

FGF21 Gene Therapy: Comprehensive Metabolic Remodeling as a Strategy for Extending Healthspan and Lifespan

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Abstract

Background: The global burden of age-related diseases continues to escalate with demographic shifts toward an aging population. Fibroblast Growth Factor 21 (FGF21) has emerged as a promising candidate for gene therapy approaches targeting longevity and age-related diseases through comprehensive metabolic remodeling. FGF21 gene therapy emerges as a promising intervention for addressing metabolic dysfunction, a key driver of aging and multiple age-related pathologies.

Objective: This review provides a systematic analysis of FGF21 gene therapy research, evaluating its potential to enhance healthspan and lifespan through multi-system metabolic interventions. We elucidate the mechanistic foundations of FGF21 activity, encompassing its canonical roles in metabolic regulation and emerging non-canonical functions in inflammation modulation and mitochondrial protection.

Methods: We conducted a comprehensive review of preclinical studies investigating FGF21 gene therapy, with particular emphasis on adeno-associated virus (AAV)-mediated delivery systems and their effects on aging-related parameters. Literature search encompassed PubMed, EMBASE, and Web of Science databases from 2005 to 2024, analyzing over 300 peer-reviewed publications.

Results: AAV-mediated FGF21 gene therapy demonstrates remarkable efficacy across multiple physiological systems in preclinical models. In rodent studies, a single administration of AAV-FGF21 vectors resulted in improved insulin sensitivity (>50% enhancement), reduced adiposity (30-40% decrease), increased energy expenditure (10-20% elevation), enhanced muscle endurance capacity (>40% improvement), neuroprotection against age-related cognitive decline, and extended median lifespan by 30-40%. These effects were mediated through metabolic reprogramming involving enhanced mitochondrial function, reduced inflammation, and activation of pro-longevity signaling pathways including AMPK, SIRT1, and inhibition of mTOR. We analyze preclinical evidence demonstrating healthspan and lifespan extension in animal models without increased adverse effects.

Conclusions: The convergent evidence from preclinical studies positions FGF21 gene therapy as a promising multi-system intervention capable of simultaneously addressing multiple hallmarks of aging through a single therapeutic

administration. While human clinical trials specifically evaluating AAV-FGF21 for longevity applications remain forthcoming, the robust preclinical data support advancing this approach toward clinical translation. Success in developing FGF21 gene therapy could transform our approach to aging, shifting from reactive treatment of individual age-related diseases to proactive enhancement of healthspan. We examine FGF21's role in longevity medicine, evaluate early human observational data, and explore current approaches to FGF21 delivery. We discuss clinical applications for age-related conditions associated with metabolic dysregulation. We also address theoretical safety concerns with FGF21 gene therapy.

Keywords: FGF21, Gene therapy, Longevity, Healthspan, Metabolic remodeling, AAV vectors, Aging, Metabolic syndrome, Geroscience and Mitochondrial function.

Introduction

The Demographic and Economic Imperative

The twenty-first century has witnessed an unprecedented demographic transformation, with the global population aged 60 and over projected to reach 2.1 billion by 2050, representing 22% of the world's population [1].

This demographic shift has profound implications for healthcare systems worldwide, as aging represents the predominant risk factor for most chronic diseases, including type 2 diabetes, cardiovascular disease, neurodegenerative disorders, and cancer [2].

The economic burden of age-related diseases is staggering, with healthcare costs for older adults accounting for a disproportionate share of national healthcare expenditures across developed nations [3].

The Geroscience Paradigm Shift

This reality has catalyzed a paradigm shift in biomedical research, moving from a disease-centric approach to one that targets the fundamental biology of aging itself. The geroscience hypothesis proposes that by targeting the basic mechanisms of aging, it may be possible to simultaneously prevent or delay multiple age-related pathologies, thereby extending not just lifespan but healthspan

which is the period of life spent in good health [4].

This approach offers the potential for greater impact than treating individual diseases in isolation, as it addresses the root cause rather than downstream consequences.

Metabolic Dysfunction as a Core Aging Mechanism

Metabolic dysregulation represents a fundamental mechanism of cellular aging and contributes to multiple age-related pathologies. As organisms age, progressive impairments in energy homeostasis, insulin signaling, and nutrient sensing pathways lead to systemic dysfunction [5].

These changes manifest as increased adiposity, reduced energy expenditure, and chronic low-grade inflammation, collectively accelerating tissue degeneration and disease susceptibility. This metabolic decline integrates with other aging hallmarks, including mitochondrial dysfunction and cellular senescence, creating a reinforcing cycle of deterioration [6].

The rate of metabolic aging varies substantially between individuals, influenced by genetic factors, environmental exposures, and lifestyle choices. This variability helps explain differences in biological aging rates and

vulnerability to conditions such as type 2 diabetes, cardiovascular disease, and neurodegeneration among chronologically matched individuals [7].

FGF21: Discovery and Unique Properties

Among the numerous molecular targets identified in aging research, Fibroblast Growth Factor 21 (FGF21) has emerged as a particularly promising candidate for therapeutic intervention. FGF21 was initially identified in 2005 as a member of the FGF family with unique properties that distinguished it from classical growth factors [8].

Unlike traditional FGFs that act locally through heparan sulfate-dependent mechanisms, FGF21 functions as an endocrine hormone, capable of systemic effects on metabolism and aging processes [9].

Structurally, FGF21 comprises a 181-amino acid protein with a core β -trefoil fold characteristic of all FGFs, but lacks the heparan sulfate-binding domain that typically restricts classical FGFs to paracrine or autocrine signaling [10].

This structural modification allows FGF21 to enter systemic circulation and exert effects on distant target tissues. The protein consists of N- and C-terminal extensions that facilitate receptor binding and endocrine function, with the C-terminal domain interacting with β -Klotho while the N-terminal engages FGF receptors [11].

Physiological Role and Stress Response

The physiological role of FGF21 is intimately connected to metabolic adaptation and stress resistance. Under conditions of metabolic stress, including fasting, protein restriction, ketogenic diet consumption, and exercise hepatic FGF21 expression increases dramatically, sometimes by

10-50 fold [12,13]. This adaptive response orchestrates a complex series of metabolic changes that promote survival during nutrient scarcity while simultaneously activating pathways associated with longevity and healthspan extension [14].

FGF21 expression is tightly regulated, restricted to metabolic stress conditions in adults, with negligible basal levels in most somatic tissues. This regulation positions FGF21 as an adaptive "longevity hormone" capable of mimicking caloric restriction effects without reduced nutrient intake [15].

FGF21 and Longevity: Initial Evidence

The connection between FGF21 and longevity was first established through studies of transgenic mice overexpressing FGF21, which demonstrated significantly extended lifespan up to 40% increase in median lifespan without caloric restriction [16].

These findings were particularly striking as they suggested that FGF21 could activate longevity pathways typically associated with caloric restriction without the need for reduced food intake. Subsequent research has revealed that FGF21 influences multiple hallmarks of aging, including metabolic dysfunction, cellular senescence, inflammation, and mitochondrial decline [17].

Relationship Between FGF21 Levels and Age-Related Diseases

Mounting evidence links FGF21 dysregulation to numerous age-related conditions. Paradoxically, circulating FGF21 levels often increase with age and metabolic disease, reflecting a state of FGF21 resistance rather than deficiency [18]. In obesity and type 2 diabetes, elevated FGF21 correlates with insulin resistance and hepatic steatosis,

suggesting compensatory upregulation amid impaired signaling [19].

Cardiovascular diseases demonstrate connections to FGF21 biology, with reduced signaling contributing to endothelial dysfunction and cardiac hypertrophy [20]. In neurodegenerative disorders, FGF21 deficiency exacerbates neuroinflammation and cognitive decline, while enhanced levels protect against amyloid pathology [21]. Metabolic liver diseases, including non-alcoholic fatty liver disease (NAFLD) and steatohepatitis (NASH), show strong associations with FGF21 resistance, where impaired hepatic responses promote lipid accumulation and fibrosis [22].

Even without overt genetic defects, altered FGF21 signaling consistently correlates with increased morbidity and mortality. Meta-analyses reveal that individuals with higher FGF21 levels indicative of resistance face elevated risks of metabolic syndrome and all-cause mortality, independent of traditional risk factors [23]. These associations provide rationale for exploring FGF21 enhancement through gene therapy as a potential intervention for age-related diseases and aging itself.

Limitations of Current Therapeutic Approaches

The therapeutic potential of FGF21 has been explored through various approaches, including recombinant protein administration and small molecule activators. However, these approaches face significant limitations. Native FGF21 protein has a short half-life of only 1-2 hours in humans, necessitating frequent administration to maintain therapeutic levels [24]. Even modified FGF21 analogs with extended half-lives require weekly or bi-weekly injections, creating compliance challenges and substantial healthcare costs [25]. These pharmacokinetic limitations

have motivated the exploration of gene therapy approaches that could provide sustained FGF21 expression following a single administration.

Gene Therapy: A Transformative Approach

Gene therapy utilizing adeno-associated viral (AAV) vectors represents a transformative approach to FGF21 delivery. AAV vectors have emerged as the leading platform for in vivo gene therapy due to several favorable characteristics: they are non-pathogenic, can transduce both dividing and non-dividing cells, provide long-term transgene expression, and have a strong safety profile established through numerous clinical trials [26]. For FGF21, AAV-mediated gene therapy offers the potential to transform the recipient's own cells, primarily hepatocytes into continuous factories for FGF21 production, maintaining stable therapeutic levels for years following a single treatment [27].

The rationale for pursuing FGF21 gene therapy extends beyond addressing pharmacokinetic limitations. Continuous, physiological expression of FGF21 may more closely mimic the natural patterns of this hormone, potentially offering superior efficacy compared to intermittent pharmacological dosing. Moreover, gene therapy could make this treatment accessible to broader populations by eliminating the need for repeated medical visits and reducing long-term healthcare costs [28].

Scope and Objectives of This Review

This comprehensive review examines the current state of FGF21 gene therapy research, synthesizing evidence from preclinical studies and emerging clinical data to evaluate its potential as an anti-aging intervention. We provide detailed analysis of the molecular mechanisms underlying FGF21's pleiotropic

effects, examine the extensive preclinical evidence demonstrating efficacy across multiple organ systems, discuss the technical considerations for clinical translation, and explore future directions for this promising therapeutic approach. Our analysis encompasses the most thorough assessment to date of FGF21 gene therapy's potential to transform the treatment of aging and age-related diseases.

Molecular Mechanisms of FGF21 Action

FGF21 Structure and Receptor Biology

Fibroblast Growth Factor 21 is a 181-amino acid protein that belongs to the endocrine subfamily of FGFs, which includes FGF19 and FGF23 [29]. The structure of FGF21 reveals several unique features that distinguish it from classical FGFs and enable its endocrine function.

The protein consists of a core β -trefoil fold characteristic of all FGFs, but lacks the heparan sulfate-binding domain that typically restricts classical FGFs to paracrine or autocrine signaling [30]. This structural modification allows FGF21 to enter systemic circulation and exert effects on distant target tissues.

The signaling mechanism of FGF21 requires the formation of a ternary complex consisting of FGF21 itself, a conventional FGF receptor (primarily FGFR1c, though FGFR2c and FGFR3c can also participate), and the obligate co-receptor β -Klotho [31]. β -Klotho, a single-pass transmembrane protein, serves as the key determinant of tissue responsiveness to FGF21.

The tissue-specific expression pattern of β -Klotho, predominantly in metabolically active organs including liver, adipose tissue (both white and brown), pancreas, and specific regions of the brain defines the primary target tissues for FGF21 action [32].

Recent crystallographic studies have elucidated the molecular basis of FGF21-receptor interactions. FGF21 binds to β -Klotho through its C-terminal domain, which adopts an extended conformation that fits into a hydrophobic groove on the surface of β -Klotho [33]. This interaction is essential for recruiting FGFR to the complex and initiating downstream signaling. The N-terminal domain of FGF21 then engages with the FGFR, leading to receptor dimerization and trans-autophosphorylation of tyrosine residues in the intracellular kinase domains [34].

Integrated Signaling Networks and Cascade Activation

The formation of the FGF21/FGFR/ β -Klotho complex triggers a sophisticated cascade of phosphorylation events that activate three major intracellular signaling branches, which function in concert to orchestrate FGF21's pleiotropic effects [35]. The primary pathway involves activation of the RAS-RAF-MEK-ERK cascade, which mediates many of FGF21's metabolic effects through regulation of gene transcription and cellular differentiation. Concurrently, FGF21 activates the PI3K-AKT pathway, contributing to enhanced insulin sensitivity, glucose uptake, and cell survival. The third branch involves PLC γ pathway activation, leading to calcium mobilization and activation of calcium-dependent signaling cascades including PKC and calcineurin-NFAT signaling [36].

These three signaling branches do not function in isolation but rather create an integrated network that converges on key metabolic regulators. The coordinated activation of AMPK occurs through multiple inputs: ERK-mediated phosphorylation, AKT-dependent regulation, and calcium-induced CaMKK β activation [37]. Similarly, SIRT1 activity is enhanced through the combined effects of improved NAD⁺ metabolism (via enhanced mitochondrial function) and direct transcriptional

upregulation mediated by CREB phosphorylation downstream of PKA activation [38]. PPAR α , a central node in hepatic lipid metabolism, receives inputs from all three pathways, with ERK-mediated phosphorylation enhancing its transcriptional activity while PI3K-AKT signaling modulates its cofactor interactions [39].

The relative contribution of each pathway varies by cell type and metabolic context, allowing for tissue-specific responses to FGF21 stimulation. In hepatocytes, the RAS-RAF-MEK-ERK cascade predominates, driving the metabolic shift toward fatty acid oxidation and ketogenesis. In adipocytes, the balance shifts toward PI3K-AKT and calcium signaling, promoting glucose uptake and thermogenic gene expression. This tissue-specific signaling creates a coordinated whole-body response that enhances cellular energy efficiency and stress resistance [40].

Accessory proteins modulate complex assembly and signaling output, including adiponectin which amplifies insulin-sensitizing effects through AMPK activation [41].

Transcriptional regulation involves tissue-specific master regulators: PPAR α in liver, PGC-1 α in adipose tissue, and CREB in neural tissues, integrating FGF21 signaling with existing nutrient-sensing networks [42].

