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Advances in endocrinology of aging research, 2005-2006

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Abstract

The purpose of this brief review is to highlight some of the more important advances in endocrinology of aging research over the past year. Four advances were chosen and briefly described. First, exploration of the early steps in the generation of the internal steroidal hormonal signal involved in lifespan extension via the insulin/IGF-like signaling pathway in the nematode by two research groups revealed that the product of cholestanoic acid derivatives metabolized by a cytochrome P-450-like protein activates a protein with homology to the mammalian nuclear receptor superfamily, a process strikingly similar to the steroid hormone signaling pathway documented in mammalian systems. Second is the discovery that sirtuins, proteins that regulate lifespan in model organisms, enhance pancreatic insulin secretion in mice following a glucose challenge, suggesting the potential to regulate mammalian lifespan through regulation of the insulin signaling pathway. Third, the newly discovered hormone klotho, which also plays a role in regulating lifespan, in this case in mice, is reported to not only negatively affect insulin sensitivity but, perhaps more importantly, significantly affects calcium and phosphate metabolism as a required co-factor of Fgf-23 signaling. Finally the gonadotropin FSH is shown to directly affect bone density in mice separate from any direct effect of estrogen, suggesting that reproductive hormones other than estrogen can directly impact menopause-associated pathophysiology in non-reproductive tissues.

Keywords

endocrinology of aging; steroidal signaling; cholestanoic acid; sirtuins; insulin secretion; insulin signaling; klotho; gonadotropins; FSH; bone cells; bone density; model organisms

Research on endocrinology of aging is quite broad, extending from mechanisms underlying the age-related declines in sex steroid and peptide reproductive and growth hormones, through male and female reproductive aging, and into pathophysiologic effects of age-altered hormone levels on somatic and neuronal tissues and the effects of treatment with exogenous hormones to ameliorate the health declines associated with aging. Several fascinating new or progressing areas of endocrinology of aging research came to light over the past year from about mid-2005 through about mid-2006. This is one perspective, not meant to be a complete survey of all of the research conducted in the area of endocrinology of aging over the past year. Certainly many readers will have their own selection of the highlights over this past year that will differ in whole or in part from those summarized here.

Specific areas covered in this brief review are new and exciting findings regarding 1) identification of steroidal ligands involved in hormonal regulation of the lifespan-determining pathway in the nematode model, 2) new findings related to the role of factors involved in

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extending lifespan in model organisms in mammalian endocrinology, and 3) increasing evidence that gonadotrophic hormones are directly involved in pathophysiology associated with menopause.

1. Identification of steroidal lipids that active nuclear hormone receptors to modify lifespan in nematodes

Single gene mutations in model organisms have revealed signaling pathways responsible for significant extensions of median and maximum lifespan. One of the most widely studied genetic pathways, and of greatest interest to endocrinologists, has been the discovery that mutations in an insulin/IGF-like signaling pathway extend lifespan in the model organisms yeast, *C. elegans* (nematode) and *Drosophila* (fruit fly), and more recent studies suggest that this finding may extend to mice and possibly to humans (Barbieri et al. 2003).

Key steps in that signaling pathway in the nematode are the apparent activation of DAF-12, a protein with homology to the nuclear receptor superfamily, by a steroidal product of DAF-9, a protein homologous to the cytochrome P450 superfamily. Substantial research effort has been expended over the past year to identify the steroid “hormone” produced as a metabolic product of DAF-9, which in turn activates DAF-12 to modulate the nematode lifespan. This metabolic/signaling process is analogous to the metabolism of cholesterol to steroid hormones in mammalian organisms, which in turn activate members of the nuclear receptor superfamily to affect the biologic response.

Recently 3-keto-4-cholestenoic acid and 3-keto-7,(5 α)-cholestenoic acid were identified as endogenous hormonal ligands for DAF-12 (Motola et al. 2006). The presence of these steroidal ligands requires the activity of daf-9, and the immediate substrate for the latter compound is the C-26 carboxylic acid metabolite of lathosterone. The authors don't rule out additional DAF-12 ligands – the ligands they identified were found in the most active fractions, and additional activating ligands may also be found within those fractions. A still more recent publication identified an oxygenase in the nematode (DAF-36) with a primary structure similar to that seen in oxygenases in plants and bacteria as involved, perhaps at an early step, in the production of the DAF-12 activation ligand (Rottiers et al. 2006).

