Plasmid delivery of follistatin gene therapy safely improves body composition and lowers extrinsic epigenetic age in sex- and age-diverse adult human subjects

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Walter Patterson and Mac Davis conceived, developed, and managed the project. Walter Patterson constructed the plasmid and performed ELISAs. Walter Patterson, Mac Davis, and Ryan Rossner wrote the manuscript. Raven Garuda analyzed the data. Glenn C. Terry administered the therapy.

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Abstract

We injected polyethyleneimine (PEI)-complexed plasmid delivering the Follistatin (FST) 344 gene to 43 adult human volunteers of both sexes, age 23-88, median age 46, to test the safety and efficacy of this delivery method and target gene as an anti-frailty longevity intervention. Patients received subcutaneous injection into abdominal fat of 50 μg FST in a plasmid vector. We evaluated several metrics immediately prior to administration and at three months post-therapy: serum FST, body composition, blood biomarkers of inflammation, glucose metabolism, lipid metabolism, and epigenetic age estimates. Serum FST, measured by enzyme-linked immunoassay, increased over twofold from a baseline mean of 8.58 ng/ml to 24.03 ng/ml ($p = 0.001$) at month three, the latter a largely supraphysiological value. Body composition, measured by dual energy x-ray absorptiometry scans, improved significantly, with a mean fat-free mass increase of 1.96 lbs ($p = 0.001$) and a mean bodyfat reduction of -0.87 % ($p = 0.01$). The maximum fat free mass increase was 12.15 lbs. High-sensitivity C-reactive protein and homocysteine, two common markers of inflammation, both showed signs of potential decrease, toward improvement. Glucometabolic markers trended toward clinically insignificant increases. Lipid panel changes were small and not statistically significant, though a minority of subjects experienced a large increase in low density lipoprotein. Intrinsic epigenetic age trended toward decrease, and extrinsic epigenetic age (EEA) showed a statistically significant mean decrease of -7.10 years ($p = 0.004$); both results were progressively more pronounced in older age brackets, with a maximum EEA decrease of -27.91 years. Telomere length trended toward increase, again progressively more so in older age brackets, but DunedinPACE rate of aging showed no clear evidence of change. Of paramount importance, no severe adverse effects related to therapy were reported. This clinical trial establishes PEI-complexed plasmid delivery of FST as a potentially safe, anti-frailty longevity therapy for both male and female human subjects across a near maximal adult age range.

Keywords: gene therapy, plasmid, follistatin, frailty, longevity

Introduction

DNA, the human genome, and gene therapy

After the discovery of the structure of DNA in 1953 [1] and the sequencing of the human genome in the early 2000s [2, 3], attention naturally turned to developing gene therapies [4–6]. Major innovations in gene therapy have included: viral vectors [7], CRISPR-Cas9 [8], and plasmids [9]. The tremendous promise of such therapies, however, was disrupted by early setbacks including a child's death in a viral vector gene therapy trial and broad safety and ethical concerns [10–13]. Our analysis of this situation lead us to develop plasmids as the therapeutic mode with the most favorable risk:reward ratio. We developed localized transfection of a non-integrating plasmid with a drug-inducible killswitch. Thus, we have three redundant safety layers: inherently transient expression, drug-inducible reversibility, and, if necessary, excision of transfected tissue.

Plasmid gene therapy

Plasmids were conceptually described in the 1920s [14] and named in the 1950s [15]. They were first isolated in 1969 [16] and first engineered in the early 1970s [17, 18]. By the late 1990s, plasmids were viewed as safe but ineffective [19]. In 1997 and 2003, smaller, higher expression [20], and longer-lasting [21] plasmids called "minicircles" were developed. Nanoplasmids [22] and mini-intronic plasmids [23] further developed this strategy over the next ten years. Despite these advances, plasmids remained out of favor generally. All FDA-approved gene therapies to date utilize viral vectors [24], and only 12% of gene therapy trials worldwide use plasmids [25].

Localized linear polyethyleneimine transfection

Polyethyleneimine (PEI) is a cationic polymer used to transfect nucleic acids into mammalian cells. PEI's positive charge enables interaction with cell membranes and subsequent endocytosis. [26]. PEI can be linear (LPEI) or branched (BPEI), and a range of molecular weights are possible for both types. There are safety concerns associated with PEI [27], but we make the case that localized LPEI in a 4:1 PEI:DNA ratio is both safe and efficacious *in vivo*. First, the toxicity associated with PEI is mostly limited to BPEI and is dependent on free, non-complexed PEI [28, 29]. The 4:1 PEI:DNA ratio we used minimizes free PEI. LPEI hydrochloride has been shown to produce neither proinflammatory cytokines nor alterations to hepatic enzyme levels *in vivo* [30, 31]. PEI-DNA administration additionally has been demonstrated as efficacious in human patients in transgenic vaccination for B-cell lymphoma [32]. Safety and efficacy advantages of lipid nanoparticles > PEI *in vitro* have been reversed *in vivo* [26]. Thus, localized LPEI at low dosages in a 4:1 ratio with DNA could be reasonably expected to be safe and efficacious.

Target genes and Geroscience

Countless genes are candidate targets for gene therapy [33]. Our approach was to develop gene therapies targeting longevity. A paradigm shift has taken place in the last ten years towards a strategy called Geroscience that treats aging itself as the biggest risk factor for the major killer diseases [34–36]. Instead of targeting each disease individually, we target their biggest shared risk factor: aging. Significant progress has been made towards understanding the molecular mechanisms of aging [37], making the Geroscience paradigm actionable.

Follistatin (FST) safety and efficacy

Follistatin (FST) was our first choice because, in both animal and human trials, it not only has an exceptionally consistent safety profile [38], but it has also been exceptionally effective at improving healthy longevity [39]. Frailty, in particular, increases with age, driven largely by late-life rapid and catastrophic declines in muscle size, muscle strength, and bone mineral density (BMD) [40–42]. FST is particularly effective at combating physical frailty [43–45].