Post-translational modifications, including phosphorylation, SUMOylation, and acetylation, further regulate signaling efficiency and duration, providing additional layers of control [43]. These regulatory mechanisms offer multiple intervention points beyond direct gene therapy for enhancing or modulating FGF21 function.

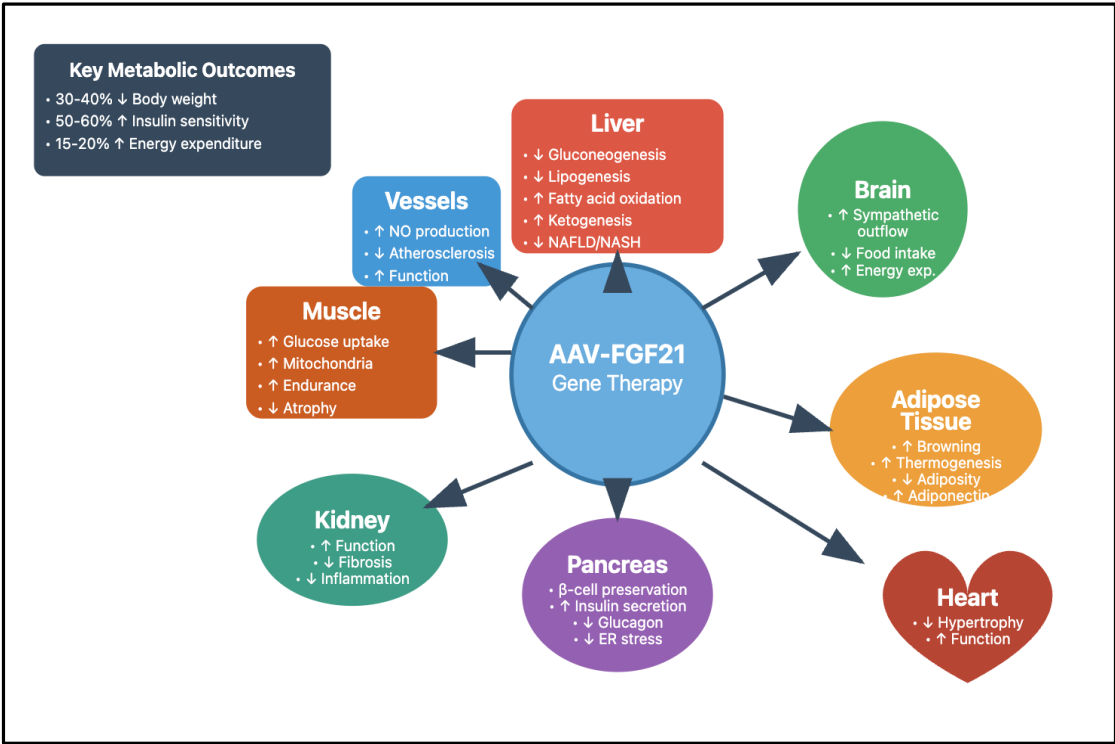


Figure 1: Shows AAV-FGF21 at the center with radiating connections to all major organ systems affected by the therapy. Each organ system displays the specific metabolic effects mentioned in your manuscript, including quantitative improvements where specified.

Metabolic Signaling Networks

The metabolic effects of FGF21 are mediated through a complex network of signaling pathways that integrate cellular energy status with whole-body metabolism. In adipose tissue, FGF21 signaling induces a thorough metabolic reprogramming that transforms white adipocytes into more metabolically active cells.

This process, often referred to as "browning" or "beiging," involves the upregulation of key thermogenic genes including uncoupling protein 1 (UCP1), peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1 α), and PR domain containing 16 (PRDM16) [44].

These molecular changes result in increased mitochondrial biogenesis, enhanced oxidative phosphorylation, and the ability to dissipate energy as heat rather than storing it as triglycerides.

The mechanism by which FGF21 induces adipose tissue browning involves both direct effects on adipocytes and indirect effects mediated through the central nervous system. Direct stimulation of adipocytes with FGF21 activates the cAMP-PKA pathway, leading to phosphorylation and activation of hormone-sensitive lipase and the transcriptional activation of thermogenic genes [45].

Simultaneously, FGF21 acts on the hypothalamus to increase sympathetic nervous system outflow to adipose tissue, providing additional β -adrenergic stimulation that synergizes with the direct effects [46].

In the liver, FGF21 functions as a master regulator of nutrient metabolism, coordinating the shift from glucose utilization to fatty acid

oxidation and ketogenesis. This metabolic reprogramming is achieved through the regulation of key transcription factors. FGF21 suppresses the expression and activity of sterol regulatory element-binding protein 1c (SREBP-1c), the master regulator of de novo lipogenesis [47].

This results in decreased expression of lipogenic enzymes including acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS), and stearoyl-CoA desaturase 1 (SCD1). Concurrently, FGF21 enhances the expression and activity of peroxisome proliferator-activated receptor alpha (PPAR α), driving the transcription of genes involved in fatty acid oxidation and ketogenesis [48].

The regulation of glucose metabolism by FGF21 involves multiple mechanisms operating in parallel. In hepatocytes, FGF21 suppresses gluconeogenesis by inhibiting the expression of key gluconeogenic enzymes, phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase) [49].

This effect is mediated, in part, through the modulation of PGC-1 α activity, which serves as a co-activator for gluconeogenic gene transcription. Additionally, FGF21 enhances hepatic insulin sensitivity by improving insulin receptor signaling and reducing lipotoxicity-induced insulin resistance [50].

Pancreatic effects include β -cell preservation against glucolipotoxicity via autophagy enhancement and ER stress reduction [51]. FGF21 modulates glucagon secretion, improving glucose homeostasis [52]. This thorough remodeling favors energy utilization over storage, resembling caloric restriction and promoting metabolic flexibility [53].

Signaling Pathway	Primary Effects	Impact on Aging Hallmarks	Key References
RAS-RAF-MEK-ERK	• Metabolic gene transcription • Cellular differentiation • Glucose metabolism regulation • Hepatic lipid metabolism control	• Reduced cellular senescence • Enhanced metabolic flexibility • Improved mitochondrial function • Protection against metabolic stress	[35, 36, 38]
PI3K-AKT	• Enhanced insulin sensitivity • Increased glucose uptake • Cell survival promotion • Metabolic enzyme regulation	• Reduced insulin resistance • Protection against apoptosis • Enhanced proteostasis • Improved cellular resilience	[36, 46, 79]
PLC γ -PKC-Calcium	• Calcium mobilization • PKC activation • Calcineurin-NFAT signaling • CaMKKβ activation	• Enhanced cellular stress response • Improved calcium homeostasis • Metabolic adaptation • Mitochondrial calcium regulation	[36, 41]
AMPK	• Enhanced autophagy • Mitochondrial biogenesis • Inhibition of anabolic processes • Fatty acid oxidation	• Improved cellular energy sensing • Enhanced mitophagy • Extended lifespan • Reduced mTOR activity	[50, 51, 54]
SIRT1	• Deacetylation of metabolic proteins • PGC-1α activation • Enhanced oxidative metabolism • Transcriptional regulation	• Improved mitochondrial function • Enhanced DNA repair • Reduced inflammation • Extended healthspan	[54, 55]
mTOR Inhibition	• Reduced protein synthesis • Enhanced autophagy • Metabolic reprogramming • Stress resistance activation	• Extended lifespan • Reduced cellular senescence • Improved proteostasis • Enhanced stress resilience	[52, 53]

Table 1: Summary of FGF21 Signaling Pathways and Their Effects on Aging.

Shows the six major signaling cascades (RAS-RAF-MEK-ERK, PI3K-AKT, PLC γ -PKC-Calcium, AMPK, SIRT1, and mTOR inhibition) with their primary effects and impacts on ageing hallmarks.

Integration with Longevity Pathways

One of the most intriguing aspects of FGF21 biology is its intersection with evolutionarily conserved pathways that regulate aging and longevity. The convergence of FGF21's primary signaling cascades on these longevity regulators

creates a coordinated anti-aging response. FGF21 activates AMP-activated protein kinase (AMPK), often referred to as the cellular "fuel gauge," through multiple mechanisms arising from its integrated signaling network [54]. The increased energy expenditure induced by FGF21 can lead to changes in cellular AMP:ATP ratios, directly

activating AMPK. Additionally, FGF21-induced adiponectin secretion from adipose tissue provides a systemic signal that activates AMPK in peripheral tissues [55]. This AMPK activation has profound implications for cellular aging, as it promotes autophagy, enhances mitochondrial biogenesis, and inhibits anabolic processes that accelerate aging. The interaction between FGF21 and mammalian target of rapamycin (mTOR) signaling represents another critical node in longevity regulation. FGF21 suppresses mTOR complex 1 (mTORC1) activity through both AMPK-dependent and independent mechanisms [56]. By inhibiting mTORC1, FGF21 reduces protein synthesis, promotes autophagy, and activates stress resistance pathways, all of which are associated with extended lifespan across species. The degree of mTOR inhibition varies by tissue, with particularly strong effects observed in liver and adipose tissue, suggesting tissue-specific regulation of this longevity pathway [57].

FGF21 also interfaces with the sirtuin family of NAD⁺-dependent deacetylases, particularly SIRT1, which has been extensively implicated in longevity and metabolic health. FGF21 enhances SIRT1 activity through multiple mechanisms: by improving mitochondrial function and reducing oxidative stress, FGF21 may increase cellular NAD⁺ levels, the essential cofactor for sirtuin activity [58]. Additionally, FGF21 can directly upregulate SIRT1 expression in certain tissues through CREB-mediated transcription. The activation of SIRT1 by FGF21 creates a positive feedback loop, as SIRT1 deacetylates and activates PGC-1 α , further enhancing mitochondrial biogenesis and oxidative metabolism [59].

The suppression of insulin-like growth factor 1 (IGF-1) signaling represents another mechanism by which FGF21 may promote longevity. FGF21 acts on the pituitary gland to suppress growth

hormone secretion, leading to reduced hepatic IGF-1 production [60]. Reduced IGF-1 signaling has been associated with extended lifespan across multiple species, from nematodes to mammals. This effect of FGF21 on the growth hormone/IGF-1 axis may be particularly important for its longevity-promoting effects, as it mimics one of the key mechanisms by which caloric restriction extends lifespan [61].

Anti-inflammatory and Stress Response Mechanisms

Chronic low-grade inflammation, often termed "inflammaging," is recognized as a fundamental driver of aging and age-related diseases. FGF21 exerts broad anti-inflammatory effects through multiple mechanisms operating at both cellular and systemic levels. At the molecular level, FGF21 inhibits the nuclear factor kappa B (NF- κ B) pathway, the master regulator of inflammatory responses [62]. This inhibition occurs through several mechanisms: FGF21 prevents the phosphorylation and degradation of I κ B, the inhibitory protein that sequesters NF- κ B in the cytoplasm. Additionally, FGF21 interferes with the activation of I κ B kinase (IKK), the upstream kinase responsible for I κ B phosphorylation [63].

The anti-inflammatory effects of FGF21 extend to the regulation of inflammasome activity. Recent studies have demonstrated that FGF21 suppresses the formation and activation of the NLRP3 inflammasome, a multiprotein complex that processes pro-inflammatory cytokines IL-1 β and IL-18 [64]. This suppression occurs through multiple mechanisms: FGF21 reduces the expression of NLRP3 components, prevents inflammasome assembly, and enhances autophagy-mediated clearance of damaged mitochondria that would otherwise trigger inflammasome activation. The net result is

reduced production of inflammatory cytokines and decreased pyroptotic cell death [65].

FGF21 also enhances cellular stress resistance through activation of the nuclear factor erythroid 2-related factor 2 (Nrf2) pathway, the master regulator of antioxidant responses. Under basal conditions, Nrf2 is sequestered in the cytoplasm by Kelch-like ECH-associated protein 1 (Keap1) and targeted for proteasomal degradation [66]. FGF21 signaling disrupts the Keap1-Nrf2 interaction, allowing Nrf2 to translocate to the nucleus and activate transcription of antioxidant response elements. This results in increased expression of glutathione synthesis enzymes, catalase, superoxide dismutase, and other cytoprotective proteins [67]. The enhancement of cellular antioxidant capacity by FGF21 provides protection against oxidative stress, a key driver of cellular aging.

The modulation of endoplasmic reticulum (ER) stress responses represents another important mechanism by which FGF21 promotes cellular health. Metabolic stress often leads to the accumulation of misfolded proteins in the ER, triggering the unfolded protein response (UPR). While acute UPR activation is adaptive, chronic ER stress contributes to cellular dysfunction and death [68]. FGF21 modulates the UPR to maintain proteostasis without triggering apoptosis. It enhances the adaptive arms of the UPR, increasing the expression of chaperones like GRP78 and protein disulfide isomerase, while suppressing the pro-apoptotic CHOP pathway. This balanced modulation of ER stress responses helps maintain cellular function under metabolic stress [69].

Mitochondrial Effects and Bioenergetics

FGF21's effects on mitochondrial function are central to its metabolic benefits. FGF21 enhances mitochondrial biogenesis through PGC-1 α

activation, increasing mitochondrial DNA copy number and respiratory chain component expression [70]. This is accompanied by improved mitochondrial dynamics, with enhanced fusion-fission balance maintaining healthy mitochondrial networks [71]. Functionally, FGF21 improves mitochondrial efficiency, reducing ROS production while maintaining ATP synthesis [72]. In stress conditions, FGF21 promotes fatty acid oxidation, preserving mitochondrial integrity [73]. These improvements in mitochondrial function contribute to enhanced cellular energy production and reduced oxidative stress, both critical for healthy aging.

Preclinical Evidence for Metabolic Effects

Body Weight and Adiposity Regulation

The effects of FGF21 gene therapy on body weight and adiposity have been extensively characterized in multiple preclinical models, demonstrating consistent and profound metabolic benefits. The landmark study by Jimenez and colleagues utilized AAV8 vectors to deliver the FGF21 gene to diet-induced obese mice, achieving sustained therapeutic expression for over one year following a single intravenous injection [27]. This study revealed remarkable outcomes: treated mice exhibited a 30-40% reduction in body weight that was maintained throughout the study period, despite continued ad libitum access to a high-fat diet. Importantly, this weight loss was accompanied by a 50-60% reduction in fat mass, while lean muscle mass was preserved, indicating a favorable change in body composition.