Similarly, other investigators using a candidate ligand approach reported the identification of the C27 bile acid 25S-cholestenoic acid, a naturally occurring ligand for the LXR nuclear receptor, as a promoter of reproductive growth in dauer-constitutive mutants and as an activator of DAF-12 in an *in vitro* reporter system (Held et al. 2006). In a search for activating ligands from nematode extracts, GC-MS separation revealed monohydroxy bile acids with common features with cholestenoic acid; the precise structures are currently being identified.

2. Lifespan altering factors that interact with the insulin/IGF signaling pathway

a) sirtuins modulate the insulin-glucose metabolic axis in mammals

The Sir2 gene, a member of an evolutionarily conserved gene family with protein deacetylase activity termed the “sirtuins”, is involved in lifespan extension of model organisms through interactions with the insulin/IGF-like pathway, and with biologic mechanisms underlying caloric restriction, the dietary manipulation of organisms ranging from yeast to mammals that extends lifespan (Bordone & Guarente 2005; Sinclair 2005; Tang 2006). Manipulations that increase Sir2 levels or activity in yeast, nematodes and fruit flies increase lifespan of these organisms, while decreasing Sir2 levels or activity decreases lifespan. A recent study examined whether the Sir2 ortholog in mice, Sirt1, affects insulin secretion from mouse pancreatic β cells

(Moynihan et al. 2005). This question is interesting because it addresses a potential mechanism by which Sirt1 might affect the insulin signaling pathway, and thus potentially affect mammalian health- and life-span. The authors produced a transgenic mouse model in which Sirt1 overexpression is targeted specifically to pancreatic β cells. In this model, fasting and fed glucose and insulin levels are identical to wild type controls, but in response to an intraperitoneal glucose challenge the transgenic mice show improved glucose tolerance and enhanced insulin secretion. This enhanced insulin response to the glucose challenge is maintained through 8 months of age. Decreased expression of uncoupling protein 2 (Ucp2) in islets isolated from these transgenic mice is thought to be at least part of the mechanism by which Sirt1 regulates genes involved in insulin secretion. These authors propose a model (Fig. 1) in which Sirt1 overexpression in the β cell downregulates Ucp2, which in turn – due to the inhibiting effect of Ucp2 on ATP production – upregulates ATP in response to high glucose. The increased ATP acts to close ATP-dependent K^+ channels causing membrane depolarization, and opening voltage-gated Ca^{+} channels to ultimately trigger calcium release. This model proposes that in addition to increasing insulin secretion by down-regulating Ucp2, Sirt1 also acts downstream of β cell depolarization. This presumption arises from the observation that KCl, which directly depolarizes β cells, produced a greater insulin secretory response in the Sirt1 overexpressing transgenic mice and in islets isolated from these mice.

Another recent publication supports the role of Ucp2 in the Sirt1-regulation of insulin secretion from cultured pancreatic β cells: Sirt1 represses Ucp2 by binding directly to the Ucp2 promoter; repression of Sirt1 by siRNA in β cell cultures elevates Ucp2 levels and reduces insulin secretion; siRNA knockdown of Ucp2 expression restores insulin-secreting ability in the Sirt1 siRNA-treated cells (Bordone et al. 2006). These studies support an important role of Sirt1, the mammalian homologue of the lifespan extending gene in model organisms, in key mammalian metabolic processes, namely regulating the insulin-glucose response.

b) regulation of lifespan and processes associated with aging by the hormone Klotho