Our system and FST secretion and expression

Additionally, FST is amenable to our plasmid delivery system. FST is a secreted protein, meaning it is made in one cell, exported to the cell surface, cleaved of its secretory sequence, and released into systemic circulation via the blood [46]. This means we could transfect a small number of cells and have them become export factories for FST. Thus, we injected the plasmid subcutaneously to transfect a small number of adipocytes capable of subsequently creating systemic effects (Fig. 1). FST counterintuitively increases with age [47], but this increase is clearly insufficient to combat aging-induced frailty.

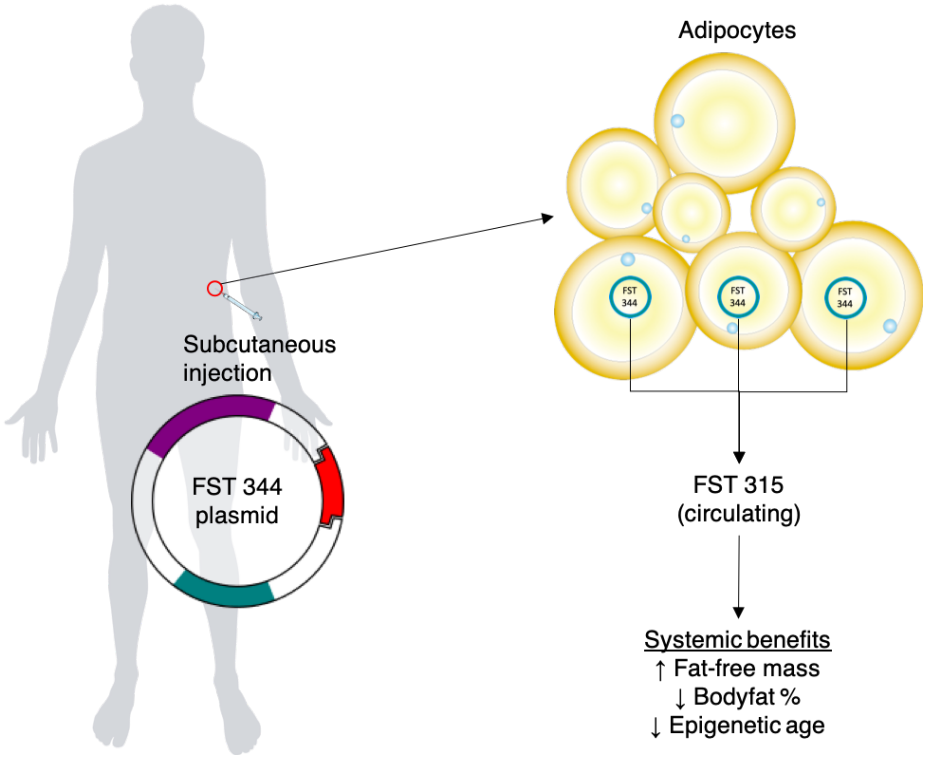


Figure 1: Injectable plasmid therapeutic strategy. Plasmid administration via subcutaneous injection into abdominal fat leads to FST export into circulation from local site and subsequent systemic benefits.

FST isoforms

FST is initially produced in two isoforms, FST 317 and FST 344 [46] (Fig. 2). Both are secreted then subsequently cleaved from their secretory signal peptide, resulting in FST 288 and FST 315, respectively. FST 344 and FST 315 contain a C-terminal acidic region that prevents FST 315 from binding to the plasma membrane's extracellular surface, subsequently allowing FST 315 to be released into circulation. FST 317 and FST 288 lack this C-terminal feature, therefore FST 288 is not the major circulating form of FST. Based on this and prior groups' research results [38], we used FST 344 to increase circulating FST 315.

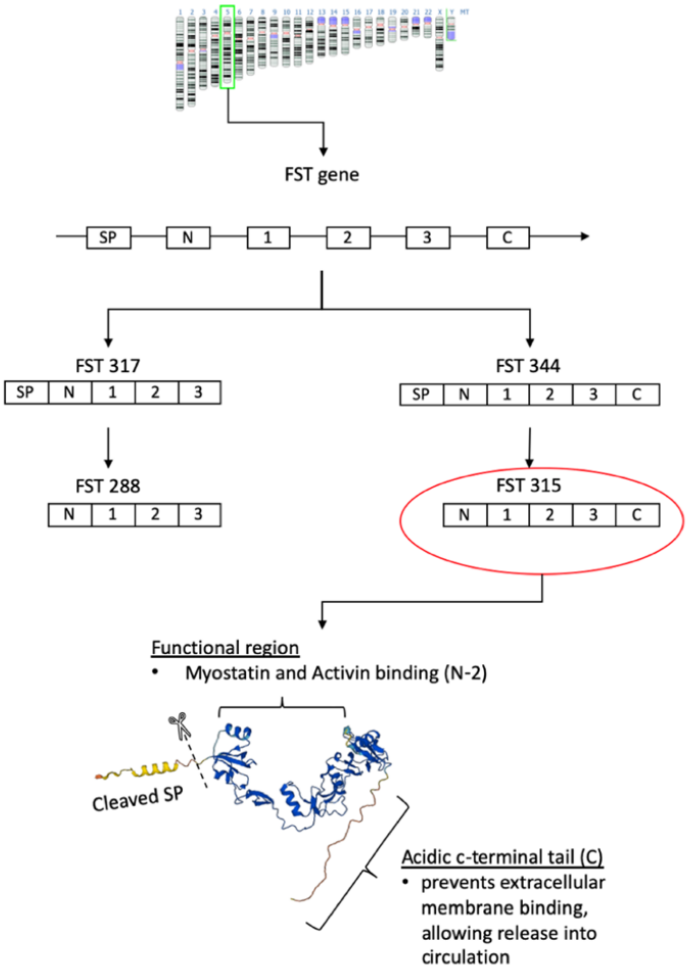


Figure 2: FST isoforms and domain functions.