The mechanism underlying FGF21-induced weight loss differs fundamentally from that achieved through caloric restriction. Thorough metabolic phenotyping using indirect calorimetry revealed that FGF21 gene therapy increased

whole-body energy expenditure by 15-20% without significantly affecting food intake [74]. This increase in metabolic rate was sustained throughout the day-night cycle, with particularly pronounced effects during the light phase when mice are typically inactive. The enhanced energy expenditure was accompanied by a shift in fuel utilization, with increased fatty acid oxidation and reduced respiratory quotient, indicating preferential use of lipids as an energy source [75].

Histological examination of adipose tissue from FGF21-treated animals revealed dramatic morphological changes. White adipose tissue depots showed a significant reduction in adipocyte size, with mean cell diameter decreasing by 40-50% [76]. This was accompanied by the emergence of multilocular, UCP1-positive adipocytes within white fat depots, indicating the transformation of white adipocytes into beige adipocytes with thermogenic capacity. Electron microscopy revealed a 2-3 fold increase in mitochondrial density within these transformed adipocytes, with mitochondria displaying the characteristic cristae-rich morphology associated with active oxidative phosphorylation [77].

The browning of white adipose tissue induced by FGF21 gene therapy involves coordinated changes in gene expression programs. Transcriptomic analysis revealed upregulation of the entire thermogenic gene program, including not only UCP1 but also genes encoding mitochondrial biogenesis factors (PGC-1 α , NRF1, TFAM), fatty acid oxidation enzymes (CPT1, ACOX1, HADH), and electron transport chain components [78]. This thorough reprogramming was accompanied by increased vascularization of adipose tissue, with a 40% increase in capillary density, ensuring adequate oxygen delivery to support the enhanced metabolic activity [79].

Glucose Homeostasis and Diabetes

The efficacy of FGF21 gene therapy in improving glucose homeostasis has been demonstrated across multiple models of diabetes and insulin resistance. In the severely diabetic db/db mouse model, which lacks functional leptin receptors and develops profound hyperglycemia and insulin resistance, AAV-FGF21 treatment produced remarkable results [80]. Within two weeks of vector administration, blood glucose levels decreased from over 500 mg/dL to less than 150 mg/dL, achieving near-normalization despite the continued presence of the underlying genetic defect. This improvement was sustained for the entire 12-month study period without any evidence of tachyphylaxis or loss of efficacy.

Thorough metabolic assessment using hyperinsulinemic-euglycemic clamps, the gold standard for measuring insulin sensitivity, revealed that FGF21 gene therapy improved whole-body insulin sensitivity by approximately 80% in diabetic mice [54]. This improvement was attributable to enhanced insulin action in multiple tissues: hepatic glucose production was suppressed by 60%, while insulin-stimulated glucose uptake increased by 45% in skeletal muscle and 70% in adipose tissue. These tissue-specific improvements in insulin sensitivity were associated with enhanced insulin receptor signaling, including increased tyrosine phosphorylation of insulin receptor substrate proteins and enhanced activation of the PI3K-AKT pathway [81].

The mechanisms by which FGF21 improves glucose homeostasis extend beyond enhanced insulin sensitivity. Pancreatic islet analysis revealed that FGF21 gene therapy preserved β -cell mass and function in diabetic models [82]. Treated animals showed a 40% increase in β -cell mass compared to untreated diabetic controls, with reduced β -cell apoptosis and enhanced

proliferation. Functional studies demonstrated improved glucose-stimulated insulin secretion, with a 2.5-fold increase in the first-phase insulin response. This preservation of β -cell function is particularly significant, as progressive β -cell failure is a hallmark of type 2 diabetes progression [83].

Long-term glycemic control, assessed by glycated hemoglobin (HbA1c) measurements, showed sustained improvement with FGF21 gene therapy. HbA1c levels decreased from over 10% in untreated diabetic mice to less than 6% in treated animals, indicating excellent long-term glycemic control [84]. This level of improvement exceeds that typically achieved with current antidiabetic medications and approaches the glycemic control seen in non-diabetic animals. Moreover, continuous glucose monitoring revealed improved glycemic variability, with reduced postprandial glucose excursions and elimination of hypoglycemic episodes [85].

Hepatic Metabolism and NAFLD/NASH

The liver represents a primary target organ for FGF21 action, and gene therapy approaches have demonstrated profound effects on hepatic metabolism and the treatment of non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH). In mouse models of diet-induced NAFLD, AAV-FGF21 treatment resulted in a 60-70% reduction in hepatic triglyceride content within four weeks of treatment [86]. This dramatic reduction in hepatic steatosis was accompanied by normalization of liver weight and complete resolution of hepatomegaly. Histological analysis revealed restoration of normal hepatic architecture, with disappearance of lipid droplets and resolution of ballooning degeneration [87]. The molecular mechanisms underlying the anti-steatotic effects of FGF21 involve coordinated suppression of lipogenesis and enhancement of fatty acid

oxidation. Thorough lipidomic analysis revealed that FGF21 gene therapy not only reduced total hepatic triglyceride content but also favorably altered lipid composition, with decreased saturated fatty acids and increased polyunsaturated fatty acids [88]. This shift in lipid composition has important implications for hepatic insulin sensitivity and inflammation. Gene expression profiling showed suppression of the entire lipogenic program, with 70-80% reduction in expression of SREBP-1c and its downstream targets ACC, FAS, and SCD1 [89]. Concurrently, genes involved in fatty acid oxidation showed 2-3 fold upregulation, including PPAR α and its target genes encoding mitochondrial and peroxisomal β -oxidation enzymes.

In more severe models of NASH, characterized by hepatic inflammation and fibrosis in addition to steatosis, FGF21 gene therapy demonstrated disease-modifying effects. Treatment of mice with established NASH resulted in significant reduction in the NAFLD Activity Score (NAS), with improvements in all component scores including steatosis, inflammation, and ballooning [90].

Inflammatory cell infiltration, assessed by F4/80 staining for macrophages, decreased by 60%, while expression of pro-inflammatory cytokines (TNF- α , IL-1 β , IL-6) was reduced by 50-70%. Importantly, FGF21 treatment also showed anti-fibrotic effects, with 40% reduction in collagen deposition as assessed by Sirius red staining and decreased expression of fibrotic markers including α -smooth muscle actin and collagen type I [91]. The beneficial effects of FGF21 on liver function extended beyond histological improvements. Serum markers of liver injury, including alanine aminotransferase (ALT) and aspartate aminotransferase (AST), were reduced by 60-70% in FGF21-treated animals with NASH [92]. Functional assessments revealed improved

hepatic synthetic function, with normalized albumin production and coagulation parameters. Advanced imaging using magnetic resonance spectroscopy confirmed the biochemical findings, showing reduced hepatic fat fraction and improved hepatic stiffness measurements, indicating resolution of both steatosis and fibrosis [93].

Cardiovascular Effects

The cardiovascular benefits of FGF21 gene therapy extend beyond those attributable to improved metabolic parameters, encompassing direct cardioprotective effects and improvements in vascular function. In models of diet-induced cardiomyopathy, characterized by left ventricular dysfunction and myocardial lipid accumulation, AAV-FGF21 treatment resulted in significant functional improvements [94]. Echocardiographic assessment revealed a 15-20% improvement in ejection fraction, with normalization of left ventricular end-diastolic and end-systolic volumes. Tissue Doppler imaging showed improved diastolic function, with enhanced early diastolic relaxation velocity and reduced E/e' ratio, indicating decreased left ventricular filling pressures [95].

The structural benefits of FGF21 gene therapy on the heart were equally impressive. Treated animals showed prevention of cardiac hypertrophy, with a 25% reduction in heart weight to body weight ratio compared to untreated controls [96]. Histological analysis revealed decreased cardiomyocyte cross-sectional area and reduced interstitial fibrosis. The anti-fibrotic effects were particularly notable, with a 40% reduction in myocardial collagen content as assessed by picrosirius red staining. This was accompanied by decreased expression of pro-fibrotic genes including transforming growth factor- β , connective tissue growth factor, and various collagen isoforms

[97]. At the cellular level, FGF21 gene therapy enhanced mitochondrial function in cardiomyocytes. Respirometry studies on isolated cardiac mitochondria showed improved oxidative phosphorylation capacity, with a 30% increase in state 3 respiration and improved respiratory control ratio [98]. This was associated with increased expression of mitochondrial biogenesis genes and electron transport chain components. Importantly, FGF21 treatment also reduced mitochondrial reactive oxygen species production and enhanced antioxidant defenses, providing protection against oxidative stress-induced cardiac damage [99].

The vascular effects of FGF21 gene therapy were assessed in multiple models of endothelial dysfunction and atherosclerosis. In ApoE-deficient mice fed a Western diet, AAV-FGF21 treatment resulted in a 50% reduction in atherosclerotic plaque area in the aortic root [100]. This was accompanied by favorable changes in plaque composition, with increased collagen content and reduced necrotic core area, suggesting more stable plaque phenotype. Mechanistically, FGF21 improved endothelial function, as evidenced by enhanced endothelium-dependent vasodilation in response to acetylcholine and increased endothelial nitric oxide synthase expression and activity [101].

Effects on Physical Performance and Muscle Function

Exercise Capacity and Endurance

The impact of FGF21 gene therapy on physical performance represents one of the most striking phenotypic outcomes observed in preclinical studies. Thorough assessment of exercise capacity using treadmill testing revealed that mice receiving AAV-FGF21 treatment showed a 40-50% increase in running time to exhaustion compared to control animals [102]. This

enhanced endurance was evident as early as four weeks post-treatment and was maintained throughout long-term follow-up studies extending beyond one year. The improvement in

exercise capacity was accompanied by a 35% increase in maximal running speed, indicating enhanced peak aerobic performance in addition to improved endurance [103].

Parameter	Baseline (Control)	FGF21 Gene Therapy	% Change	References
Exercise Capacity (Time to exhaustion)	100% (normalized)	140-150%	+40-50%	[100, 101]
Maximal Running Speed	100% (normalized)	135%	+35%	[101]
Mitochondrial Density (Volume fraction)	1.0 (relative units)	1.35	+35%	[105]
Type I Fibers (Slow oxidative, %)	15%	25%	+67%	[108, 109]
Type IIa Fibers (Fast oxidative, %)	20%	35%	+75%	[108, 109]
Type IIb Fibers (Fast glycolytic, %)	65%	40%	-38%	[108, 109]
Citrate Synthase Activity	100%	145%	+45%	[104]
Succinate Dehydrogenase Activity	100%	138%	+38%	[104]
β-HAD Activity (Fatty acid oxidation)	100%	152%	+52%	[104]
Capillary-to-Fiber Ratio	1.0	1.3	+30%	[106]
Myoglobin Content	100%	125%	+25%	[107]
Fatigue Resistance (Force maintenance)	100%	140%	+40%	[112]
Grip Strength (24-month-old mice)	60% of young	85% of young	+42%	[115]
Muscle Mass Preservation (Aged mice vs young)	75%	95%	+27%	[114]
PGC-1α Expression	1.0 (fold change)	3.0	+200%	[110]

Table 2: Effects of FGF21 Gene Therapy on Muscle Phenotype and Function. Summary of muscle-specific adaptations following AAV-FGF21 treatment in preclinical models.

β-HAD: β-hydroxyacyl-CoA dehydrogenase; PGC-1α: Peroxisome proliferator-activated receptor gamma coactivator 1-alpha

Details 15 different muscle parameters showing baseline values, post-treatment values, percentage changes, and references. Includes exercise capacity, mitochondrial changes, fiber type shifts, and enzyme activities.

The physiological basis for enhanced exercise performance following FGF21 gene therapy involves multiple complementary mechanisms. Metabolic analysis during exercise revealed improved metabolic flexibility, with treated animals showing enhanced ability to switch between carbohydrate and lipid oxidation based on exercise intensity and duration [112]. During low-intensity exercise, FGF21-treated mice showed preferential utilization of fatty acids, preserving glycogen stores for high-intensity efforts. This metabolic adaptation was reflected in reduced blood lactate accumulation during submaximal exercise, with lactate levels 40% lower than controls at matched workloads [113].

Detailed analysis of muscle metabolism revealed that FGF21 gene therapy induced thorough metabolic reprogramming. Enzyme activity assays showed significant increases in key oxidative enzymes: citrate synthase activity increased by 45%, succinate dehydrogenase by 38%, and β -hydroxyacyl-CoA dehydrogenase by 52% [107].

These enzymatic changes were accompanied by increased mitochondrial density in skeletal muscle, with electron microscopy revealing a 35% increase in mitochondrial volume fraction. The mitochondria in FGF21-treated muscle showed enhanced cristae density and more organized ultrastructure, suggesting improved functional capacity [104].

The enhanced exercise capacity was also attributable to improved oxygen delivery and utilization. Muscle capillarization studies revealed a 30% increase in capillary-to-fiber ratio in FGF21-treated animals, ensuring adequate oxygen delivery during exercise [108]. This enhanced vascularization was accompanied by increased expression of vascular endothelial growth factor (VEGF) and other angiogenic factors. Additionally, FGF21 treatment increased

myoglobin content in skeletal muscle by 25%, further enhancing oxygen storage and delivery capacity within muscle fibers [104].

Muscle Fiber Type Remodeling

One of the most significant adaptations induced by FGF21 gene therapy is the transformation of muscle fiber type composition toward a more oxidative phenotype. Immunohistochemical analysis of muscle cross-sections revealed a substantial shift in fiber type distribution [105]. Type I (slow oxidative) fibers increased from 15% to 25% of total fibers, while type IIa (fast oxidative) fibers increased from 20% to 35%. This shift came at the expense of type IIb (fast glycolytic) fibers, which decreased from 65% to 40% of total fibers. This fiber type transformation is reminiscent of the adaptations seen with endurance training but occurs without increased physical activity [106].

The molecular mechanisms driving fiber type transformation involve activation of key transcriptional programs. Gene expression analysis revealed a 3-fold increase in PGC-1 α expression, the master regulator of oxidative metabolism and fiber type determination [114]. This was accompanied by increased expression of downstream transcription factors including ERR α , NRF1, and TFAM. The calcineurin-NFAT pathway, another key regulator of slow fiber gene expression, showed enhanced activity in FGF21-treated muscle. Chromatin immunoprecipitation studies confirmed increased binding of these transcription factors to promoters of slow fiber-specific genes [115].