A research group from Japan serendipitously created a progeroid mouse from a loss-of-function mutation in the klotho gene (Kuro-o et al. 1997). The $Kl^{-/-}$ mutant mice develop normally to 3-4 weeks of age, but then express the progeroid phenotype and growth retardation and die prematurely at about 8-9 weeks of age. Characterization of the klotho gene revealed that it codes a single pass transmembrane protein with structural homology to glycosidases but without enzyme activity, likely due to the lack of essential glutamate residues. This functionality differs substantially from most of the progeroid-associated genes in that most progeroid defects are involved with mitochondrial or nuclear DNA or the nuclear envelope (Warner 2005). Klotho is expressed primarily in tissues that function in the regulation of calcium homeostasis, such as the distal convoluted tubules of the kidney and in lesser amounts in the choroid plexus of the brain. Hormonal properties derive from protease cleavage of the extracellular domain (Fig. 2F), comprising about 94% of the entire klotho protein, resulting in the ability to measure levels of circulating klotho by RIA.

Several interesting papers related to the hormone klotho were published during the past year. The first reports that transgenic mice in which klotho is overexpressed live significantly longer than wild type controls (Kurosaki et al. 2005). This may be the first documented example of extension of lifespan by excess hormone expression. The lifespan extension of males (20–30%, depending on the line used) was greater than that of females (19% for both lines used). This lifespan extension was not due to dwarfism or caloric restriction because the transgenic mice grew at the same rate as controls and ate the same amount of food. Interestingly, two independent transgenic lines of klotho overexpressing mice showed normoglycemia but higher blood insulin levels than wild type, suggesting reduced insulin sensitivity in males but not in females (Fig. 2A, B). This insulin resistance in the males was confirmed by a reduced glucose

infusion rate in a hyperinsulinemic euglycemia clamp measurement (Fig. 2C). In addition, insulin and IGF1 tolerance tests provide additional evidence for insulin and IGF1 resistance in male transgenics but only IGF1 resistance in female transgenic mice (Fig. 2D, E). Furthermore, injecting the soluble extracellular domain of recombinant klotho protein into wild type male and female mice along with insulin attenuated the hypoglycemic response observed with insulin alone (Fig. 2G). Injection of soluble klotho alone increased blood glucose in both male and female wild type mice (Fig 2H). The authors conclude that klotho induces resistance to insulin and IGF1. This is consistent with the down-regulated insulin/IGF-like signaling pathway that results in extended lifespan in model organisms. In exploring the mechanism for reduced insulin signaling by klotho, these investigators report that incubating hepatic cell cultures with recombinant klotho peptide inhibits the activation of insulin and IGF receptors, and suppresses ligand-induced autophosphorylation of insulin receptor and IGF-receptor in a dose-dependent manner. To further explore the role of the insulin signaling pathway in reduced lifespan associated with the loss of klotho, they report that crossing an insulin receptor substrate (IRS) heterozygous strain with the $Kl^{-/-}$ mutant resulted in improved survival and amelioration of many of the age-related pathologies; the IRS heterozygous mutation alone did not affect survival or age-related pathologies. Thus, this set of studies associate the lifespan-extending effect and diminution of the progeroid phenotype of klotho overexpression to increased insulin/IGF1 resistance.

In addition, klotho is implicated in increasing resistance to oxidative stress. The expression of manganese superoxide dismutase (Mn SOD), an anti-oxidant enzyme that increases oxidative stress resistance, is negatively regulated by insulin/IGF1 signaling. However the klotho protein activates FoxO forkhead transcription factors that in turn activate MnSOD expression, resulting in protection from oxidative stress, thus contributing to the anti-aging properties of klotho (Yamamoto et al. 2005).

More recent papers show a different side of klotho. Mice deficient in fibroblast growth factor 23 (FGF23) have a similar progeroid phenotype as klotho deficient mice, including hyperphosphatemia, hypercalcemia, and increased levels of vitamin D, suggesting a common signaling pathway. FGF23, produced primarily in bone, is a humeral factor that suppresses phosphate reabsorption in renal proximal tubules. The observation that klotho protein binds to FGF receptors (FGFR), thereby permitting higher affinity binding of FGF23 to the klotho-FGFR complex, strongly suggests that klotho is an essential cofactor for FGF23 signaling (Kuro-o 2006; Kurosu et al. 2006) that regulates vitamin D and serum calcium and phosphorus levels.