FST mechanisms (Myostatin, Activin, ActRIIB)

The mechanisms by which FST mediates its anti-frailty and anti-inflammatory effects are reasonably well understood [48]. FST binds to Transforming Growth Factor beta (TGFβ) proteins in circulation, primarily Activin and Myostatin, and subsequently prevents their binding to and activation of the Activin Receptor Type II B (ActRIIB) cell surface receptor [49]. FST improvement of lean mass and strength requires both Activin and Myostatin inhibition [43, 50], but FST-mediated improvement of inflammation is thought to primarily require Activin inhibition [48].

Materials & Methods

Plasmid Construct

A plasmid was constructed delivering the 344 amino acid secreted isoform of the human follistatin gene. Transgene expression was driven by a CMV promotor. Scaffold/Matrix attachment regions (S/MARs) were included in order to enhance tethering of the plasmid to the nuclear matrix, thus promoting nuclear retention.

Vector Purification and Characterization

The sequence was verified by third party synthesis post-oligo ligation via m-13 reverse and forward primers as well as next-generation sequencing by GeneWiz. Endotoxin concentration determined to conform to FDA Guidance for Industry recommendations via kinetic LAL assay performed by third-party CRO Charles River [51].

Transformation Technique

Transfection was achieved by means of 40 kDA linear polyethyleneimine (Polysciences catalog no. 24765-1) coadministration with plasmid. A 4:1 ratio of transfection agent to plasmid was used as suggested by the manufacturer, Polysciences.

Plasmid administration

Two sterile vials, one containing plasmid and one containing PEI, were mixed and incubated at room temperature for 30 minutes prior to administration. After incubation, 400 μl of of saline solution containing 50 μg of plasmid was injected into subjects' abdominal subcutaneous fat. The primary physician observed patients for 15 minutes post-injection in case of allergic reactions or other complications.

Serum FST ELISA

The FST 344 concentration was determined using the Invitrogen Follistatin (FST) Human ELISA Kit. This assay kit is a colorimetric sandwich-type ELISA provided by ThermoFisher, catalog #EHFST. Serum was isolated prior to administration as well as three months post administration.

Cohort selection

Subjects volunteered for the trial without receiving any financial compensation for participation in the study. Ethics approval for this Phase I research study was obtained from the Global Alliance for Regenerative Medicine (GARM) IRB committee.

Body composition

DEXA scans were used to measure body composition of subjects.

Blood biomarkers

Blood draws were performed according to a defined panel of desired values, and providers directly emailed to study organizers.

Individual Data

Each participant had a set of measurements per variable: pre-treatment (months -1 and 0 and treatment (months 1-3). Months were numbered relative to FST administration date. Baseline measurements were calculated by taking the mean of the pre-treatment measurements. Change in baseline measurements were calculated by subtracting the baseline from the treatment timepoints.

Group Trends

To analyze a measurement for significant changes within a group, only participants with a baseline and a month 3 measurement were included. Distributions were checked for normality and extreme outliers (≥

3.0 SD from the mean). Shapiro-Wilk test was performed on change from baseline (month 3 – baseline) measurements, with $p < 0.05$ indicating a violation of the assumption of normality. A paired Student's *t*-test was conducted to compare baseline to month 3 measurements in normally distributed measurements with no extreme outliers. A Wilcoxon signed-rank test was performed on measurements which violated at least one of the assumptions. The following variables violated the assumption of normality: fat free mass, bone density, extrinsic epigenetic age (EEA), DunedinPACE, FST, FSH, LH, total cholesterol, HDL cholesterol, triglycerides, insulin, and hsCRP, and homocysteine. All other variables were normally distributed with no extreme outliers.

Results

Over twofold increase in serum FST

We measured serum FST by enzyme-linked immunoassay (ELISA) immediately prior to plasmid administration and three months post-administration. Mean serum FST increased over twofold (Fig. 3), the latter value being largely supraphysiological.

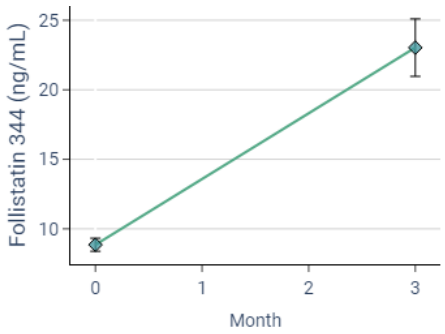


Figure 3: Serum FST increased over twofold to a largely supraphysiological value.

Body composition improved in all age groups and both sexes

We measured body composition via dual-energy X-ray absorptiometry (DEXA) scans before and after treatment at one month intervals through three months. By three months, mean fat-free mass (FFM) increased (Fig. 4(A)), and mean body fat % (BF%) decreased (Fig. 4(B)). Both results were directionally consistent between both sexes (Fig. 4(C), Fig. 4(D)) and across all age groups (Fig. 4(E), Fig. 4(F)). At three months, android to gynoid fat ratio (A/G) was trending downward, toward improvement (Fig. 4(G)), and bone mineral density (BMD) was trending upward slightly (Fig. 4(H)).