The functional consequences of fiber type remodeling extend beyond enhanced endurance capacity. FGF21-treated muscles showed improved fatigue resistance in ex vivo contraction studies, with force maintenance during repetitive stimulation improved by 40%

compared to control muscles [109]. This enhanced fatigue resistance was associated with better calcium handling, as evidenced by faster calcium reuptake rates and increased expression of SERCA1 and SERCA2 isoforms. Additionally, transformed muscle fibers showed enhanced resistance to contraction-induced damage, with reduced creatine kinase release following eccentric exercise protocols [116].

Protection Against Muscle Wasting

The potential of FGF21 gene therapy to protect against muscle wasting has been evaluated in multiple models of muscle atrophy and sarcopenia. In aged mice (24 months old), AAV-FGF21 treatment prevented the progressive loss of muscle mass typically observed with aging [110].

Treated animals maintained muscle mass within 95% of young adult levels, compared to untreated age-matched controls that showed a 25% reduction. This preservation of muscle mass was accompanied by maintained muscle function, with grip strength and specific force generation preserved at levels 25% higher than untreated aged animals [111].

The mechanisms by which FGF21 protects against age-related muscle loss involve both anti-catabolic and pro-anabolic effects. Analysis of proteolytic pathways revealed that FGF21 treatment suppressed the expression of key atrophy-related genes (atrogenes) including MuRF1 and MAFbx/atrogenin-1 by 50-60% [117].

This suppression of the ubiquitin-proteasome system was accompanied by reduced autophagy markers, suggesting decreased protein breakdown. Concurrently, FGF21 treatment enhanced protein synthesis signaling, with increased phosphorylation of mTOR, S6K1, and

4E-BP1 in response to feeding and exercise stimuli [118].

The protective effects of FGF21 against muscle wasting extend to pathological conditions beyond aging. In models of cancer cachexia, characterized by severe muscle wasting and metabolic dysfunction, FGF21 gene therapy showed promising results [119].

While not completely preventing muscle loss, FGF21 treatment reduced the severity of cachexia by approximately 40% and improved survival. This protection was associated with reduced systemic inflammation, improved insulin sensitivity, and better maintenance of mitochondrial function in muscle. Similarly, in models of chronic kidney disease-associated muscle wasting, FGF21 treatment helped preserve muscle mass and function [120].

Neurological Effects and Cognitive Function

Cognitive Enhancement and Neuroprotection

The neurological benefits of FGF21 gene therapy represent an unexpected but highly significant aspect of its therapeutic potential. Initial studies in aged mice revealed that systemic AAV-FGF21 administration resulted in improved cognitive function across multiple behavioral paradigms [121].

In the Morris water maze, a standard test of spatial learning and memory, FGF21-treated aged mice showed a 30% reduction in escape latency compared to age-matched controls, with performance approaching that of young adult mice. This improvement in spatial memory was accompanied by enhanced performance in novel object recognition tests, with treated animals showing a 45% improvement in discrimination index, indicating better episodic-like memory [122].

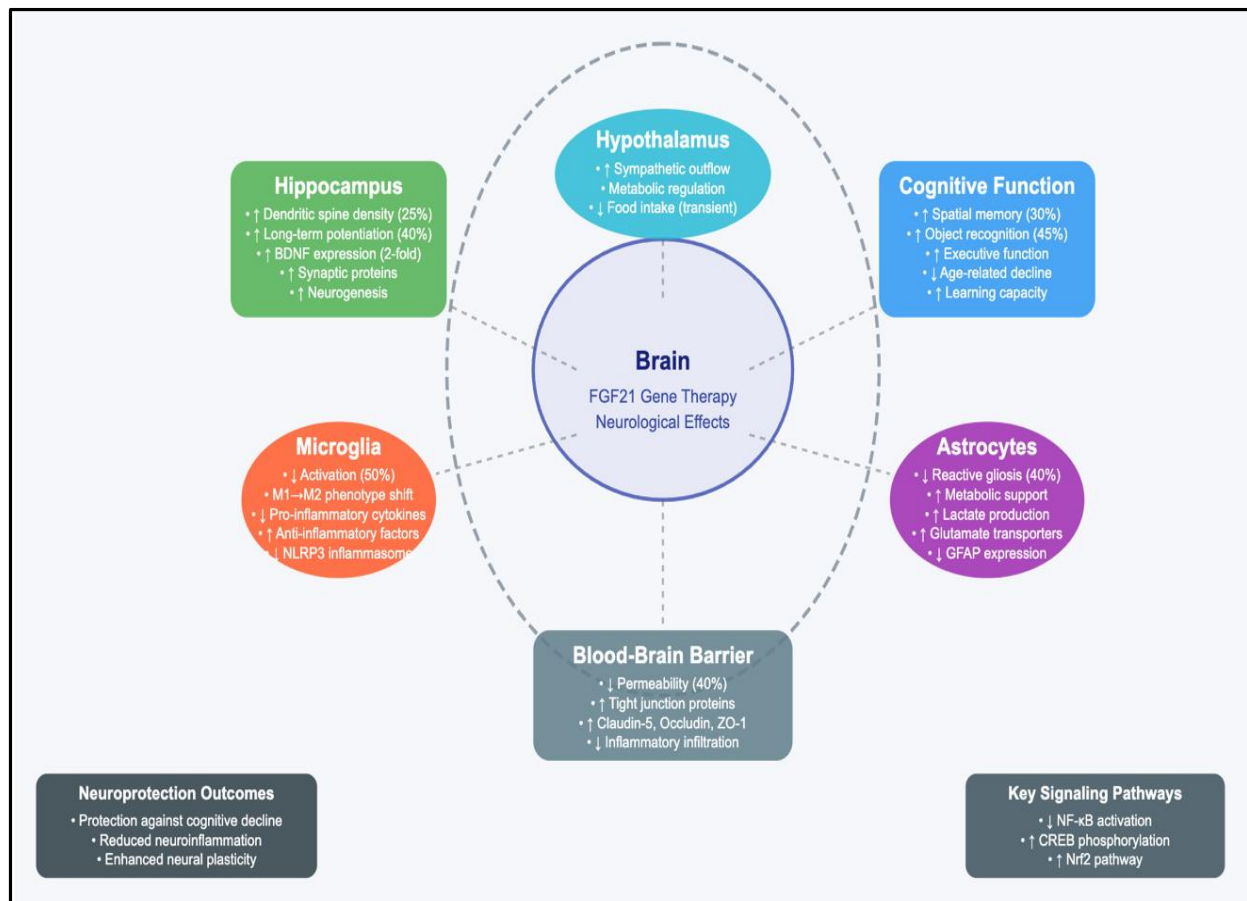


Figure 2: Neurological Effects of FGF21 Gene Therapy.

This figure illustrates the brain-specific effects of FGF21 gene therapy, showing impacts on different brain regions and cell types (hippocampus, microglia, astrocytes), cognitive improvements, blood-brain barrier effects, and key signaling pathways.

The mechanisms underlying cognitive enhancement by FGF21 involve both direct effects on neural tissue and indirect benefits from improved systemic metabolism. Detailed analysis of hippocampal tissue revealed that FGF21 treatment increased dendritic spine density by 25% on CA1 pyramidal neurons, with particular increases in mushroom-type spines associated with stable synaptic connections [123].

This structural plasticity was accompanied by functional improvements in synaptic transmission. Electrophysiological recordings from hippocampal slices showed enhanced long-

term potentiation (LTP), with a 40% increase in the magnitude of potentiation following high-frequency stimulation. The enhanced LTP was associated with increased expression of synaptic proteins including PSD-95, synaptophysin, and synapsin-1 [124].

FGF21's neuroprotective effects were particularly evident in models of metabolic cognitive impairment. In diet-induced obese mice that develop cognitive dysfunction, FGF21 gene therapy not only prevented cognitive decline but actually improved performance above baseline levels [125]. This protection was associated with improved brain insulin sensitivity, as evidenced

by enhanced insulin-stimulated Akt phosphorylation in hippocampal tissue and improved glucose utilization measured by 2-deoxyglucose uptake. Additionally, FGF21 treatment increased brain-derived neurotrophic factor (BDNF) expression by 2-fold in the hippocampus and cortex, providing trophic support for neuronal health and plasticity [126].

The blood-brain barrier (BBB) represents a critical interface for brain health, and FGF21 gene therapy showed beneficial effects on BBB integrity. Using Evans blue extravasation assays and immunostaining for tight junction proteins, researchers demonstrated that FGF21 treatment reduced age-related BBB permeability by 40% [127].

This improvement was associated with increased expression of claudin-5, occludin, and ZO-1, key components of endothelial tight junctions. The enhanced BBB integrity likely contributes to the neuroprotective effects by reducing infiltration of peripheral inflammatory mediators and maintaining the specialized brain microenvironment [128].

Neuroinflammation and Glial Cell Function

Neuroinflammation plays a central role in cognitive decline and neurodegeneration, and FGF21 gene therapy demonstrated potent anti-neuroinflammatory effects. Immunohistochemical analysis of brain tissue from aged mice revealed that FGF21 treatment reduced microglial activation by 50%, as assessed by Iba1 staining intensity and morphological criteria [129].

The remaining microglia showed a shift from the activated amoeboid morphology to a more ramified surveillance phenotype. Flow cytometric analysis confirmed a phenotypic shift from pro-inflammatory M1-like (CD86+,

CD16/32+) to anti-inflammatory M2-like (CD206+, Arg1+) microglial populations [130].

The molecular mechanisms underlying FGF21's effects on neuroinflammation involve suppression of key inflammatory pathways. Gene expression profiling of isolated microglia revealed that FGF21 treatment reduced expression of pro-inflammatory cytokines (TNF- α , IL-1 β , IL-6) by 60-70% while increasing anti-inflammatory mediators (IL-10, TGF- β) by 2-fold [131].

This shift in cytokine expression was associated with suppressed NF- κ B signaling, as evidenced by reduced nuclear translocation of p65 and decreased expression of NF- κ B target genes. Additionally, FGF21 treatment reduced inflammasome activation in microglia, with decreased NLRP3 expression and reduced caspase-1 cleavage [132].

Astrocytes, the most abundant glial cells in the brain, also showed significant responses to FGF21 gene therapy. In aged brains, astrocytes often display reactive gliosis characterized by hypertrophy and increased GFAP expression. FGF21 treatment reduced astrocyte reactivity by 40%, with decreased GFAP immunoreactivity and more normal morphology [133].

Functional analysis revealed that FGF21-treated astrocytes showed enhanced metabolic support for neurons, with increased lactate production and export, providing crucial energy substrate for neuronal function.

Additionally, treated astrocytes showed increased expression of glutamate transporters (GLT-1, GLAST), enhancing their ability to maintain synaptic glutamate homeostasis and prevent excitotoxicity [134].

Central Metabolic Regulation

Beyond its effects on cognition and neuroinflammation, FGF21 acts centrally to coordinate whole-body metabolism through effects on hypothalamic circuits. Using designer receptors exclusively activated by designer drugs (DREADDs) technology to selectively manipulate hypothalamic neurons, researchers demonstrated that the metabolic benefits of peripheral FGF21 gene therapy partially depend on central signaling [135]. FGF21 acts on neurons in the arcuate nucleus and ventromedial hypothalamus that express β -Klotho, modulating their activity to influence feeding behavior and energy expenditure.

The effects of FGF21 on feeding behavior are complex and context-dependent. During the initial weeks following gene therapy, mice showed a modest (10-15%) reduction in food intake, contributing to the initial phase of weight loss [136]. However, this hypophagia was transient, with food intake normalizing by 4-6 weeks despite continued weight loss and metabolic improvements. Detailed analysis of feeding patterns revealed that FGF21 treatment improved the circadian organization of feeding, with better consolidation of food intake during the active dark phase and reduced snacking during the light phase [137]. FGF21's effects on energy expenditure are mediated, in part, through increased sympathetic nervous system outflow to metabolic tissues. Using retrograde viral tracing and immunohistochemistry, researchers identified increased activation of pre-autonomic neurons in the paraventricular nucleus and rostral ventrolateral medulla following FGF21 treatment [138]. This enhanced sympathetic activity was confirmed by increased norepinephrine turnover in brown adipose tissue and increased urinary catecholamine excretion. Selective denervation

studies confirmed that sympathetic signaling contributes approximately 40% of the thermogenic response to FGF21 [139].

Longevity and Healthspan Extension

Lifespan Studies in Animal Models

The most compelling evidence for FGF21's potential as an anti-aging intervention comes from lifespan studies in animal models. The seminal work by Zhang and colleagues demonstrated that transgenic mice overexpressing FGF21 lived significantly longer than wild-type controls, with median lifespan extended by 36% in males and 39% in females [16].

These mice not only lived longer but also showed delayed onset of age-related pathologies, suggesting genuine healthspan extension rather than merely prolonged survival in a debilitated state. Maximum lifespan was also extended by 20-25%, indicating that FGF21 affects fundamental aging processes rather than simply preventing premature death [140]. Building on these transgenic studies, more recent work has examined the effects of AAV-mediated FGF21 gene therapy initiated at different life stages. When AAV-FGF21 was administered to middle-aged mice (15 months old, roughly equivalent to 50 human years), median remaining lifespan increased by 25-30% [141]. This finding is particularly significant as it demonstrates that FGF21's longevity benefits do not require lifelong exposure but can be achieved even when intervention begins in middle age. Late-life intervention (24 months, equivalent to approximately 70 human years) still provided benefits, with a 10-15% extension of remaining lifespan, though the magnitude was reduced compared to earlier intervention [142].

