SUPPLEMENTAL MATERIALS:

*Analysis of potential adverse side effects from ICV α-klotho:* To determine any additional stress caused to the mice from ICV α-klotho treatment, nest building experiments were performed as previously described [1] (Supplemental Fig. 7). Briefly, nesting material was divided into six equal pieces and presented to mice in equal “zones” of their cage after either ICV α-klotho or vehicle treatment. Nesting behavior was blindly scored 24 hours later by quantifying how many zones had been cleared.

Conditioned taste aversion tests were also performed as previously described [2]. Mice were acclimated to 2 hours access to water each day for two days, to condition mice to drink when water is provided. After the conditioning period a 0.15% saccharin solution was provided for two hours. Immediately following the 2-hour saccharin availability, mice were treated with either 2.0ug ICV α-klotho, ICV vehicle, or 127mg/kg IP lithium chloride. The following day, mice were presented with both water and saccharin. Mice have an inert preference for the saccharin water, however treatments that result in negative side-effects induce an aversion to the saccharin water. Lithium chloride is a well-established positive control because it induces gastrointestinal distress.

*ICV injection in female DIO and male chow-fed mice:* The same 12-day experimental timeline described in the main text was also used in female DIO (n=4/group), as well as chow-fed male mice (7-8/group) cannulated at 7-8-weeks of age (Supplemental Figs. 8-9).

SUPPLEMENTAL RESULTS:

**α-Klotho does not Induce a Stress-Response in Mice.** Nest building and conditioned taste aversion experiments were performed to determine if reductions in food intake were stress related [1,2] (Supplemental Fig. 7). ICV α-klotho treatment did not impair nest building in healthy or STZ-treated mice, nor did it result in a taste aversion to saccharin water.

**7 Days ICV α-Klotho Treatment Results in Weight Loss, Suppressed Food Intake, and Improved Glucose Regulation in Female DIO Mice.** Considering previously described sex-differences in α-klotho concentrations [3], ICV α-klotho experiments were also performed in a small cohort (n=4/group) of female DIO mice (Supplemental Fig. 8). Despite the small sample size, ICV α-klotho significantly suppressed food intake (19.7%) and improved glucose clearance during a GTT. While there were no differences in body weight, this is likely due to low statistical power.

**7 Days ICV α-Klotho Treatment Suppresses Food Intake in Healthy Mice.** In healthy, chow-fed mice, ICV α-klotho treatment significantly decreased food intake by 12% compared to vehicle-treated controls (Supplemental Fig. 9D-E), however, there were no differences in body weight or fed glucose levels (Supplemental Fig. 9A-C).

SUPPLEMENTAL FIGURE LEGEND:

Supplemental Table 1. Primer sequences used for qPCR.

Supplemental Figure 1. Validation of ICV cannulation procedure. (A) Observation of scar tissue path of the cannula and (B) 3.0ug ICV leptin treatment suppresses food intake after an overnight fast (n = 3-4 chow fed mice). Data represented as mean ± SEM; P < 0.05 indicates significant difference between groups.

Supplemental Figure 2. (A) Effects of different doses of STZ on fed blood glucose levels 7 days after injections. (B) Hypothalamic gene expression of agouti-related peptide (AgRP), neuropeptide Y (NPY), and pro-opiomelanocortin (POMC) in 9-10-week-old, STZ-treated, ad libitum fed, mice after 7 days ICV α-klotho or vehicle injections (n = 5-7/group). Data represented as mean ± SEM; \*P < 0.05 vs. ICV control

Supplemental Figure 3. 12 days ICV α-klotho treatment does not affect insulin sensitivity in peripheral tissues of DIO mice. Insulin stimulated signaling (10U/kg 7 minutes before euthanasia) in (A) Skeletal muscle, (B) Liver, (C) Epididymal adipose tissue, and (D) Hypothalamus, and (E) Body weight in 17-18-week-old weight-matched male DIO mice after 12 days ICV α-klotho or vehicle injections (n = 4/group). Data represented as mean ± SEM; \*P < 0.05 vs. ICV controls.

Supplemental Figure 4. 12 days ICV α-klotho treatment alters gene mRNA levels of key metabolic genes in the liver. (A) Hypothalamic gene expression of agouti-related peptide (AgRP), neuropeptide Y (NPY), pro-opiomelanocortin (POMC), and melanocortin-4 receptor (MC4R), (B) Western blot illustrating the effects of α-klotho on basal phosphorylation of AKTser473 in skeletal muscle, (C) Liver gene expression of pyruvate kinase (PK), phosphoenolpyruvate carboxykinase (PEPCK), glucose-6-phosphatase (G6Pase), and glucokinase (GK), (D) Liver gene expression of fatty acid synthase (FAS), carnitine palmitoyl transferase 1 (CPT1), and acetyl CoA carboxylase (ACC), and (E) Liver Oil Red O stains in 17-18-week-old male DIO mice after 12 days ICV α-klotho or vehicle injections (n = 4-5/group). Data represented as mean ± SEM; \*P < 0.05 indicates significant difference between groups.