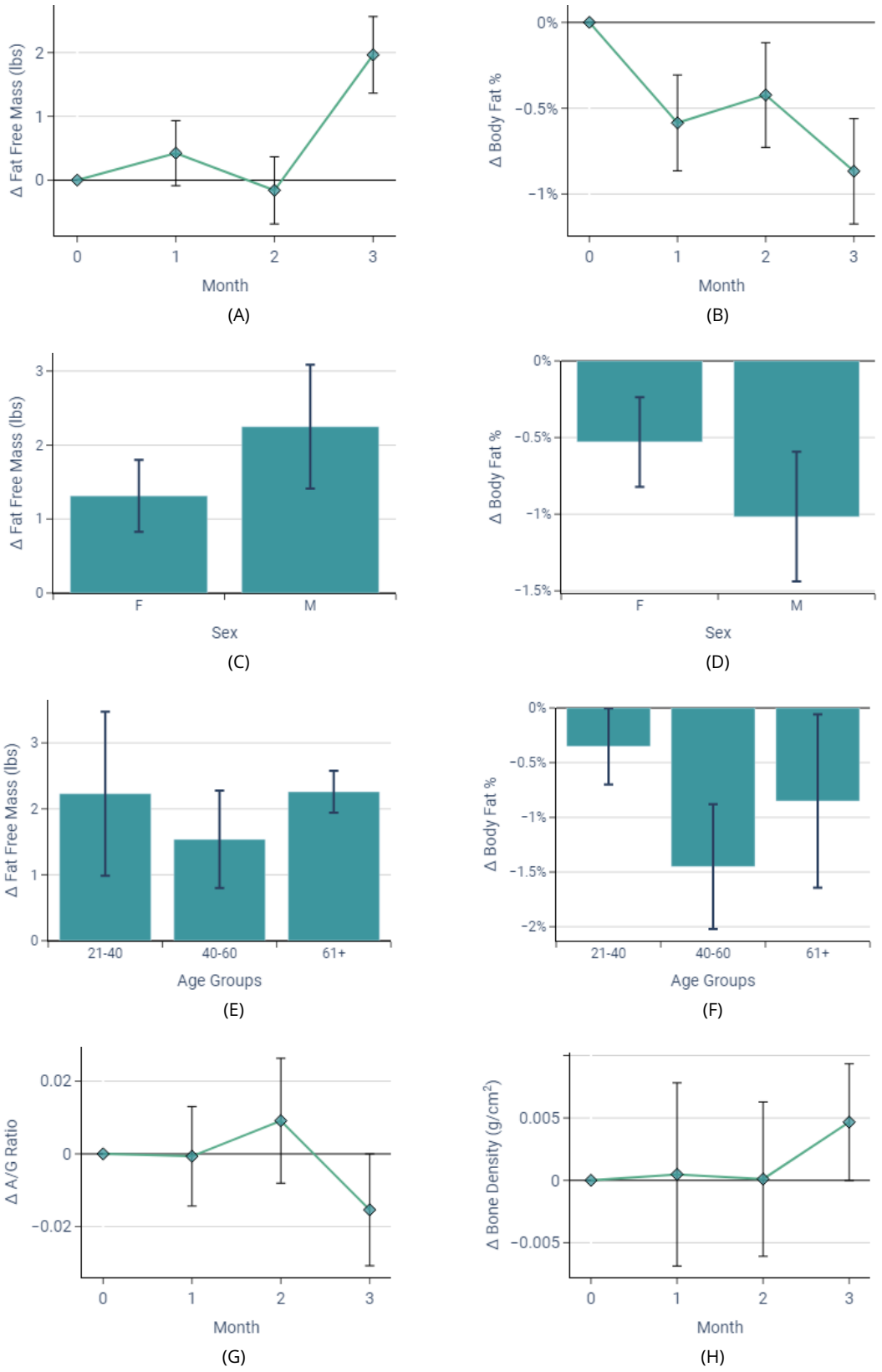


Figure 4: Body composition improved in both sexes and all age groups. (A) FFM increased and (B) BF% decreased. (C) FFM and BF% directionality were conserved in (C,D) both sexes and (E,F) across all age groups. (G) A/G ratio exhibited a potential trend downward, toward improvement, at three months. (H) BMD was trending upward very slightly at three months.

Markers of inflammation

High-sensitivity measurement of C-reactive protein (hsCRP) (Fig. 5(A)) and homocysteine (Hcy) (Fig. 5(B)), two common markers of systemic inflammation, both showed signs of potential decrease, toward improvement.

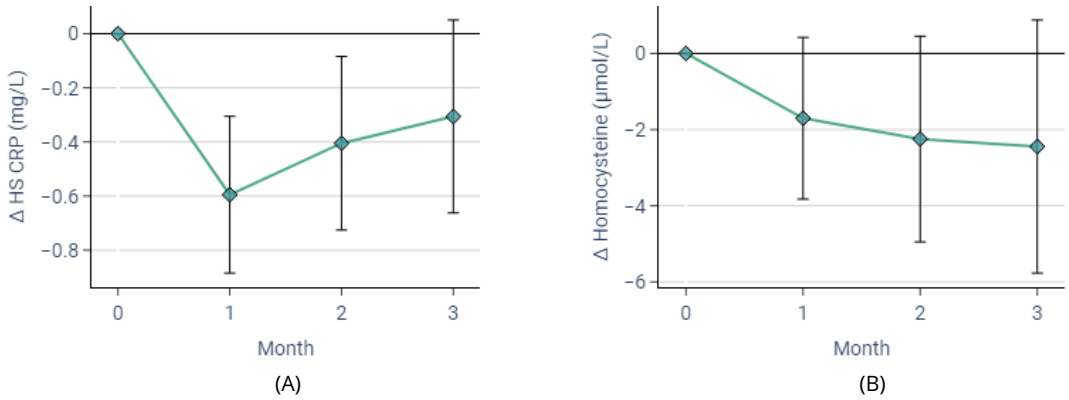


Figure 5: Markers of inflammation showed signs of potential decrease. (A) hsCRP and (B) Hcy both showed signs of potential decrease.