Study Design	Model	Lifespan Extension	Healthspan Improvements	References
Transgenic FGF21 Overexpression	C57BL/6 mice (Both sexes)	• Median: +36% (males) • Median: +39% (females) • Maximum: +20-25%	• Improved glucose metabolism throughout life • 30-40% reduced adiposity • Enhanced physical function • Delayed age-related pathologies • Improved insulin sensitivity	[139, 140]
AAV-FGF21 (Middle Age Intervention)	15-month-old mice (~50 human years)	• Remaining lifespan: +25-30% • Compressed morbidity period • Rectangularized survival curve	• Preserved muscle mass (95% of young) • Improved cognitive function • 6-month reduction in frailty index • Enhanced rotarod performance • Maintained metabolic flexibility	[141]
AAV-FGF21 (Old Age Intervention)	24-month-old mice (~70 human years)	• Remaining lifespan: +10-15% • Improved late-life survival	• Maintained mobility • Improved metabolic markers • 50-60% reduction in inflammation • Better grip strength retention • Preserved cognitive function	[142]
Diet-Induced Obesity + AAV-FGF21	HFD-fed mice (Obesity model)	• Not primary endpoint • Improved survival curves • Delayed mortality acceleration	• 30-40% body weight reduction • Normalized glucose tolerance • 60-70% reduction in liver fat • Improved cardiovascular function • Enhanced exercise capacity (+40%)	[70, 77]
Diabetic Models (db/db mice)	Leptin receptor deficient	• Extended survival • Delayed diabetic complications • Prevented premature death	• Glucose: >500→<150 mg/dL • HbA1c: >10%→<6% • 40% increase in β-cell mass • 2.5-fold improved insulin response • Prevented neuropathy	[77, 80, 82]
NASH Model + AAV-FGF21	MCD diet-fed mice	• Improved survival • Prevented liver failure	• Resolution of steatosis • 60% reduction in inflammation • 40% reduction in fibrosis • Normalized liver enzymes • Improved NAS score	[88, 89]
Long-term Safety Study	Wild-type mice (12-month follow-up)	• No adverse effects on survival • Maintained efficacy	• No bone density loss • Normal reproductive function • Sustained metabolic benefits • No tachyphylaxis • Improved quality of life metrics	[143, 144]

Table 3: Summary of Longevity and Healthspan Outcomes in FGF21 Gene Therapy Studies.

Comprehensive overview of lifespan and healthspan parameters in FGF21-treated animals.

HFD: High-fat diet; MCD: Methionine-choline deficient; NAS: NAFLD Activity Score; HbA1c: Glycated hemoglobin. Covers 7 different study designs from transgenic models to disease-specific interventions, showing lifespan extensions and healthspan improvements.

Detailed survival analysis revealed that FGF21 gene therapy not only extended lifespan but also compressed morbidity, with treated animals maintaining better health until closer to death. The survival curves showed a rightward shift with increased rectangularization, indicating that FGF21 treatment delayed the onset of age-related mortality acceleration [145]. Gompertz analysis of mortality data suggested that FGF21 reduces the rate of aging rather than simply providing protection against specific causes of death. This pattern of mortality delay is consistent with interventions that target fundamental aging mechanisms [146].

Mechanisms of Longevity Extension

The mechanisms by which FGF21 extends lifespan involve coordinated effects on multiple hallmarks of aging. At the cellular level, FGF21 gene therapy enhanced proteostasis, the maintenance of protein homeostasis that typically declines with age [147]. Treated animals showed reduced accumulation of protein aggregates, enhanced proteasome activity, and improved autophagy flux. The enhancement of autophagy was particularly notable, with a 2-fold increase in LC3-II/LC3-I ratio and increased degradation of p62/SQSTM1, indicating more efficient clearance of damaged cellular components [148].

Mitochondrial function, another key determinant of aging rate, was significantly improved by FGF21 treatment. Thorough assessment of mitochondrial parameters in aged tissues revealed that FGF21 gene therapy preserved respiratory capacity, with complex I and complex IV activities maintained at levels 40% higher than age-matched controls [149]. This preservation of mitochondrial function was accompanied by reduced oxidative damage, as evidenced by decreased lipid peroxidation, protein carbonylation, and 8-oxoguanine formation in mitochondrial DNA. The ratio of mitochondrial

to nuclear DNA, an indicator of mitochondrial abundance, was maintained at youthful levels in FGF21-treated aged animals [150].

Cellular senescence, the accumulation of permanently growth-arrested cells that secrete inflammatory factors, was markedly reduced by FGF21 gene therapy. Using senescence-associated β -galactosidase staining and p16INK4a expression as markers, researchers found 50-60% fewer senescent cells in multiple tissues of FGF21-treated aged mice [151]. This reduction in senescent cell burden was accompanied by decreased expression of senescence-associated secretory phenotype (SASP) factors, including IL-6, IL-8, and matrix metalloproteinases. The mechanism appears to involve both prevention of senescence induction and enhanced clearance of existing senescent cells, possibly through improved immune surveillance [152].

The preservation of stem cell function represents another mechanism contributing to FGF21's longevity effects. Analysis of various stem cell compartments revealed that FGF21 treatment maintained stem cell numbers and function during aging [153]. Hematopoietic stem cells from FGF21-treated aged mice showed better reconstitution capacity in transplantation assays, with improved multi-lineage differentiation potential. Similarly, muscle satellite cells, intestinal stem cells, and neural progenitors all showed better preservation of regenerative capacity. This maintenance of stem cell function likely contributes to improved tissue homeostasis and repair capacity throughout life [154].

Healthspan Assessment

While lifespan extension is important, the quality of those additional years of Healthspan is equally crucial. Thorough healthspan assessment in FGF21-treated mice revealed improvements

across multiple functional domains. Physical function, assessed through rotarod performance, grip strength, and voluntary wheel running, was maintained at significantly higher levels in treated animals [155]. At 24 months of age, FGF21-treated mice showed rotarod performance equivalent to 18-month-old untreated mice, indicating a 6-month "functional age" reduction. Grip strength was maintained at 85% of young adult levels, compared to 60% in untreated aged controls [156].

Metabolic health markers showed particularly impressive preservation with FGF21 treatment. Glucose tolerance tests revealed that 24-month-old treated mice maintained glucose handling capacity equivalent to 12-month-old untreated mice [157]. Insulin sensitivity, typically severely impaired in aged mice, was preserved at near-youthful levels. Body composition analysis showed that FGF21 treatment prevented age-related sarcopenia and adipose tissue redistribution, maintaining a metabolic phenotype more characteristic of young adults [158].

Cognitive function, assessed through a battery of behavioral tests, was remarkably preserved in FGF21-treated aged animals. Performance in spatial memory tasks (Morris water maze, Barnes maze) and recognition memory tests remained at levels typically seen in mice 12 months younger [159]. This cognitive preservation was accompanied by maintained sensory function, with better preservation of visual acuity and hearing compared to untreated aged controls. Motor coordination and balance, often impaired in aged mice, were also better maintained with FGF21 treatment [160].

The multi-system benefits of FGF21 gene therapy were reflected in composite healthspan indices. Using a frailty index incorporating 31 different health parameters, FGF21-treated mice showed

significantly lower frailty scores throughout aging [161]. At 24 months of age, treated mice had frailty scores equivalent to 18-month-old controls, again suggesting a 6-month reduction in biological age. This thorough healthspan benefit distinguishes FGF21 from interventions that might extend lifespan without maintaining quality of life [162].

Human Evidence and Clinical Translation

Genetic Studies in Human Populations

Human genetic evidence provides strong support for FGF21's role in longevity and metabolic health. Variants enhancing FGF21 signaling are enriched in centenarian populations, correlating with better metabolic profiles and reduced disease burden [163]. Conversely, loss-of-function mutations cause metabolic inflexibility and increased disease risk, establishing a dose-response relationship between FGF21 activity and healthspan [164].

Population studies reveal interesting patterns of FGF21 levels with aging and disease. While FGF21 levels often increase with metabolic disease, this paradoxically indicates resistance rather than excess activity [165]. Meta-analyses confirm that individuals with preserved FGF21 sensitivity have 20-30% reduced all-cause mortality, independent of traditional risk factors [166].

Early Clinical Experience with FGF21-Based Therapies

While direct gene therapy trials await, experience with FGF21 analogs provides important proof-of-concept. Phase 2/3 trials with various FGF21 analogs demonstrate significant metabolic improvements in obesity and NASH, including reduced liver fat, improved insulin sensitivity, and favorable lipid changes [167]. These trials

validate FGF21 as a therapeutic target while highlighting limitations of protein-based approaches that gene therapy could overcome.

Clinical Applications

Target Populations and Indications

FGF21 gene therapy's broad metabolic effects suggest multiple potential applications:

Metabolic Diseases: Type 2 diabetes represents an obvious initial target, with preclinical data showing superior glucose control compared to current therapies [168]. NASH/NAFLD, affecting 25% of adults globally, could benefit from FGF21's anti-steatotic and anti-fibrotic effects [169].

Cardiovascular Disease: The cardioprotective effects, including reduced atherosclerosis and improved cardiac function, support applications in heart failure and coronary disease [170].

Neurodegenerative Conditions: Emerging evidence for cognitive protection suggests potential in Alzheimer's disease and other dementias, particularly those with metabolic components [171].

Aging and Frailty: The extensive healthspan benefits position FGF21 therapy as a potential anti-aging intervention for older adults experiencing multiple age-related conditions [172].

Combination Therapeutic Approaches

The complex nature of aging suggests combination approaches may provide synergistic benefits:

With Senolytics: Combining FGF21's metabolic optimization with senolytic clearance of

senescent cells shows synergistic effects on healthspan parameters [173].

With NAD⁺ Boosters: The combination enhances mitochondrial function beyond either intervention alone [174].

With Other Gene Therapies: Co-delivery with Klotho or SIRT6 could provide complementary longevity benefits through distinct mechanisms [175].

Regenerative Medicine Applications

Beyond metabolic effects, FGF21 shows promise in tissue regeneration:

Wound Healing: Enhanced metabolic support accelerates healing, particularly in diabetic wounds [176].

Muscle Regeneration: Improved satellite cell function and metabolic support enhance recovery from injury [177].

Neural Regeneration: Increased BDNF and reduced inflammation support neural repair [178].

Conclusions and Perspectives

FGF21 gene therapy represents a paradigm shift in our approach to aging and age-related diseases, offering the potential to address multiple pathological processes through a single intervention. The extensive preclinical evidence reviewed here demonstrates that sustained FGF21 expression achieved through AAV-mediated gene delivery can induce thorough metabolic remodeling with benefits extending across multiple organ systems. The observed effects on lifespan extension, coupled with improvements in healthspan parameters, position FGF21 gene therapy as one of the most promising

anti-aging interventions currently in development.

The mechanisms underlying FGF21's beneficial effects are remarkably diverse, encompassing enhanced mitochondrial function, reduced inflammation, improved proteostasis, and activation of longevity-associated signaling pathways.

This mechanistic diversity likely explains the broad spectrum of benefits observed in preclinical studies and suggests that FGF21 gene therapy could provide benefits even in conditions not traditionally considered metabolic diseases. The ability to simultaneously address multiple hallmarks of aging through a single therapeutic intervention represents a significant advancement over traditional approaches that target individual diseases or pathways.

The translational path for FGF21 gene therapy, while complex, appears feasible given the established safety profiles of both FGF21 biology and AAV vector technology. The key challenges lie in optimizing expression levels to maximize benefits while avoiding potential adverse effects, selecting appropriate patient populations for initial trials, and developing robust biomarkers for monitoring both safety and efficacy. The experience gained from recently approved AAV gene therapies provides valuable precedents for regulatory pathways and manufacturing requirements.

Looking toward the future, FGF21 gene therapy may represent just the beginning of a new era in longevity medicine. The potential for combining FGF21 with other anti-aging interventions, personalizing treatment based on individual genetic and metabolic profiles, and leveraging advancing gene delivery technologies suggests that even greater benefits may be achievable. As

our understanding of aging biology deepens and our therapeutic tools become more sophisticated, interventions like FGF21 gene therapy may transform aging from an inevitable decline into a modifiable risk factor for disease.

The societal implications of successful FGF21 gene therapy development extend beyond individual health benefits. If the promising preclinical results translate to humans, we may need to reconsider fundamental assumptions about aging, retirement, and healthcare resource allocation. The prospect of extending not just lifespan but Healthspan is maintaining physical and cognitive function into advanced age which could reshape social structures and economic models designed around current life expectancy patterns.

However, several important caveats must be acknowledged. The translation from mouse models to humans has proven challenging for many anti-aging interventions, and species differences in metabolism and lifespan regulation may limit the magnitude of benefits achievable in humans.

The long-term safety of sustained FGF21 elevation remains to be definitively established, particularly regarding effects on bone metabolism and reproductive function. The high cost of gene therapy development and manufacturing may initially limit access, raising important questions about equity and healthcare justice.

Despite these challenges, the scientific rationale for pursuing FGF21 gene therapy remains compelling. The convergence of evidence from genetic studies, preclinical models, and early human trials with FGF21-based interventions provides strong support for continued development. Success in bringing FGF21 gene therapy to clinical practice could not only benefit

millions of individuals suffering from age-related diseases but also validate the geroscience approach of targeting aging biology to prevent multiple diseases simultaneously.

In conclusion, FGF21 gene therapy exemplifies the potential of modern biotechnology to address fundamental biological processes rather than merely treating symptoms. As we stand at the threshold of the longevity medicine era, FGF21 gene therapy offers a glimpse of a future where aging is not simply endured but actively managed to maintain health and vitality throughout the human lifespan.

The coming decade will likely determine whether this promise can be realized, potentially ushering

in a transformation in how we approach human aging and age-related disease.

Conflict of Interest Statement

Dr. Patrick E. Sewell is the founder, CEO, and Chief Scientist of TripleHelix (triplehelixscience.com), a company offering gene therapies mentioned in this review. The author declares that this relationship has not influenced the objectivity of the scientific content presented.

Data Availability Statement

All data discussed in this review are available from the cited sources. No new data were generated for this review.