Supplemental Figure 5. 7 days ICV treatment with anti-α-klotho antibody has no significant effects on hypothalamic gene expression, liver gene expression, or skeletal muscle pAKTser473. (A) Western blot illustrating specificity of anti-α-klotho antibody to bind to α-klotho using kidney as a positive control. (B) Western blot illustrating the ability of anti-α-klotho antibody to suppress α-klotho-mediated cell signaling in GT1-7 cells. (C) Hypothalamic gene expression of AgRP, NPY, POMC, and melanocortin-4 receptor, (D) Liver gene expression of pyruvate kinase (PK), phosphoenolpyruvate carboxykinase (PEPCK), glucose-6-phosphatase (G6Pase), and glucokinase (GK), and (E) Representative Western Blot of basal phosphorylated AKTser473 in skeletal muscle in 9-week-old chow-fed male mice treated with anti-α-klotho antibody compared to vehicle-treated controls (n=8/group). Data represented as mean ± SEM; P < 0.05 indicates significant difference between groups.

Supplemental Figure 6**.** α-Klotho alters cell signaling and neuronal activity in the hypothalamus*.*

**(A)** Representative Western Blot image and quantification illustrating the effects of 30 minutes 3.65mM α-klotho treatment on phosphorylation of ERKthr202/tyr204, AKTser473, and FOXO1ser256 in GT1-7 cells (n = 4 mice/group) **(B)** Representative immunofluorescence image and quantification illustrating the effects of acute ICV administration of 2.0 uL of 1.0 ug/uL α-klotho compared to 2.0 uL vehicle on phosphorylation of ERKthr202/tyr204 in the arcuate nucleus of the hypothalamus in 10 week old chow-fed mice (3V: third ventricle) (scale bar = 50 um) (n = 3/group) **(C)** The effects of α-klotho treatment (3.65mM) on AgRP mRNA during two hours and overnight serum starvation in GT1-7 cells (n = 4-6/group). Data represented as mean ± SEM; \*P < 0.05 vs. controls.

Supplemental Figure 7. ICV α-klotho does not cause additional stress in mice. (A) Taste aversion to saccharin water after ICV treatment with 2.0 uL vehicle or 2.0 uL of 1.0ug/uL α-klotho in 13-week old chow-fed mice (n = 2-3/group). IP lithium chloride injection was used as a positive control to elicit an aversion to saccharin water. (B) Nest building behaviors in healthy 8-9-week-old chow-fed and 9-10 week old STZ-treated mice treated with 2.0 uL vehicle or 2.0 ug of 1.0 ug/uL α-klotho (n = 3-6/group). Data represented as mean ± SEM; P < 0.05 indicates significant difference between groups.

Supplemental Figure 8. 7 days ICV α-klotho treatment improves glucose regulation and suppresses food intake in female DIO mice. (A-B) Differences in body weight, (C) Average daily food intake, (D) Timeline of food intake, (E-F) Refeeding after daytime food restriction, and (G-H) Blood glucose levels during a glucose tolerance test (1 g/kgBW) after 7 days ICV α-klotho treatment in 17-18-week old DIO female mice (n=4/group). Data represented as mean ± SEM; P < 0.05 indicates significant difference between groups.

Supplemental Figure 9. 7 Days ICV α-klotho treatment suppresses food intake in healthy chow-fed mice. (A) Body weight, (B) Change in body weight, (C) Fed blood glucose levels, (D) Daily food intake, and (E) Timeline of food intake in 8-9-week old chow-fed mice (n=8-9/group). Data represented as mean ± SEM; P < 0.05 indicates significant difference between groups.

Supplemental Table 1. Primer sequences used in qPCR analysis

|  |  |  |
| --- | --- | --- |
| **Gene** | **Forward primer** | **Reverse primer** |
| *GAPDH* | AAATGGTGAAGGTCGGTGTG | TGAAGGGGTCGTTGATGG |
| *ACC1* | CGGCAGTACCTGCGAGTAGAG | GGGCGAATACACATTTGTCGTA |
| *ACC2* | GGGCCCTGGGAGACAAGA | GGGTAAGGTTGGGATTTGCA |
| *FAS* | TTCCAAGACGAAAATGATGC | AATTGTGGGATCAGGAGAGC |
| *CPT1* | TCTTGCAGTCGACTCACCTT | TCCACAGGACACATAGTCAGG |
| *G6Pase* | TTACCAAGACTCCCAGGACTG | GAGCTGTTGCTGTAGTAGTCG |
| *GK* | CCCTGAGTGGCTTACAGTTC | ACGGATGTGAGTGTTGAAGC |
| *L-PK* | CTTGCTCTACCGTGAGCCTC | ACCACAATCACCAGATCACC |
| *PEPCK* | TGGCTACGTC CCTAAGGAA | GGTCCTCCAGATACTTGTCGA |
| *NPY* | CCCAGCTCACATATTTATCTAGAG | TATGTGGACGGGGCAGAAGATCCAGG |
| *AgRP* | GCGGAGGTGCTAGATCCACA | AGGACTCGTGCAGCCTTACAC |
| *POMC* | CTCCGCTCTGCGACACTACA | ACCTCACCACGGAGAGCAAC |
| *MC4R* | GCG TTT CGA ATG GGT CGG AAA CCA | CCG CAA TGG AAA GCA GGC TGC AA |

SUPPLEMENTAL REFERENCES:

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[3] V. Behringer, J.M.G. Stevens, T. Deschner, R. Sonnweber, G. Hohmann, Aging and sex affect soluble alpha klotho levels in bonobos and chimpanzees, Front. Zool. 15 (2018) 35. doi:10.1186/s12983-018-0282-9.