LSH and FH

Neither LH nor FSH decreased on average (Fig. 6(A), Fig. 6(B)), and this absence of decrease was consistent between both sexes (Fig. 6(C), Fig. 6(D)) and across all age groups (Fig. 6(E), Fig. 6(F)).

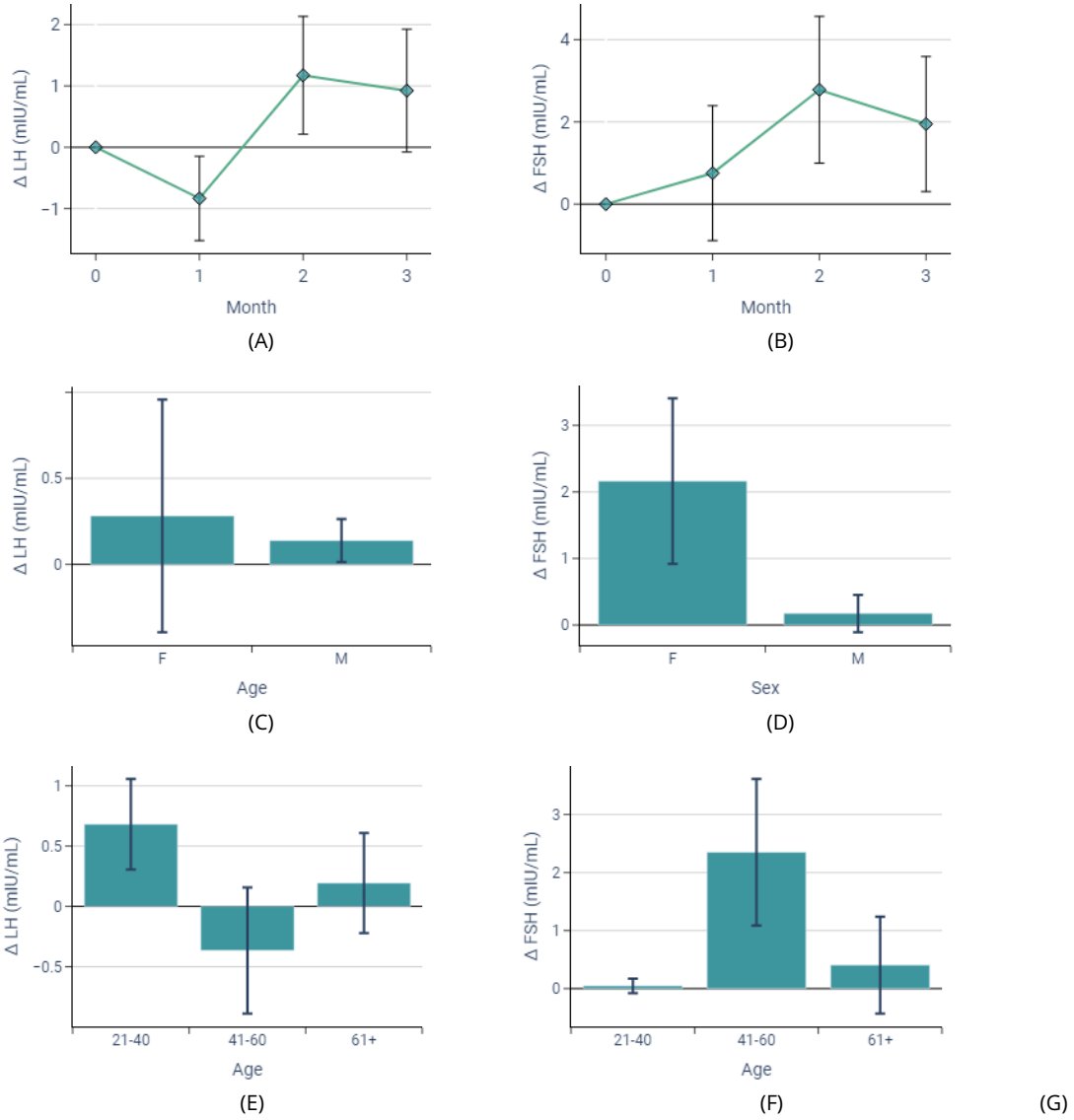


Figure 6: No decrease in LH or FSH in any group. (A) LH and (B) FSH both trended toward increase. Neither sex showed a decrease in (C) LH or (D) FSH, and no age group showed a decrease in (E) LH or (F) FSH.

Glucometabolic markers

Fasting glucose (Fig. 7(A)), HbA1C (Fig. 7(B)), fasting insulin (Fig. 7(C)), and leptin (Fig. 7(D)) all showed signs of a small potential trend upward at month three. The consistency across these values may be noteworthy, but the changes' magnitude at three months suggests they are unlikely clinically significant.

Lipids

Total cholesterol trended toward an increase (Fig. 8(A)), and most of this was driven by a trend toward increased low-density lipoprotein (LDL) (Fig. 8(B)). The majority of subjects, however, experienced no change or some improvement in LDL, while a minority, roughly one third, experienced a mean increase (Fig. 8(C)). Median LDL levels remained stable, consistent with the majority of participants not experiencing an increase (Fig. 8(D)). Subjects clustered into two response groups, with two subjects' values being extremely elevated (Fig. 8(E)). HDL was potentially trending upward (Fig. 8(F)), and triglycerides were potentially trending downward (Fig. 8(G)).

Epigenetic age decrease and telomere length increase

Intrinsic epigenetic age (IEA) trended toward modest decrease (Fig. 9(A)), but extrinsic epigenetic age (EEA) experienced a larger, statistically significant increase (Fig. 9(B)). Both results were directionally consistent between both sexes (Fig. 9(C), Fig. 9(D)) and both were largely directionally conserved across all age groups and progressively stronger in older age groups (Fig. 9(E), Fig. 9(F)). Telomere length trended toward increase (Fig. 9(G)), but DunedinPACE [52] showed no clear evidence of change (Fig. 9(H)). The telomere length trend, like IEA and EEA changes, was directionally conserved in both sexes (Fig. 9(I)) and progressively stronger in older age brackets (Fig. 9(J)). The maximum reduction in EEA was an impressive -27.91 years (Fig. 9(K)).

