old patient with tumoural calcinosis was associated with significantly raised serum levels of phosphate, despite the presence of increased serum levels of FGF-23.[45] Our understanding of the critical role of the kidney in the regulation of systemic phosphate metabolism has been improved by the identification of this relationship between FGF-23 and Klotho.[19]

The kidney also has an important role in maintaining systemic glucose metabolism by influencing gluconeogenesis, partly through controlling glucose filtration and reabsorption.[128–130] Further studies are needed to determine whether altered phosphate metabolism can directly affect systemic glucose metabolism and vice-versa. Existing human studies provide some support for this association.[131–134] Low serum phosphate levels are associated with reduced insulin activity.[135–137] In a study of 298 children and adolescents aged 6–12 years old, in which 190 individuals with obesity and 108 controls without obesity were compared, reduced phosphate serum levels were significantly associated with the development of insulin resistance in children with obesity.[138] Moreover, studies in humans show that the glucose infusion rate needed to maintain hyperglycaemia (blood glucose levels 7.0 mmol/l) was 36% lower in individuals with chronic hypophosphataemia but without diabetes mellitus than in controls. Similarly, when exogenous insulin was infused at a constant rate to maintain serum insulin levels at approximately 100 μU/ml above basal levels, the glucose infusion rate required to maintain fasting glucose levels was 43% lower in hypophosphataemic participants than in control individuals.[139] These results, therefore, indicate an association between an altered phosphate balance and impaired glucose metabolism in both hyperglycaemic and euglycaemic states.[139] Serum phosphate levels were positively correlated with insulin sensitivity (but not with insulin secretion) in a separate study conducted in 881 individuals without diabetes mellitus.[140] This correlation was independent of age, sex, proportion of body adipose content, serum calcium and serum creatinine levels.[140] Whether low insulin sensitivity and impaired glucose tolerance are the cause or the consequence of hypophosphataemia requires further investigation at the

molecular level. Understanding the effects of increased serum phosphate levels on the hypoglycaemic phenotype in *Kl*-knockout or *Kl*-knockdown mice could help reveal the effects of phosphate metabolism on glucose metabolism.

Conclusions

The increasing occurrence of obesity and its related complications, including T2DM and cardiovascular anomalies, is alarming and becoming a major public health problem. Over the past decade, numerous *in vitro* and *in vivo* experiments have helped to elucidate the various steps of adipose tissue remodelling that are relevant and important to human pathophysiology. Although Klotho expression is restricted to a few organs and circulating levels of this enzyme are quite low, Klotho affects numerous important biological functions, ranging from phosphate metabolism to energy metabolism (Box 2).[16,17,19,45,61–81] Dysregulation of these functions has many physiological effects and can lead to disease (Box 1).[20–48]

The identification of Klotho as a possible adipocyte maturation-promoting factor and its interactions with other known adipogenic factors, such as PPAR-γ and the CCAAT–enhancer binding proteins, suggest a potentially important role for Klotho in adipocyte turnover.[13,61] However, additional studies are needed to determine the precise role of Klotho in lipid synthesis. The lean phenotype of the *Kl*-knockout and *Kl*-knockdown mice is due to reduced white adipose tissue accumulation, and their resistance to gaining body weight while on a high-fat diet implies a potential *in vivo* role for Klotho in adipocyte differentiation and maturation. Consistent with this observation, eliminating Klotho activity from obese $Lep^{ob/ob}$ mice results in reduced weight gain in double-mutant ($Kl^{-/-}Lep^{ob/ob}$) mice. The *in vivo* findings that Klotho influences adipose tissue generation and glucose metabolism are likely to serve as the basis for further studies to delineate the biological and therapeutic importance of this enzyme in the control of energy homeostasis.

At present, however, whether Klotho affects the functionality of β-Klotho (which influences energy metabolism by mediating the functions of FGF-19 and FGF-21) is not clearly understood.[14,51–53,93] Dissociating the *in vivo* functions of Klotho and β-Klotho will be a challenging task, but one that could have important clinical benefits, such as enabling the development of therapies to combat obesity and its related complications.