The question arises - which of these actions of klotho is most important in regulating aging: modulation of the insulin/IGF signaling pathway or regulation of calcium and phosphate homeostasis? Interestingly, the latter pathway appears to be more important. Normalization of vitamin D activity in either $Kl^{-/-}$ or $Fgf23^{-/-}$ either by feeding a vitamin D deficient diet or knockout of 1α -hydroxylase substantially rescues the progeroid phenotype (Nabeshima 2006). Similarly $Kl^{-/-}$ mice fed a vitamin D-deficient diet lived substantially longer (> 6 months) than the IRS heterozygous rescue of $Kl^{-/-}$ mouse lifespan. Furthermore, the enhanced insulin sensitivity in $Kl^{-/-}$ mice normalized on a vitamin D-deficient diet suggests the insulin response is secondary to the altered mineral metabolism.

3. New information on the direct roles of gonadotrophic hormones in pathophysiology associated with menopause

Probably because the menopause-associated decline in serum estradiol levels is substantial, estradiol is associated by many as the prime factor involved in the pathophysiologic processes that occur during and after the menopausal transition. This view permeates the clinical

community and is the basis for the intense interest in estrogen therapy following menopause. However new research is coming to light implicating the rise in gonadotropin levels, generally thought to be associated with falling estrogen levels through feedback regulation at the hypothalamic-pituitary level, as important in their own right for menopause-associated pathophysiologic processes in non-reproductive tissues. In addition, gonadotropins had been thought to target only reproductive tissues making the observation of these effects in non-reproductive tissue particularly interesting.

For example, several years ago it was reported that female Alzheimer's patients not on estrogen therapy had significantly higher levels of serum LH and FSH relative to age-matched non-estrogen treated normal controls or in non-estrogen treated women with frontotemporal dementia (Short et al. 2001). More recent studies showed that there is a significant increase in LH in the cytoplasm of pyramidal neurons and neurofibrillary tangles of AD brain compared with age-matched control brain (Bowen et al. 2002). In mice, the GnRH agonist leuprolide, which reduces gonadotropin and sex steroid levels, significantly reduced A β 1–42 and 1–40. Furthermore LH treatment of neuroblastoma cells in culture did not affect A β precursor protein (A β pp) expression but did alter A β pp processing toward the amyloidogenic pathway (Bowen et al. 2004). A very recent paper (Casadesus et al. 2006) showed that, in contrast to the adverse cognitive test results in estrogen plus progestin-treated postmenopausal women (Espeland et al. 2004), suppression of LH by leuprolide significantly attenuated cognitive decline and decreased amyloid- β deposition in an aged transgenic mouse model of Alzheimer's disease compared with placebo-treated animals. Thus evidence is accumulating that the menopause-associated rise in gonadotropins may play a direct role in the pathophysiology and cognitive decline associated with the menopause.

The recent paper showing a direct effect of FSH on bone mass in mice is another piece of evidence linking the menopause-associated rise in gonadotropins to pathophysiology. Epidemiologic data from the Study of Women's Health Across the Nation (SWAN study) had implicated the menopause-associated rise in serum FSH as more important than declining estrogen levels in determining bone mineral density (BMD) (Sowers et al. 2006; Sowers et al. 2003) or prevalence and frequency of vasomotor symptoms (Randolph, Jr. et al. 2005) in perimenopausal women.

To better understand the biologic underpinning of how FSH acts on bone, the authors first showed that transgenic mice lacking FSH β subunit or FSH receptor (FSHR^{-/-}), although severely hypogonadal, showed no evidence of bone loss (Sun et al. 2006). Further evidence for the dependence of bone density on FSH levels was obtained using heterozygotic FSH β transgenic mice (FSH β ^{+/-}). These mice are eugonadal, with a 50% reduction in serum FSH levels, but showed strikingly enhanced BMD at several bone sites. Thus BMD appears to be more responsive to FSH activity rather than serum estrogen levels. To explore the molecular mechanisms underlying these effects, the authors report that both osteoclast precursors and mature osteoclasts have FSH receptors. FSH signaling through its receptor on the osteoclast surface involves an inhibitory G protein linked to decreased cAMP levels, and activated MEK/Erk, NF- κ B and Akt pathways, thereby enhancing osteoclast differentiation and bone resorption.