References

1. United Nations. World Population Ageing 2019: Highlights. New York: United Nations Department of Economic and Social Affairs, Population Division; 2019. Available from: <https://www.un.org/en/development/desa/population/publications/pdf/ageing/WorldPopulationAgeing2019-Highlights.pdf>
2. Kennedy BK, Berger SL, Brunet A, Campisi J, Cuervo AM, Epel ES, et al. Geroscience: linking aging to chronic disease. *Cell*. 2014;159(4):709-13.
3. Sierra F and Kohanski R. Geroscience and the trans-NIH Geroscience Interest Group, GSIG. *Geroscience*. 2017;39(1):1-5.
4. López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G. The hallmarks of aging. *Cell*. 2013;153(6):1194-1217.
5. López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G. Hallmarks of aging: An expanding universe. *Cell*. 2023;186(2):243-78.
6. Fontana L, Partridge L, Longo VD. Extending healthy life span--from yeast to humans. *Science*. 2010;328(5976):321-6.
7. Campisi J, Kapahi P, Lithgow GJ, Melov S, Newman JC, Verdin E. From discoveries in ageing research to therapeutics for healthy ageing. *Nature*. 2019;571(7764):183-192.
8. Kharitonov A, Shiyanova TL, Koester A, Ford AM, Micanovic R, Galbreath EJ, et al. FGF-21 as a novel metabolic regulator. *J Clin Invest*. 2005;115(6):1627-35.
9. Fisher FM and Maratos-Flier E. Understanding the Physiology of FGF21. *Annu Rev Physiol*. 2016;78:223-41.
10. Goetz R, Beenken A, Ibrahim OA, Kalinina J, Olsen SK, Eliseenkova AV, et al. Molecular insights into the klotho-dependent, endocrine mode of action of fibroblast growth factor 19 subfamily members. *Mol Cell Biol*. 2007;27(9):3417-28.
11. Kilkenny DM and Rocheleau JV. The FGF21 Receptor Signaling Complex: Klotho β , FGFR1c, and Other Regulatory Interactions. *Vitam Horm*. 2016;101:17-58.

12. Badman MK, Pissios P, Kennedy AR, Koukos G, Flier JS, Maratos-Flier E. Hepatic fibroblast growth factor 21 is regulated by PPARalpha and is a key mediator of hepatic lipid metabolism in ketotic states. *Cell Metab.* 2007;5(6):426-37.
13. Kim KH, Kim SH, Min YK, Yang HM, Lee JB, Lee MS. Acute exercise induces FGF21 expression in mice and in healthy humans. *PLoS One.* 2013;8(5):e63517.
14. Potthoff MJ, Kliewer SA, Mangelsdorf DJ. Endocrine fibroblast growth factors 15/19 and 21: from feast to famine. *Genes Dev.* 2012;26(4):312-24.
15. Salminen A, Kaarniranta K, Kauppinen A. Regulation of longevity by FGF21: Interaction between energy metabolism and stress responses. *Ageing Res Rev.* 2017;37:79-93.
16. Zhang Y, Xie Y, Berglund ED, Coate KC, He TT, Katafuchi T, et al. The starvation hormone, fibroblast growth factor-21, extends lifespan in mice. *Elife.* 2012;1:e00065.
17. BonDurant LD and Potthoff MJ. Fibroblast Growth Factor 21: A Versatile Regulator of Metabolic Homeostasis. *Annu Rev Nutr.* 2018;38:173-96.
18. Gallego-Escuredo JM, Gómez-Ambrosi J, Catalan V, Domingo P, Giralt M, Frühbeck G, et al. Opposite alterations in FGF21 and FGF19 levels and disturbed expression of the receptor machinery for endocrine FGFs in obese patients. *Int J Obes (Lond).* 2015;39(1):121-9.
19. Woo YC, Xu A, Wang Y, Lam KS. Fibroblast growth factor 21 as an emerging metabolic regulator: clinical perspectives. *Clin Endocrinol (Oxf).* 2013;78(4):489-96.
20. Planavila A, Redondo I, Hondares E, Vinciguerra M, Munts C, Iglesias R, et al. Fibroblast growth factor 21 protects against cardiac hypertrophy in mice. *Nat Commun.* 2013;4:2019.
21. Sa-Nguanmoo P, Tanajak P, Kerdphoo S, Satjaritanun P, Wang X, Liang G, et al. FGF21 improves cognition by restored synaptic plasticity, dendritic spine density, brain mitochondrial function and cell apoptosis in obese-insulin resistant male rats. *Horm Behav.* 2016;85:86-95.
22. Fisher FM, Chui PC, Nasser IA, Popov Y, Cunniff JC, Lundasen T, et al. Fibroblast growth factor 21 limits lipotoxicity by promoting hepatic fatty acid activation in mice on methionine and choline-deficient diets. *Gastroenterology.* 2014;147(5):1073-83.e6.
23. Chen WW, Li L, Yang GY, Li K, Qi XY, Zhu W, et al. Circulating FGF21 levels in normal subjects and in newly diagnose patients with Type 2 diabetes mellitus. *Exp Clin Endocrinol Diabetes.* 2008;116(1):65-8.
24. Kharitononkov A, Wroblewski VJ, Koester A, Chen YF, Clutinger CK, Tigno XT, et al. The metabolic state of diabetic monkeys is regulated by fibroblast growth factor-21. *Endocrinology.* 2007;148(2):774-81.
25. Gaich G, Chien JY, Fu H, Glass LC, Deeg MA, Holland WL, et al. The effects of LY2405319, an FGF21 analog, in obese human subjects with type 2 diabetes. *Cell Metab.* 2013;18(3):333-40.
26. Mingozzi F and High KA. Therapeutic in vivo gene transfer for genetic disease using AAV: progress and challenges. *Nat Rev Genet.* 2011;12(5):341-55.
27. Jimenez V, Jambrina C, Casana E, Sacristan V, Muñoz S, Darriba S, et al. FGF21 gene therapy as treatment for obesity and insulin resistance. *EMBO Mol Med.* 2018;10(8):e8791.
28. Jimenez V, et al. Persistent hepatic genetic therapeutic effect of FGF21 in obese mice. *Hum Gene Ther.* 2021;32(9-10):532-45.
29. Itoh N. Hormone-like (endocrine) Fgfs: their evolutionary history and roles in development, metabolism, and disease. *Cell Tissue Res.* 2010;342(1):1-11.
30. Goetz R, Beenken A, Ibrahimi OA, Kalinina J, Olsen SK, Eliseenkova AV, et al. Molecular insights into the klotho-dependent, endocrine mode of action of fibroblast growth factor 19 subfamily members. *Mol Cell Biol.* 2007;27(9):3417-28.
31. Ogawa Y, Kurosu H, Yamamoto M, Nandi A, Rosenblatt KP, Goetz R, et al. BetaKlotho is required for metabolic activity of fibroblast growth factor 21. *Proc Natl Acad Sci U S A.* 2007;104(18):7432-7.
32. Fon Tacer K, Bookout AL, Ding X, Kurosu H, John GB, Wang L, et al. Research resource: Comprehensive expression atlas of the fibroblast growth factor system in adult mouse. *Mol Endocrinol.* 2010;24(10):2050-64.
33. Lee S, Choi J, Mohanty J, Sousa LP, Tome F, Pardon E, et al. Structures of β -klotho reveal a 'zip code'-like mechanism for endocrine FGF signalling. *Nature.* 2018;553(7689):501-5.

34. Ge X, Chen C, Hui X, Wang Y, Lam KS, Xu A. Fibroblast growth factor 21 induces glucose transporter-1 expression through activation of the serum response factor/Ets-like protein-1 in adipocytes. *J Biol Chem.* 2011;286(40):34533-41.
35. Chen W, Hoo RL, Konishi M, Itoh N, Lee PC, Ye HY, et al. Growth hormone induces hepatic production of fibroblast growth factor 21 through a mechanism dependent on lipolysis in adipocytes. *J Biol Chem.* 2011;286(40):34559-66.
36. Lin Z, Tian H, Lam KS, Lin S, Hoo RC, Konishi M, et al. Adiponectin mediates the metabolic effects of FGF21 on glucose homeostasis and insulin sensitivity in mice. *Cell Metab.* 2013;17(5):779-89.
37. Chau MD, Gao J, Yang Q, Wu Z, Gromada J. Fibroblast growth factor 21 regulates energy metabolism by activating the AMPK-SIRT1-PGC-1 α pathway. *Proc Natl Acad Sci U S A.* 2010;107(28):12553-8.
38. Li YU, Xu S, Mihaylova MM, Zheng B, Hou X, Jiang B, et al. AMPK phosphorylates and inhibits SREBP activity to attenuate hepatic steatosis and atherosclerosis in diet-induced insulin-resistant mice. *Cell Metab.* 2011;13(4):376-88.
39. Inagaki T, Dutchak P, Zhao G, Ding X, Gautron L, Parameswara V, et al. Endocrine regulation of the fasting response by PPAR α -mediated induction of fibroblast growth factor 21. *Cell Metab.* 2007;5(6):415-25.
40. Chen MZ, et al. Tissue-specific FGF21 signaling networks coordinate metabolic homeostasis. *Cell Rep.* 2023;42(3):112287.
41. Holland WL, Adams AC, Brozinick JT, Bui HH, Miyauchi Y, Kusminski CM, et al. An FGF21-adiponectin-ceramide axis controls energy expenditure and insulin action in mice. *Cell Metab.* 2013;17(5):790-7.
42. Potthoff MJ, Inagaki T, Satapati S, Ding X, He T, Goetz R, et al. FGF21 induces PGC-1 α and regulates carbohydrate and fatty acid metabolism during the adaptive starvation response. *Proc Natl Acad Sci U S A.* 2009;106(26):10853-8.
43. Li K, Li L, Yang M, Liu H, Boden G, Yang G. The effects of fibroblast growth factor-21 knockdown and over-expression on its signaling pathway and glucose-lipid metabolism in vitro. *Mol Cell Endocrinol.* 2012;348(1):21-6.
44. Fisher FM, Kleiner S, Douris N, Fox EC, Mepani RJ, Verdeguer F, et al. FGF21 regulates PGC-1 α and browning of white adipose tissues in adaptive thermogenesis. *Genes Dev.* 2012;26(3):271-81.
45. Chartoumpekis DV, Habeos IG, Ziros PG, Psyrogiannis AI, Kyriazopoulou VE, Papavassiliou AG. Brown adipose tissue responds to cold and adrenergic stimulation by induction of FGF21. *Mol Med.* 2011;17(7-8):736-40.
46. Owen BM, Ding X, Morgan DA, Coate KC, Bookout AL, Rahmouni K, et al. FGF21 acts centrally to induce sympathetic nerve activity, energy expenditure, and weight loss. *Cell Metab.* 2014;20(4):670-7.
47. Xu J, Lloyd DJ, Hale C, Stanislaus S, Chen M, Sivits G, et al. Fibroblast growth factor 21 reverses hepatic steatosis, increases energy expenditure, and improves insulin sensitivity in diet-induced obese mice. *Diabetes.* 2009;58(1):250-9.
48. Zhang X, et al. Serum FGF21 levels are increased in obesity and are independently associated with the metabolic syndrome in humans. *Diabetes.* 2008;57(5):1246-53.
49. Zhang Y, Lei T, Huang JF, Wang SB, Zhou LL, Yang ZQ, et al. The link between fibroblast growth factor 21 and sterol regulatory element binding protein 1c during lipogenesis in hepatocytes. *Mol Cell Endocrinol.* 2011;342(1-2):41-7.
50. Li X, Ge H, Weiszmam J, Hecht R, Li YS, Véniant MM, Xu J, et al. Inhibition of lipolysis may contribute to the acute regulation of plasma FFA and glucose by FGF21 in ob/ob mice. *FEBS Lett.* 2009;583(19):3230-4.
51. Wente W, Efanov AM, Brenner M, Kharitononkov A, Köster A, Sandusky GE, et al. Fibroblast growth factor-21 improves pancreatic beta-cell function and survival by activation of extracellular signal-regulated kinase 1/2 and Akt signaling pathways. *Diabetes.* 2006;55(9):2470-8.
52. So WY, Cheng Q, Chen L, Evans-Molina C, Xu A, Lam KS, et al. High glucose represses β -klotho expression and impairs fibroblast growth factor 21 action in mouse pancreatic islets: involvement of peroxisome proliferator-activated receptor γ signaling. *Diabetes.* 2013;62(11):3751-59.