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Key points

- Klotho exists in membrane-bound and secreted forms; the secreted form is generated both by alternative splicing and by shedding of the extracellular domain of the transmembrane form
- Klotho increases organ-specific FGF-23 function in the systemic regulation of phosphate metabolism in the kidney
- Klotho might also have a role in energy metabolism
- Reducing or eliminating Klotho activity in mice results in reduced white adipose tissue accumulation
- *Kl*-mutant mice (both $Kl^{kl/kl}$ and $Kl^{-/-}$) are hypoglycaemic (owing to increased insulin sensitivity) and resistant to obesity induced by a high-fat diet
- Reducing or eliminating Klotho activity from $Kl^{-/-}Lep^{ob/ob}$ mice results in mice with decreased obesity and increased insulin sensitivity

Box 1 | Klotho in human physiology and disease

Physiological processes linked to Klotho

- Aging[20,21]
- Control of blood pressure[22–25]
- Regulation of bone mineral density[23,26–30]
- Glucose metabolism[23,31]
- Regulation of HDL, LDL, cholesterol and uric acid levels[21,32]

Diseases linked to Klotho

- Atherosclerosis[24]
- Breast cancer[33]
- Coronary artery disease[34–36]
- Ischaemic stroke[37,38]
- Kidney stones[39]
- Mortality in patients on hemodialysis[40,41]
- Osteoarthritis[42]
- Rickets[43]
- Sickle-cell anaemia[44]
- Tumoural calcinosis[45]

Diseases and physiological factors lacking any confirmed association with Klotho

- Valvular, vascular[46] and coronary[36] calcification
- Leukocyte telomere length (a marker of cell senescence)[47]
- Type 2 diabetes mellitus[48]

Box 2 | Functions of Klotho[16,17,19,45,61–81]

- Adipogenesis[61,62]
- Angiogenesis[63]
- Antiageing effects[64]
- Antioxidant effects[65]
- Calcium metabolism[66–68]
- FGF signaling[17,69–71]*
- Glucose metabolism[72,73]
- Insulin signaling[64]
- Phosphate metabolism[74,75]
- Potassium metabolism[76]

The above functions were documented in experimental studies.

*The role of Klotho in FGF-23 signalling has also been validated in human studies.[45,77–79]

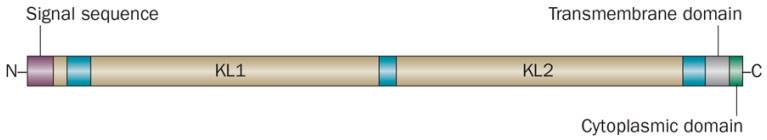

Figure 1.

Schematic diagram of the Klotho protein structure. Klotho is 1,014 amino acids in length and possesses a putative signal sequence at its N-terminus and a transmembrane domain with a short cytoplasmic domain at the C-terminus. The extracellular domain of the Klotho protein consists of two internal repeats (KL1 and KL2) that share sequence homology with β-glucosidase.

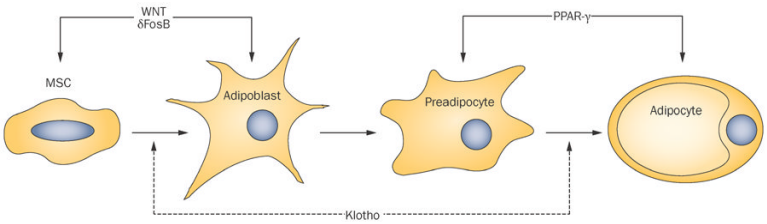

Figure 2.

Potential influence of Klotho on adipocyte development. Adipocytes develop from MSCs through two stages of maturation. WNT and δFosB are involved in the differentiation of MSCs to adipoblasts, whereas PPAR-γ drives differentiation of preadipocytes to adipocytes. Klotho could potentially influence both the adipogenic lineage commitment of MSCs and adipocytic maturation, but these roles remain to be confirmed experimentally. Abbreviation: MSC, mesenchymal stem cell.