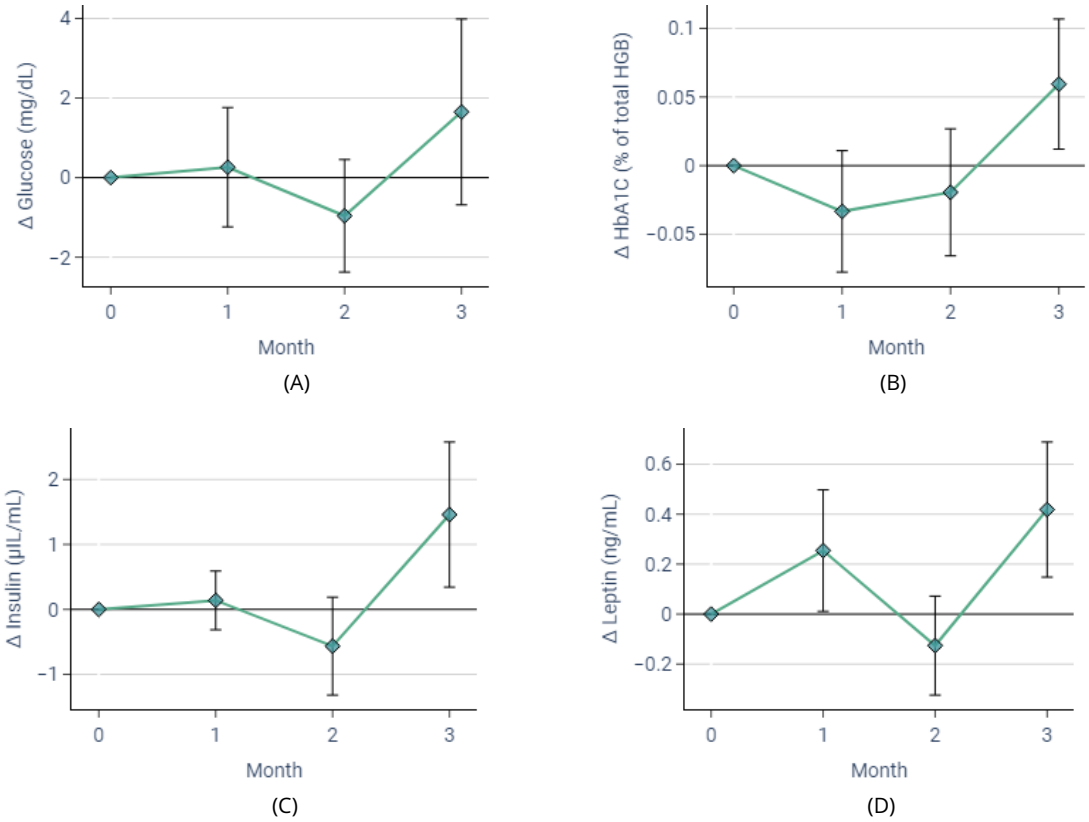


Figure 7: Trend toward slightly elevated glucometabolic markers. (A) Fasting blood glucose, (B) HbA1c, (C) fasting insulin, and (D) leptin all trended toward small, likely clinically insignificant, increases.