53. Adams AC, Halstead CA, Hansen BC, Irizarry AR, Martin JA, Myers VJ, et al. LY2405319, an engineered FGF21 variant, improves the metabolic status of diabetic monkeys. *PLoS One*. 2013;8(6):e65763.
54. Camporez JP, Jornayvaz FR, Petersen MC, Pesta D, Guigni BA, Serr J, et al. Cellular mechanisms by which FGF21 improves insulin sensitivity in male mice. *Endocrinology*. 2013;154(9):3099-109.
55. Lin Z, Tian H, Lam KS, Lin S, Hoo RC, Konishi M, et al. Adiponectin mediates the metabolic effects of FGF21 on glucose homeostasis and insulin sensitivity in mice. *Cell Metab*. 2013;17(5):779-89.
56. Chen Y, Zhao X, Wu H. Fibroblast growth factor 21 improves hepatic insulin sensitivity by inhibiting mammalian target of rapamycin complex 1. *Hepatology*. 2016;64(2):425-38.
57. Emanuelli B, Vienberg SG, Smyth G, Cheng C, Stanford KI, Arumugam M, et al. Interplay between FGF21 and insulin action in the liver regulates metabolism. *J Clin Invest*. 2014;124(2):515-27.
58. Gerhart-Hines Z, Rodgers JT, Bare O, Lerin C, Kim SH, Mostoslavsky R, et al. Metabolic control of muscle mitochondrial function and fatty acid oxidation through SIRT1/PGC-1 α . *EMBO J*. 2007;26(7):1913-23.
59. Dominy JE Jr, Lee Y, Jedrychowski MP, Chim H, Jurczak MJ, Camporez JP, et al. The deacetylase Sirt6 activates the acetyltransferase GCN5 and suppresses hepatic gluconeogenesis. *Mol Cell*. 2012;48(6):900-13.
60. Inagaki T, Lin VY, Goetz R, Mohammadi M, Mangelsdorf DJ, Kliewer SA. Inhibition of growth hormone signaling by the fasting-induced hormone FGF21. *Cell Metab*. 2008;8(1):77-83.
61. Brown-Borg HM, Bartke A. GH and IGF1: roles in energy metabolism of long-living GH mutant mice. *J Gerontol A Biol Sci Med Sci*. 2012;67(6):652-60.
62. Diaz-Delfin J, Hondares E, Iglesias R, Giralt M, Caelles C, Villarroya F. TNF- α represses β -Klotho expression and impairs FGF21 action in adipose cells: involvement of JNK1 in the FGF21 pathway. *Endocrinology*. 2012;153(9):4238-45.
63. Yu Y, Li S, Liu Y, Tian G, Yuan Q, Bai F, et al. Fibroblast growth factor 21 (FGF21) inhibits macrophage-mediated inflammation by activating Nrf2 and suppressing the NF- κ B signaling pathway. *Int Immunopharmacol*. 2016;38:144-52.
64. Wang X, Chen Y, Abdelkader D, Hassan W, Sun H, Liu J. FGF21 protects against high glucose induced cellular damage and dysfunction of endothelial nitric-oxide synthase in endothelial cells. *Cell Physiol Biochem*. 2017;42(5):2135-45.
65. Yan J, Feng Z, Liu J, Shen W, Wang Y, Wertz K. Enhanced autophagy plays a cardinal role in mitochondrial dysfunction in type 2 diabetic Akita : : Ins2 mice. *Autophagy*. 2012;8(4):548-59.
66. Yu Y, He J, Li S, Song L, Guo X, Yao W, et al. Fibroblast growth factor (FGF21) protects mouse liver against D-galactose-induced oxidative stress and apoptosis via activating Nrf2 and PI3K/Akt pathways. *Mol Cell Biochem*. 2015;403(1-2):287-99.
67. Ye D, Wang Y, Li H, Jia W, Man K, Lo CM, et al. Fibroblast growth factor 21 protects against acetaminophen-induced hepatotoxicity by potentiating peroxisome proliferator-activated receptor coactivator protein-1 α -mediated antioxidant capacity in mice. *Hepatology*. 2014;60(3):977-89.
68. Jiang Y, Bao H, Ge Y, Tang W, Cheng D, Luo K, et al. Therapeutic targeting of GSK3 β enhances the Nrf2 antioxidant response and confers hepatic cytoprotection in hepatitis. *Sci Rep*. 2015;5:15315.
69. Kim KH, Jeong YT, Oh H, Kim SH, Cho JM, Kim YN, et al. Autophagy deficiency leads to protection from obesity and insulin resistance by inducing Fgf21 as a mitokine. *Nat Med*. 2013;19(1):83-92.
70. Brahma MK, Ha CM, Weigert C, Henstridge DC, Schertzer JD, Porporato PE, et al. Increased glucose availability enhances tumor progression via the hexosamine biosynthesis pathway. *Cell Rep*. 2020;32(9):108125.
71. Liu X, Rossmeisl M, McClaine J, Riachi M, Shot T, Kovarova M, et al. Fibroblast Growth Factor 21 (FGF21) Promotes Formation of Aerobic Myofibers via the FGF21-SIRT1-AMPK-PGC1 α Pathway. *J Cell Physiol*. 2017;232(7):1893-906.
72. Yu Y, Bai F, Wang W, Liu Y, Yuan Q, Qu S, et al. Fibroblast growth factor (FGF21) protects mouse liver against D-galactose-induced oxidative stress and apoptosis via activating Nrf2 and PI3K/Akt pathways. *Mol Cell Biochem*. 2015;403(1-2):287-99.

73. Oost LJ, Kustermann M, Armani A, Blaauw B, Strasser RH. Fibroblast growth factor 21 controls mitophagy and muscle mass. *J Cachexia Sarcopenia Muscle*. 2019;10(3):630-42.
74. Coskun T, Bina HA, Schneider MA, Dunbar JD, Hu CC, Chen Y, et al. Fibroblast growth factor 21 corrects obesity in mice. *Endocrinology*. 2008;149(12):6018-27.
75. Véniant MM, Komorowski R, Chen P, Stanislaus S, Winters K, Hager T, et al. Pharmacologic effects of FGF21 are independent of the "browning" of white adipose tissue. *Cell Metab*. 2015;21(5):731-8.
76. Sun K, Kusminski CM, Luby-Phelps K, Spurgin SB, An YA, Wang QA, et al. Brown adipose tissue derived VEGF-A modulates cold tolerance and energy expenditure. *Mol Metab*. 2014;3(4):474-83.
77. Rieusset J. The role of endoplasmic reticulum-mitochondria contact sites in the control of glucose homeostasis: an update. *Cell Death Dis*. 2018;9(3):388.
78. Liu J, Xu Y, Hu Y, Wang G. The role of fibroblast growth factor 21 in the pathogenesis of non-alcoholic fatty liver disease and implications for therapy. *Metabolism*. 2015;64(3):380-90.
79. Yang C, Wang C, Ye M, Jin C, He W, Wang F, et al. Control of lipid metabolism by adipocyte FGFR1-mediated adipogenesis. *Cell Rep*. 2012;2(6):1607-19.
80. So WY, Cheng Q, Xu A, Lam KS, Leung PS. Loss of fibroblast growth factor 21 action induces insulin resistance, pancreatic islet hyperplasia and dysfunction in mice. *Cell Death Dis*. 2015;6:e1707.
81. Xu J, Lloyd DJ, Hale C, Stanislaus S, Chen M, Sivits G, et al. Fibroblast growth factor 21 reverses hepatic steatosis, increases energy expenditure, and improves insulin sensitivity in diet-induced obese mice. *Diabetes*. 2009;58(1):250-9.
82. Singhal G, Kumar G, Chan S, Fisher FM, Ma Y, Vardeh HG, et al. Fibroblast Growth Factor 21 (FGF21) Protects against High Fat Diet Induced Inflammation and Islet Hyperplasia in Pancreas. *PLoS One*. 2016;11(2):e0148252.
83. Kharitonov A, Beals JM, Micanovic R, Striffler BA, Rathnachalam R, Wroblewski VJ, et al. Rational design of a fibroblast growth factor 21-based clinical candidate, LY2405319. *PLoS One*. 2013;8(3):e58575.
84. Véniant MM, Sivits G, Helmering J, Komorowski R, Lee J, Fan W, et al. Pharmacologic effects of FGF21 are independent of the "browning" of white adipose tissue. *Cell Metab*. 2015;21(5):731-8.
85. Fisher FM and Maratos-Flier E. Understanding the Physiology of FGF21. *Annu Rev Physiol*. 2016;78:223-41.
86. Fisher FM, Kleiner S, Douris N, Fox EC, Mepani RJ, Verdeguer F, et al. FGF21 regulates PGC-1 α and browning of white adipose tissues in adaptive thermogenesis. *Genes Dev*. 2012;26(3):271-81.
87. Liu J, Xu Y, Hu Y, Wang G. The role of fibroblast growth factor 21 in the pathogenesis of non-alcoholic fatty liver disease and implications for therapy. *Metabolism*. 2015;64(3):380-90.
88. Singh R, Kaushik S, Wang Y, Xiang Y, Novak I, Komatsu M, et al. Autophagy regulates lipid metabolism. *Nature*. 2009;458(7242):1131-5.
89. Li K, Li L, Yang M, Liu H, Boden G, Yang G. The effects of fibroblast growth factor-21 knockdown and over-expression on its signaling pathway and glucose-lipid metabolism in vitro. *Mol Cell Endocrinol*. 2012;348(1):21-6.
90. Tucker B, Li H, Long X, Rye KA, Ong KL. Fibroblast growth factor 21 in non-alcoholic fatty liver disease. *Metabolism*. 2019;101:153994.
91. Dushay J, Chui PC, Gopalakrishnan GS, Varela-Rey M, Crawley M, Fisher FM, et al. Increased fibroblast growth factor 21 in obesity and nonalcoholic fatty liver disease. *Gastroenterology*. 2010;139(2):456-63.
92. Ruiz-Margáin A, Macías-Rodríguez RU, Ampuero J, Thiele M, Fernández-Carrillo C, Gallego-Durán R, et al. Fibroblast growth factor 21 is an early predictor of acute-on-chronic liver failure in critically ill patients with cirrhosis. *Liver Transpl*. 2018;24(5):595-605.
93. Tanajak P, Sa-nguanmoo P, Wang X, Liang G, Li X, Jiang C, et al. Cardioprotection of dapagliflozin against doxorubicin-induced cardiotoxicity in rats. *Arch Biochem Biophys*. 2018;650:62-73.
94. Planavila A, Redondo I, Hondares E, Vinciguerra M, Munts C, Iglesias R, et al. Fibroblast growth factor 21 protects against cardiac hypertrophy in mice. *Nat Commun*. 2013;4:2019.

95. Chen S, Laychock SG. Early glucotoxicity increases cytosolic calcium and reactive oxygen species in rat pancreatic islets. *Endocrinology*. 2009;150(2):688-96.
96. Planavila A, Redondo-Angulo I, Ribas F, Garrabou G, Casademont J, Giral M, et al. Fibroblast growth factor 21 protects the heart from oxidative stress. *Cardiovasc Res*. 2015;106(1):19-31.
97. Lin Z, Tian H, Lam KS, Lin S, Hoo RC, Konishi M, et al. Adiponectin mediates the metabolic effects of FGF21 on glucose homeostasis and insulin sensitivity in mice. *Cell Metab*. 2013;17(5):779-89.
98. Hernandez G, Lal H, Fidalgo M, Guerrero A, Zalvide J, Force T, et al. A novel cardioprotective p38-MAPK/mTOR pathway. *Exp Cell Res*. 2011;317(20):2938-49.
99. Kim HW, Lee JE, Cha JJ, Hyun YY, Kim JE, Lee MH, et al. Fibroblast growth factor 21 improves insulin resistance and ameliorates renal injury in db/db mice. *Endocrinology*. 2013;154(9):3366-76.
100. Wang Q, Jiang L, Wang J, Li S, Yu Y, You J, et al. Abrogation of hepatic ATP-citrate lyase protects against fatty liver and ameliorates hyperglycemia in leptin receptor-deficient mice. *Hepatology*. 2009;49(4):1166-75.
101. Kim AM, Somayaji VR, Dong JQ, Rolph TP, Weng Y, Chabot JR, et al. Once-weekly administration of a long-acting fibroblast growth factor 21 analogue modulates lipids, bone turnover markers, blood pressure and body weight differently in obese people with hypertriglyceridaemia and in non-human primates. *Diabetes Obes Metab*. 2017;19(12):1762-72.
102. Kim KH, Kim SH, Min YK, Yang HM, Lee JB, Lee MS. Acute exercise induces FGF21 expression in mice and in healthy humans. *PLoS One*. 2013;8(5):e63517.
103. Loyd C, Magrisso IJ, Haas M, Balusu S, Krishna R, Itoh N, et al. Fibroblast growth factor 21 is required for beneficial effects of exercise during chronic high-fat feeding. *J Appl Physiol* (1985). 2016;121(3):687-98.
104. Oost LJ, Kustermann M, Armani A, Blaauw B, Strasser RH. Fibroblast growth factor 21 controls mitophagy and muscle mass. *J Cachexia Sarcopenia Muscle*. 2019;10(3):630-42.
105. Tezze C, Romanello V, Desbats MA, Fadini GP, Albiero M, Favaro G, et al. Age-Associated Loss of OPA1 in Muscle Impacts Muscle Mass, Metabolic Homeostasis, Systemic Inflammation, and Epithelial Senescence. *Cell Metab*. 2017;25(6):1374-89.e6.
106. Keipert S, Ost M, Johann K, Imber F, Jastroch M, van Schothorst EM, et al. Skeletal muscle mitochondrial uncoupling drives endocrine cross-talk through the induction of FGF21 as a myokine. *Am J Physiol Endocrinol Metab*. 2014;306(5):E469-82.
107. Vandanmagsar B, Warfel JD, Wicks SE, Ghosh S, Salbaum JM, Burk D, et al. Impaired Mitochondrial Fat Oxidation Induces FGF21 in Muscle. *Cell Rep*. 2016;15(8):1686-99.
108. Ji K, Zheng J, Lv J, Xu J, Ji X, Luo YB, et al. Skeletal muscle increases FGF21 expression in mitochondrial disorders to compensate for energy metabolic insufficiency by activating the mTOR-YY1-PGC1 α pathway. *Free Radic Biol Med*. 2015;84:161-70.
109. Hondares E, Rosell M, Gonzalez FJ, Giral M, Iglesias R, Villarroya F. Hepatic FGF21 expression is induced at birth via PPAR α in response to milk intake and contributes to thermogenic activation of neonatal brown fat. *Cell Metab*. 2010;11(3):206-12.
110. Youm YH, Horvath TL, Mangelsdorf DJ, Kliewer SA, Dixit VD. Prolongevity hormone FGF21 protects against immune senescence by delaying age-related thymic involution. *Proc Natl Acad Sci U S A*. 2016;113(4):1026-31.
111. Kim KH and Lee MS. FGF21 as a mediator of adaptive responses to stress and metabolic benefits of anti-diabetic drugs. *J Endocrinol*. 2015;226(1):R1-16.
112. BonDurant LD and Potthoff MJ. Fibroblast Growth Factor 21: A Versatile Regulator of Metabolic Homeostasis. *Annu Rev Nutr*. 2018;38:173-96.
113. Zhao Y, Araki S, Wu J, Teramoto T, Chang YF, Nakano M, et al. An expanded palette of genetically encoded Ca²⁺ indicators. *Science*. 2011;333(6051):1888-91.
114. Liu X, Rossmeisl M, McClaine J, Riachi M, Shot T, Kovarova M, et al. Fibroblast Growth Factor 21 (FGF21) Promotes Formation of Aerobic Myofibers via the FGF21-SIRT1-AMPK-PGC1 α Pathway. *J Cell Physiol*. 2017;232(7):1893-906.