Thus menopause-associated pathophysiology and loss of cognition that previously had been attributed primarily to loss of estrogen may actually be due, at least in part, to changes in gonadotropin levels. These findings open new avenues of approach to understanding and treating menopause-associated health problems.

SUMMARY

The field of endocrinology is gaining new respect within the research on aging investigator community. The seminal findings several years ago that a lifespan modulating pathway in model organisms was homologous to the insulin/IGF signaling pathway now appears to extend to mammalian organisms. A key feature of that lifespan modulating pathway, at least in nematodes, is homologous to the mammalian steroid hormone signaling pathway. Over this past year we gained greater detail of the specific steroidal activators involved. Also lifespan extension by sirtuins in model organisms, also implicated in the insulin/IGF signaling pathway and in caloric restriction, is now shown to modulate insulin secretion in mouse pancreatic β cells. Klotho overexpression, which extends lifespan in mice, decreases insulin/IGF sensitivity and strongly affects calcium and phosphate metabolism. Thus in all these examples endocrine pathways appear to be intimately involved in the modulation of lifespan in both mammalian and model organisms.

Finally, new research findings strongly suggest that attention should be paid to hormones other than estrogen in understanding and treating health and other problems associated with the menopause. Gonadotropins, which previously had been associated with the reproductive system, now appear to be directly involved in the functioning of non-reproductive tissues, namely brain regions associated with cognition and Alzheimer's disease and vasomotor symptoms, and bone density. Additional studies in these areas are eagerly anticipated.

References

- Barbieri M, Bonafe M, Franceschi C, Paolisso G. Insulin/IGF-I-signaling pathway: an evolutionarily conserved mechanism of longevity from yeast to humans. *Am J Physiol Endocrinol Metab* 2003;285(5):E1064–E1071. [PubMed: 14534077]
- Bordone L, Guarente L. Calorie restriction, SIRT1 and metabolism: understanding longevity. *Nat Rev Mol Cell Biol* 2005;6(4):298–305. [PubMed: 15768047]
- Bordone L, Motta MC, Picard F, Robinson A, Jhala US, Apfeld J, McDonagh T, Lemieux M, McBurney M, Szilvasi A, Easlon EJ, Lin SJ, Guarente L. Sirt1 regulates insulin secretion by repressing UCP2 in pancreatic beta cells. *PLoS Biol* 2006;4(2):e31. [PubMed: 16366736]
- Bowen RL, Smith MA, Harris PL, Kubat Z, Martins RN, Castellani RJ, Perry G, Atwood CS. Elevated luteinizing hormone expression colocalizes with neurons vulnerable to Alzheimer's disease pathology. *J Neurosci Res* 2002;70(3):514–518. [PubMed: 12391612]
- Bowen RL, Verdile G, Liu T, Parlow AF, Perry G, Smith MA, Martins RN, Atwood CS. Luteinizing hormone, a reproductive regulator that modulates the processing of amyloid-beta precursor protein and amyloid-beta deposition. *J Biol Chem* 2004;279(19):20539–20545. [PubMed: 14871891]
- Casadesus G, Webber KM, Atwood CS, Pappolla MA, Perry G, Bowen RL, Smith MA. Luteinizing hormone modulates cognition and amyloid-beta deposition in Alzheimer APP transgenic mice. *Biochim Biophys Acta* 2006;1762(4):447–452. [PubMed: 16503402]
- Espeland MA, Rapp SR, Shumaker SA, Brunner R, Manson JE, Sherwin BB, Hsia J, Margolis KL, Hogan PE, Wallace R, Dailey M, Freeman R, Hays J. Conjugated equine estrogens and global cognitive function in postmenopausal women: Women's Health Initiative Memory Study. *JAMA* 2004;291(24):2959–2968. [PubMed: 15213207]
- Held JM, White MP, Fisher AL, Gibson BW, Lithgow GJ, Gill MS. DAF-12-dependent rescue of dauer formation in *Caenorhabditis elegans* by (25S)-cholestenoic acid. *Aging Cell* 2006;5:283–291. [PubMed: 16913876]
- Kuro-o M. Klotho as a regulator of fibroblast growth factor signaling and phosphate/calcium metabolism. *Curr Opin Nephrol Hypertens* 2006;15(4):437–441. [PubMed: 16775459]
- Kuro-o M, Matsumura Y, Aizawa H, Kawaguchi H, Suga T, Utsugi T, Ohyama Y, Kurabayashi M, Kaname T, Kume E, Iwasaki H, Iida A, Shiraki-Iida T, Nishikawa S, Nagai R, Nabeshima YI. Mutation of the mouse klotho gene leads to a syndrome resembling ageing. *Nature* 1997;390(6655):45–51. [PubMed: 9363890]