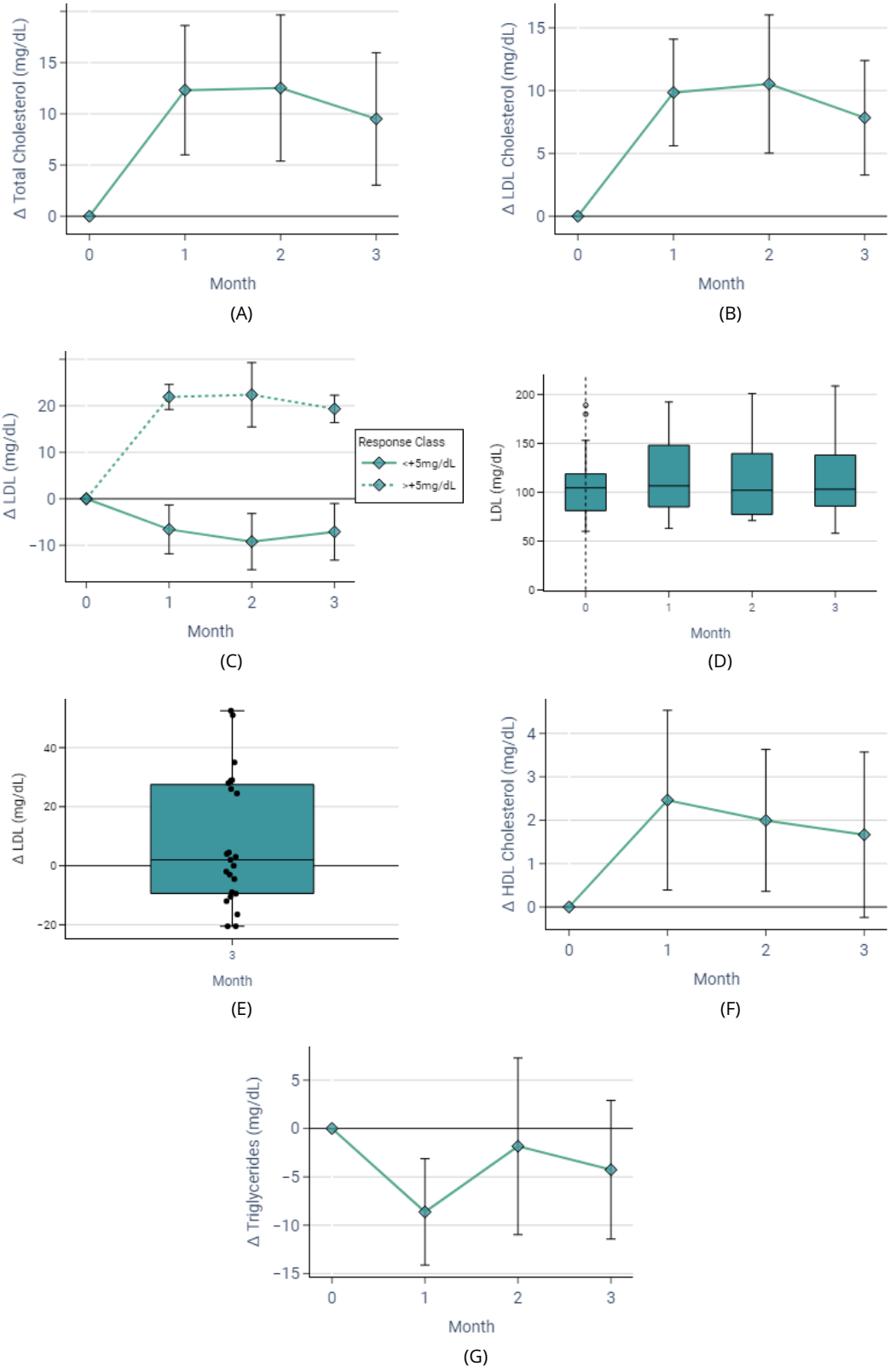
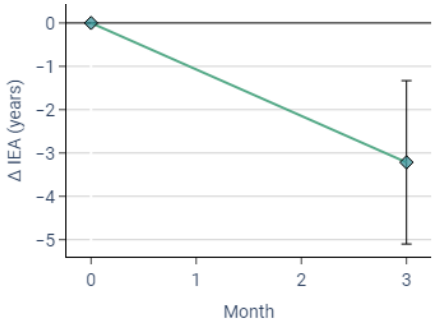
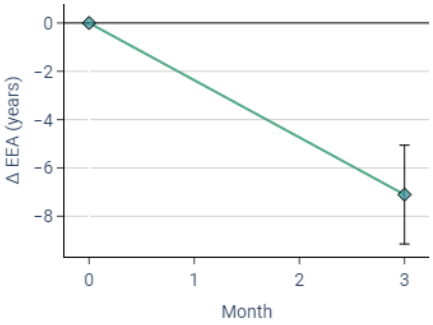


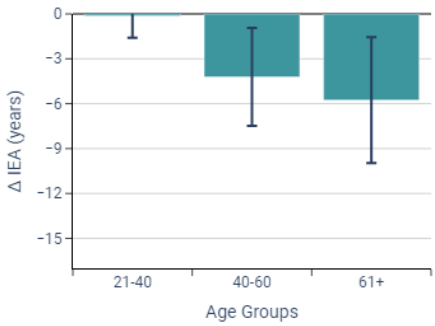
Figure 8: Lipid changes. (A) Total cholesterol trended toward an increase, and (B) most of this was driven by a trend toward increased LDL. (C) The majority of subjects, however, experienced no change or some improvement in LDL, while a minority, roughly one third, experienced a mean increase of 19 mg/dL. (D) Median LDL levels remained stable, consistent with the majority of participants not experiencing an increase. (E) Subjects clustered into two response groups, with two subjects' values being extremely elevated. (F) HDL was trended upward, and (G) triglycerides trended downward.


Δ IEA (years)
0
−1
−2
−3
−4
−5
0
1
2
3
Month

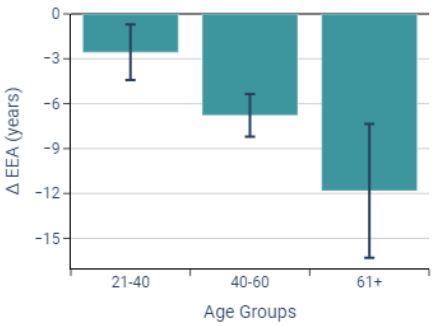
(A)


Δ EEA (years)
0
−2
−4
−6
−8
0
1
2
3
Month

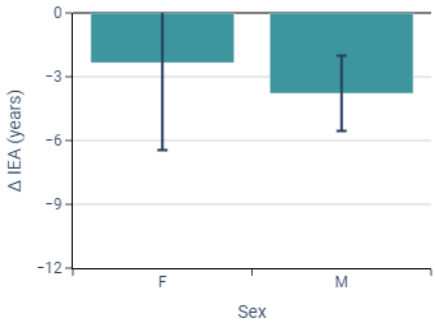
(B)


Δ IEA (years)
0
−3
−6
−9
−12
−15
21-40
40-60
61+
Age Groups

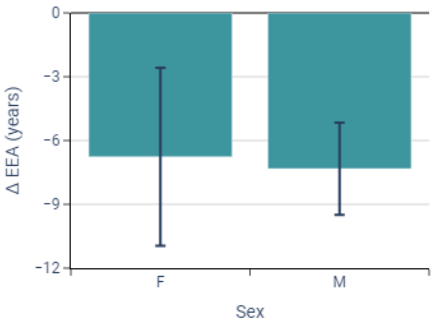
(C)


Δ EEA (years)
0
−3
−6
−9
−12
−15
21-40
40-60
61+
Age Groups

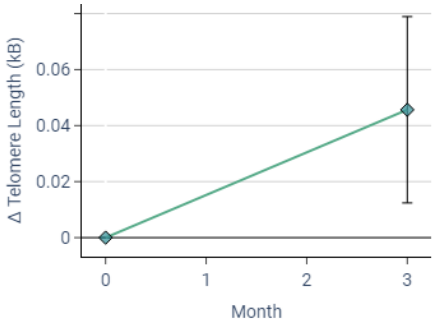
(D)