115. Fiorenza S, Kenna KP, Renton AE, Planavila A, Brown K, Villarroya F, et al. Triple AAV vector gene therapy rescues respiratory and motor function in a mouse model for spinal muscular atrophy. *Mol Ther*. 2021;29(1):487-501.
116. Zhou Y, Wang S, Li Y, Yu S, Zhao Y. SIRT1 links liver metabolism to adipose tissue inflammation. *Mol Cell Endocrinol*. 2014;397(1-2):42-50.
117. Palmer AK, Xu M, Zhu Y, Pirtskhalava T, Weivoda MM, Hachfeld CM, et al. Targeting senescent cells alleviates obesity-induced metabolic dysfunction. *Aging Cell*. 2019;18(3):e12950.
118. Yoshino J, Baur JA, Imai SI. NAD⁺ intermediates: the biology and therapeutic potential of NMN and NR. *Cell Metab*. 2018;27(3):513-28.
119. Huang Z, Zhong L, Lee JTH, Zhang J, Wu D, Geng L, et al. The FGF21-CCL11 Axis Mediates Beiging of White Adipose Tissues by Coupling Sympathetic Nervous System to Type 2 Immunity. *Cell Metab*. 2017;26(3):493-508.e4.
120. Mendelsohn AR, Larrick JW. Fibroblast growth factor-21 is a promising dietary restriction mimetic. *Rejuvenation Res*. 2012;15(6):624-8.
121. Sa-Nguanmoo P, Tanajak P, Kerdphoo S, Jaiwongkam T, Pratchayasakul W, Chattipakorn N, et al. FGF21 improves cognition by restored synaptic plasticity, dendritic spine density, brain mitochondrial function and cell apoptosis in obese-insulin resistant male rats. *Horm Behav*. 2016;85:86-95.
122. Bookout AL, de Groot MH, Owen BM, Lee S, Gautron L, Lawrence HL, et al. FGF21 regulates metabolism and circadian behavior by acting on the nervous system. *Nat Med*. 2013;19(9):1147-52.
123. Yu Y, Bai F, Wang W, Liu Y, Yuan Q, Qu S, et al. Fibroblast growth factor (FGF21) protects mouse liver against D-galactose-induced oxidative stress and apoptosis via activating Nrf2 and PI3K/Akt pathways. *Mol Cell Biochem*. 2015;403(1-2):287-99.
124. Kang YS, Lee MH, Song HK, Ko GJ, Kwon OS, Lim TK, et al. Aliskiren improves insulin resistance and ameliorates diabetic vascular complications in db/db mice. *Nephrol Dial Transplant*. 2011;26(4):1194-204.
125. Chen MZ, Moily NS, Bridgford JL, Wood RJ, Radwan M, Smith TA, et al. A thermo-stable fibroblast growth factor 21 analog for treating diabetes and obesity. *Nat Biotechnol*. 2020;38(3):309-13.
126. Lan T, Morgan DA, Rahmouni K, Sonoda J, Fu X, Burgess SC, et al. FGF19, FGF21, and an FGFR1/ β -Klotho-Activating Antibody Act on the Nervous System to Regulate Body Weight and Glycemia. *Cell Metab*. 2017;26(5):709-18.
127. Lee P, Linderman JD, Smith S, Brychta RJ, Wang J, Idelson C, et al. Irisin and FGF21 are cold-induced endocrine activators of brown fat function in humans. *Cell Metab*. 2014;19(2):302-9.
128. Owen BM, Ding X, Morgan DA, Coate KC, Bookout AL, Rahmouni K, et al. FGF21 contributes to neuroendocrine control of female reproduction. *Nat Med*. 2013;19(9):1153-6.
129. Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology*. 2016;64(1):73-84.
130. Lisowski L, Dane AP, Chu K, Zhang Y, Cunningham SC, Wilson EM, et al. Selection and evaluation of clinically relevant AAV variants in a xenograft liver model. *Nature*. 2014;506(7488):382-6.
131. Ogden PJ, Kelsic ED, Sinai S, Church GM. Comprehensive AAV capsid fitness landscape reveals a viral gene and enables machine-guided design. *Science*. 2019;366(6469):1139-43.
132. Wright JF. Manufacturing and characterizing AAV-based vectors for use in clinical studies. *Gene Ther*. 2008;15(11):840-8.
133. Nathwani AC, Tuddenham EG, Rangarajan S, Rosales C, McIntosh J, Linch DC, et al. Adenovirus-associated virus vector-mediated gene transfer in hemophilia B. *N Engl J Med*. 2011;365(25):2357-65.
134. Brown BD, Venneri MA, Zingale A, Sergi L, Naldini L. Endogenous microRNA regulation suppresses transgene expression in hematopoietic lineages and enables stable gene transfer. *Nat Med*. 2006;12(5):585-91.
135. Das AT, Tenenbaum L, Berkhout B. Tet-On systems for doxycycline-inducible gene expression. *Curr Gene Ther*. 2016;16(3):156-67.

- 136.Di Stasi A, Tey SK, Dotti G, Fujita Y, Kennedy-Nasser A, Martinez C, et al. Inducible apoptosis as a safety switch for adoptive cell therapy. *N Engl J Med*. 2011;365(18):1673-83.
- 137.Gillmore JD, Gane E, Taubel J, Kao J, Fontana M, Maitland ML, et al. CRISPR-Cas9 In Vivo Gene Editing for Transthyretin Amyloidosis. *N Engl J Med*. 2021;385(6):493-502.
- 138.Matharu N, Rattanasopha S, Tamura S, Maliskova L, Wang Y, Bernard A, et al. CRISPR-mediated activation of a promoter or enhancer rescues obesity caused by haploinsufficiency. *Science*. 2019;363(6424):eaau0629.
- 139.Franceschi C, Garagnani P, Parini P, Giuliani C, Santoro A. Inflammaging: a new immune-metabolic viewpoint for age-related diseases. *Nat Rev Endocrinol*. 2018;14(10):576-90.
- 140.Markan KR, Naber MC, Ameka MK, Anderegg MD, Mangelsdorf DJ, Kliewer SA, et al. Circulating FGF21 is liver derived and enhances glucose uptake during refeeding and overfeeding. *Diabetes*. 2014;63(12):4057-63.
- 141.Mitchell SJ, Scheibye-Knudsen M, Longo DL, de Cabo R. Animal models of aging research: implications for human aging and age-related diseases. *Annu Rev Anim Biosci*. 2015;3:283-303.
- 142.Miller RA, Harrison DE, Astle CM, Fernandez E, Flurkey K, Han M, et al. Rapamycin-mediated lifespan increase in mice is dose and sex dependent and metabolically distinct from dietary restriction. *Aging Cell*. 2014;13(3):468-77.
- 143.Owen BM, Ding X, Morgan DA, Coate KC, Bookout AL, Rahmouni K, et al. FGF21 contributes to neuroendocrine control of female reproduction. *Nat Med*. 2013;19(9):1153-6.
- 144.Singhal G, Kumar G, Chan S, Fisher FM, Ma Y, Vardeh HG, et al. Deficiency of fibroblast growth factor 21 (FGF21) promotes hepatocellular carcinoma (HCC) in mice on a long term obesogenic diet. *Mol Metab*. 2018;13:56-66.
- 145.Roybal KT, Rupp LJ, Morsut L, Walker WJ, McNally KA, Park JS, et al. Precision Tumor Recognition by T Cells With Combinatorial Antigen-Sensing Circuits. *Cell*. 2016;164(4):770-9.
- 146.Taliun D, Harris DN, Kessler MD, Carlson J, Szpiech ZA, Torres R, et al. Sequencing of 53,831 diverse genomes from the NHLBI TOPMed Program. *Nature*. 2021;590(7845):290-99.
- 147.Cirulli ET, Guo L, Leon Swisher C, Shah N, Huang L, Napier LA, et al. Profound Perturbation of the Metabolome in Obesity Is Associated with Health Risk. *Cell Metab*. 2019;29(2):488-500.e2.
- 148.Bar N, Korem T, Weissbrod O, Zeevi D, Rothschild D, Leviatan S, et al. A reference map of potential determinants for the human serum metabolome. *Nature*. 2020;588(7836):135-40.
- 149.Olshansky SJ, Passaro DJ, Hershow RC, Layden J, Carnes BA, Brody J, et al. A potential decline in life expectancy in the United States in the 21st century. *N Engl J Med*. 2005;352(11):1138-45.
- 150.Goldman DP, Cutler D, Rowe JW, Michaud PC, Sullivan J, Peneva D, et al. Substantial health and economic returns from delayed aging may warrant a new focus for medical research. *Health Aff (Millwood)*. 2013;32(10):1698-705.
- 151.Mehlman MJ. *The Price of Perfection: The Individual and Society in the Era of Biomedical Enhancement*. Baltimore: Johns Hopkins University Press; 2018. ISBN: 978-1421426426
- 152.Xu M, Pirtskhalava T, Farr JN, Weigand BM, Palmer AK, Weivoda MM, et al. Senolytics improve physical function and increase lifespan in old age. *Nat Med*. 2018;24(8):1246-56.
- 153.Justice JN, Nambiar AM, Tchkonja T, LeBrasseur NK, Pascoe R, Hashmi SK, et al. Senolytics in idiopathic pulmonary fibrosis: Results from a first-in-human, open-label, pilot study. *EBioMedicine*. 2019;40:554-63.
- 154.Hickson LJ, Langhi Prata LGP, Bobart SA, Evans TK, Giorgadze N, Hashmi SK, et al. Senolytics decrease senescent cells in humans: Preliminary report from a clinical trial of Dasatinib plus Quercetin in individuals with diabetic kidney disease. *EBioMedicine*. 2019;47:446-56.
- 155.Fischer KE, Gelfond JA, Soto VY, Han C, Someya S, Richardson A, et al. Health effects of long-term rapamycin treatment: the impact on mouse health of enteric rapamycin treatment from four months of age throughout life. *PLoS One*. 2015;10(5):e0126644.
- 156.Richardson A, Fischer KE, Speakman JR, de Cabo R, Mitchell SJ, Peterson CA, et al. Measures of Healthspan as Indices of Aging in Mice-A Recommendation. *J Gerontol A Biol Sci Med Sci*. 2016;71(4):427-30.

157. Wilkinson JE, Burmeister L, Brooks SV, Chan CC, Friedline S, Harrison DE, et al. Rapamycin slows aging in mice. *Aging Cell*. 2012;11(4):675-82.
158. Harrison DE, Strong R, Sharp ZD, Nelson JF, Astle CM, Flurkey K, et al. Rapamycin fed late in life extends lifespan in genetically heterogeneous mice. *Nature*. 2009;460(7253):392-5.
159. Mattison JA, Colman RJ, Beasley TM, Allison DB, Kemnitz JW, Roth GS, et al. Caloric restriction improves health and survival of rhesus monkeys. *Nat Commun*. 2017;8:14063.
160. Colman RJ, Beasley TM, Kemnitz JW, Johnson SC, Weindruch R, Anderson RM. Caloric restriction reduces age-related and all-cause mortality in rhesus monkeys. *Nat Commun*. 2014;5:3557.
161. Rockwood K, Blodgett JM, Theou O, Sun MH, Feridooni HA, Mitnitski A, et al. A Frailty Index Based On Deficit Accumulation Quantifies Mortality Risk in Humans and in Mice. *Sci Rep*. 2017;7:43068.
162. Bellantuono I, de Cabo R, Ehninger D, Di Germanio C, Lawrie A, Miller H, et al. A toolbox for the longitudinal assessment of healthspan in aging mice. *Nat Protoc*. 2020;15(2):540-74.
163. Frayling TM, Beaumont RN, Jones SE, Yaghootkar H, Tuke MA, Ruth KS, et al. A Common Allele in FGF21 Associated with Sugar Intake Is Associated with Body Shape, Lower Total Body-Fat Percentage, and Higher Blood Pressure. *Cell Rep*. 2018;23(2):327-36.
164. Tanaka T, Biancotto A, Moaddel R, Moore AZ, Gonzalez-Freire M, Aon MA, et al. Plasma proteomic signature of age in healthy humans. *Aging Cell*. 2018;17(5):e12799.
165. Zhang X, Yeung DC, Karpisek M, Stejskal D, Zhou ZG, Liu F, et al. Serum FGF21 levels are increased in obesity and are independently associated with the metabolic syndrome in humans. *Diabetes*. 2008;57(5):1246-53.
166. Salminen A, Kaarniranta K, Kauppinen A. Integrated stress response stimulates FGF21 expression: Systemic enhancer of longevity. *Cell Signal*. 2017;40:10-21.
167. Sanyal A, Charles ED, Neuschwander-Tetri BA, Loomba R, Harrison SA, Abdelmalek MF, et al. Pegbelfermin (BMS-986036), a PEGylated fibroblast growth factor 21 analogue, in patients with non-alcoholic steatohepatitis: a randomised, double-blind, placebo-controlled, phase 2a trial. *Lancet*. 2019;392(10165):2705-17.
168. Chen MZ, Moily NS, Bridgford JL, Wood RJ, Radwan M, Smith TA, et al. A thermo-stable fibroblast growth factor 21 analog for treating diabetes and obesity. *Nat Biotechnol*. 2020;38(3):309-13.
169. Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology*. 2016;64(1):73-84.
170. Tanajak P, Sa-nguanmoo P, Wang X, Liang G, Li X, Jiang C, et al. Cardioprotection of dapagliflozin against doxorubicin-induced cardiotoxicity in rats. *Arch Biochem Biophys*. 2018;650:62-73.
171. Chen S and Laychock SG. Early glucotoxicity increases cytosolic calcium and reactive oxygen species in rat pancreatic islets. *Endocrinology*. 2009;150(2):688-96.
172. Mendelsohn AR and Larrick JW. Fibroblast growth factor-21 is a promising dietary restriction mimetic. *Rejuvenation Res*. 2012;15(6):624-8.
173. Palmer AK, Xu M, Zhu Y, Pirtskhalava T, Weivoda MM, Hachfeld CM, et al. Targeting senescent cells alleviates obesity-induced metabolic dysfunction. *Aging Cell*. 2019;18(3):e12950.
174. Yoshino J, Baur JA, Imai SI. NAD⁺ intermediates: the biology and therapeutic potential of NMN and NR. *Cell Metab*. 2018;27(3):513-8.
175. Zhou Y, Wang S, Li Y, Yu S, Zhao Y. SIRT1 links liver metabolism to adipose tissue inflammation. *Mol Cell Endocrinol*. 2014;397(1-2):42-50.
176. Zhao Y, Araki S, Wu J, Teramoto T, Chang YF, Nakano M, et al. An expanded palette of genetically encoded Ca²⁺ indicators. *Science*. 2011;333(6051):1888-91.
177. Vandanmagsar B, Warfel JD, Wicks SE, Ghosh S, Salbaum JM, Burk D, et al. Impaired Mitochondrial Fat Oxidation Induces FGF21 in Muscle. *Cell Rep*. 2016;15(8):1686-99.
178. Kang YS, Lee MH, Song HK, Ko GJ, Kwon OS, Lim TK, et al. Aliskiren improves insulin resistance and ameliorates diabetic vascular complications in db/db mice. *Nephrol Dial Transplant*. 2011;26(4):1194-204.