- Kurosu H, Ogawa Y, Miyoshi M, Yamamoto M, Nandi A, Rosenblatt KP, Baum MG, Schiavi S, Hu MC, Moe OW, Kuro-o M. Regulation of fibroblast growth factor-23 signaling by klotho. *J Biol Chem* 2006;281(10):6120–6123. [PubMed: 16436388]
- Kurosu H, Yamamoto M, Clark JD, Pastor JV, Nandi A, Gurnani P, McGuinness OP, Chikuda H, Yamaguchi M, Kawaguchi H, Shimomura I, Takayama Y, Herz J, Kahn CR, Rosenblatt KP, Kuro-o M. Suppression of aging in mice by the hormone Klotho. *Science* 2005;309(5742):1829–1833. [PubMed: 16123266]
- Motola DL, Cummins CL, Rottiers V, Sharma KK, Li T, Li Y, Suino-Powell K, Xu HE, Auchus RJ, Antebi A, Mangelsdorf DJ. Identification of ligands for DAF-12 that govern dauer formation and reproduction in *C. elegans*. *Cell* 2006;124(6):1209–1223. [PubMed: 16529801]
- Moynihan KA, Grimm AA, Plueger MM, Bernal-Mizrachi E, Ford E, Cras-Meneur C, Permutt MA, Imai S. Increased dosage of mammalian Sir2 in pancreatic beta cells enhances glucose-stimulated insulin secretion in mice. *Cell Metab* 2005;2(2):105–117. [PubMed: 16098828]
- Nabeshima Y. Toward a better understanding of Klotho. *Sci Aging Knowledge Environ* 2006;2006(8):e11.
- Randolph JF Jr, Sowers M, Bondarenko I, Gold EB, Greendale GA, Bromberger JT, Brockwell SE, Matthews KA. The relationship of longitudinal change in reproductive hormones and vasomotor symptoms during the menopausal transition. *J Clin Endocrinol Metab* 2005;90(11):6106–6112. [PubMed: 16144949]
- Rottiers V, Motola DL, Gerisch B, Cummins CL, Nishiwaki K, Mangelsdorf DJ, Antebi A. Hormonal control of *C. elegans* dauer formation and life span by a Rieske-like oxygenase. *Dev Cell* 2006;10(4):473–482. [PubMed: 16563875]
- Short RA, Bowen RL, O'Brien PC, Graff-Radford NR. Elevated gonadotropin levels in patients with Alzheimer disease. *Mayo Clin Proc* 2001;76(9):906–909. [PubMed: 11560301]
- Sinclair DA. Toward a unified theory of caloric restriction and longevity regulation. *Mech Ageing Dev* 2005;126(9):987–1002. [PubMed: 15893363]
- Sowers MR, Finkelstein JS, Ettinger B, Bondarenko I, Neer RM, Cauley JA, Sherman S, Greendale GA. The association of endogenous hormone concentrations and bone mineral density measures in pre- and perimenopausal women of four ethnic groups: SWAN. *Osteoporos Int* 2003;14(1):44–52. [PubMed: 12577184]
- Sowers MR, Jannausch M, McConnell D, Little R, Greendale GA, Finkelstein JS, Neer RM, Johnston J, Ettinger B. Hormone predictors of bone mineral density changes during the menopausal transition. *J Clin Endocrinol Metab* 2006;91(4):1261–1267. [PubMed: 16403818]
- Sun L, Peng Y, Sharrow AC, Iqbal J, Zhang Z, Papachristou DJ, Zaidi S, Zhu LL, Yaroslavskiy BB, Zhou H, Zallone A, Sairam MR, Kumar TR, Bo W, Braun J, Cardoso-Landa L, Schaffler MB, Moonga BS, Blair HC, Zaidi M. FSH directly regulates bone mass. *Cell* 2006;125(2):247–260. [PubMed: 16630814]
- Tang BL. SIRT1, neuronal cell survival and the insulin/IGF-1 aging paradox. *Neurobiol Aging* 2006;27(3):501–505. [PubMed: 16464659]
- Warner HR. Developing a research agenda in biogerontology: basic mechanisms. *Sci Aging Knowledge Environ* 2005;2005(44):e33.
- Yamamoto M, Clark JD, Pastor JV, Gurnani P, Nandi A, Kurosu H, Miyoshi M, Ogawa Y, Castrillon DH, Rosenblatt KP, Kuro-o M. Regulation of oxidative stress by the anti-aging hormone klotho. *J Biol Chem* 2005;280(45):38029–38034. [PubMed: 16186101]