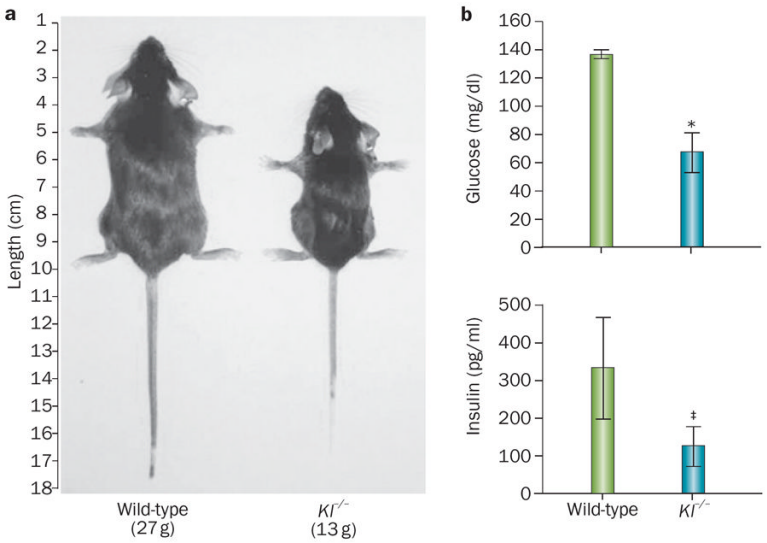

Figure 3.

Physiological effects of Klotho inactivation. **a** | *Kl*-knockout mice ($Kl^{-/-}$) are smaller in size and weight than wild-type littermates, partly as a result of a lack of adipose tissue accumulation. **b** | *Kl*-knockout mice ($Kl^{-/-}$) are hypoglycaemic $*P<0.001$ despite having lower insulin concentrations. $^{‡}P<0.05$ than wild-type littermates, indicating that they have increased insulin sensitivity (100 mg/dl glucose = 5.55 mmol/l; 100 pg/ml insulin = 0.02 pmol/l).

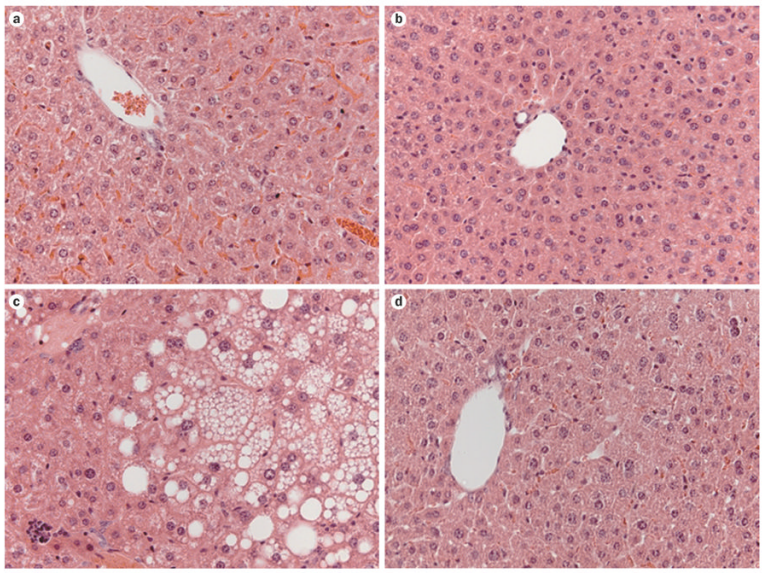

Figure 4.
Effects of Klotho on liver structure. Liver sections stained with haematoxylin and eosin (magnification ×40). **a** | Wild-type mice. **b** | $Kl^{-/-}$ mice. **c** | Leptin-deficient $Lep^{ob/ob}$ obese mice. **d** | $Kl^{-/-}Lep^{ob/ob}$ double-knockout mice. Steatosis was not seen in liver sections from either of the groups of mice that were lacking *Kl*.