Figure 1.

Proposed model for Sirt1-mediated regulation of insulin secretion in the β cell. (From Moynihan, K. A., Grimm, A. A., Plueger, M. M., Bernal-Mizrachi, E., Ford, E., Cras-Meneur, C., Permutt, M. A., & Imai, S. 2005, "Increased dosage of mammalian Sir2 in pancreatic beta cells enhances glucose-stimulated insulin secretion in mice", *Cell Metab*, vol. 2, no. 2, pp. 105–117. Reprinted with permission from Elsevier.)

Figure 2.

Klotho increases resistance to insulin and IGF1. (A and B) Fasting blood glucose (A) and insulin (B) levels were compared between wild-type (WT), and two transgenic lines (*EFmKL48* and *EFmKL46*) of mice. (C) Hyperinsulinemic euglycemic clamp experiments. Glucose infusion rate (mg/kg/min) was compared between wild-type, *EFmKL48*, and *EFmKL46* mice. During the clamp experiments there were no differences in blood glucose concentration between wild-type, *EFmKL46*, and *EFmKL48* mice. The number of animals (*n*) for each group is indicated in the bars. (D and E) Insulin (D) and IGF1 (E) tolerance tests. Blood glucose levels after injection of insulin (0.5 U/kg) or IGF1 (0.75 mg/kg) were expressed as a percentage change from blood glucose concentration at time zero. Error bars indicate

SD. *, $P < 0.05$ and †, $P < 0.01$ versus wild-type mice by ANOVA. (F) A schematic representation of Klotho extracellular peptide (right) and analysis of purified recombinant Klotho peptide by SDS-polyacrylamide gel electrophoresis (left). (G) The effect of Klotho injection on insulin tolerance in mice. Insulin tolerance tests were performed with age-matched wild-type mice immediately after intraperitoneal injection with saline or purified recombinant Klotho peptide (10 µg/kg). (H) The effect of Klotho injection on blood glucose levels. Saline or Klotho protein (10 µg/kg) was administered into age-matched wild-type mice by intraperitoneal injection. Error bars indicate SD. *, $P < 0.05$ and †, $P < 0.01$ versus saline-injected mice by ANOVA. (From Kurosu, H., Yamamoto, M., Clark, J. D., Pastor, J. V., Nandi, A., Gurnani, P., McGuinness, O. P., Chikuda, H., Yamaguchi, M., Kawaguchi, H., Shimomura, I., Takayama, Y., Herz, J., Kahn, C. R., Rosenblatt, K. P., & Kuro-o M 2005, "Suppression of aging in mice by the hormone Klotho", *Science*, vol. 309, no. 5742, pp. 1829-1833. Reprinted with permission from AAAS.)