Δ IEA (years)
0
−3
−6
−9
−12
F
M
Sex

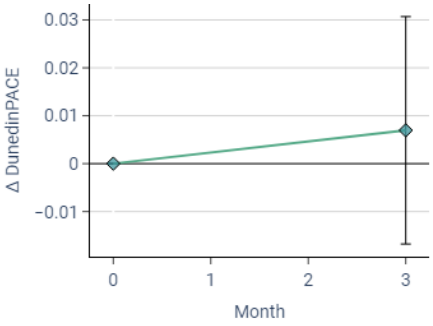
(E)


Δ EEA (years)
0
−3
−6
−9
−12
F
M
Sex

(F)


Δ Telomere Length (kB)
0.06
0.04
0.02
0
0
1
2
3
Month

(G)


Δ DunedinPACE
0.03
0.02
0.01
0
−0.01
0
1
2
3
Month

(H)

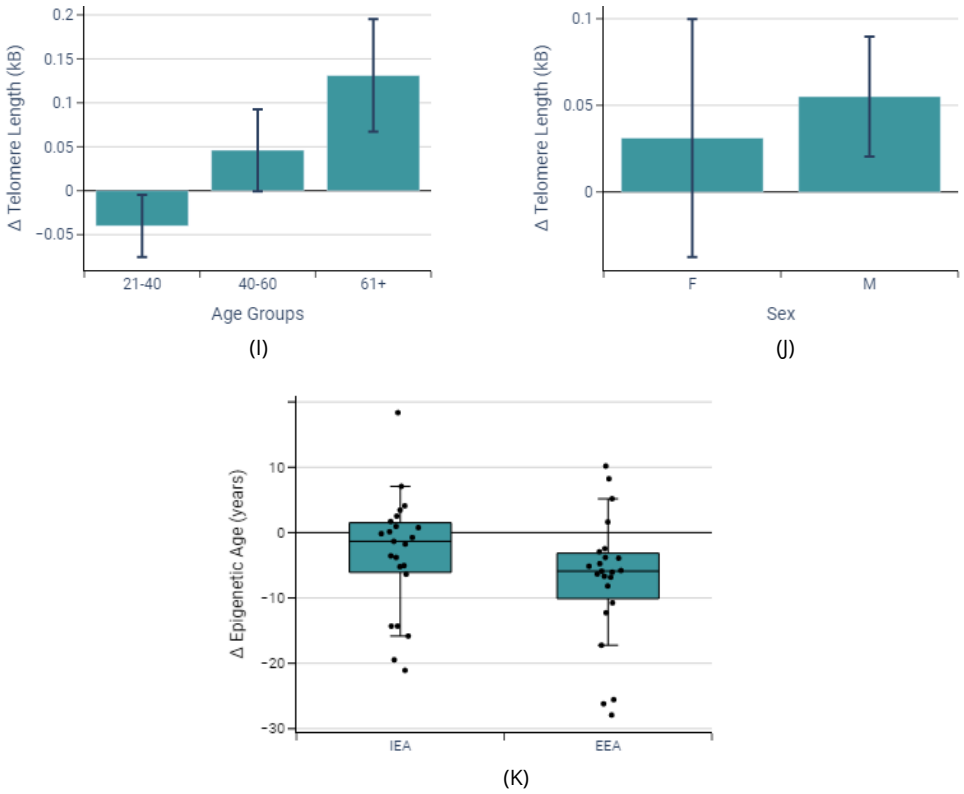


Figure 9: Decreased extrinsic epigenetic age estimates and progressively stronger trends toward improvement in older age brackets. (A) IEA trended toward decrease, and (B) EEA decreased significantly. Both results were largely conserved across (C,D) both sexes and (E,F) all age groups, with older age brackets showing progressively more pronounced responses. (G) Average telomere length trended toward increase. (H) DunedinPACE rate of aging showed no clear evidence of change. Telomere length trend toward increase, like IEA and EEA, (I) was progressively stronger in older age brackets and (J) was directionally conserved between both sexes. (K) Scatter plot showing distribution of IEA and EEA values.

Discussion

Safety

The paramount concern of any Phase 1 clinical trial is safety. This concern is elevated for gene therapy trials because of their novelty, intrinsic biological power, and historical adverse effects. Thus, it is critical to note that none of our subjects, who represent both sexes and a near-maximal adult age range, reported severe adverse effects related to therapy.

Serum FST increase

Our plasmid was very effective at increasing serum FST to largely supraphysiological levels [47]. Human biological homeostatic systems have evolved over tens of thousands of years, so it is not obvious that supraphysiological values would be tolerated. Here, however, supraphysiological FST was not disruptive. Counterintuitively, FST increases naturally with age [47], but such increase is clearly insufficient to alleviate age-induced frailty. Thus, a supraphysiological increase is conceivably required to confer meaningful benefits.

Body composition improvement

Our primary goal in terms of efficacy was improved body composition. As hoped, body composition improved considerably, with mean FFM gain of 1.96 lbs. and BF% loss of -0.87 %. Importantly, both changes were directionally conserved across both sexes and all age groups. Body composition is especially important for elderly people, because age-related frailty can tremendously decrease quality of life and increase

risk of catastrophic adverse health events [40, 42]. Falls that lead to fractures and other injuries are often especially harmful [53]. FST gene therapy might be a novel, powerful, safe way to combat age-induced frailty.

Fat loss is intrinsically good until a healthy value is reached, but fat-free mass is a surrogate metric for the more functional metric, strength. Our trial produced extraordinary case studies of subjectively assessed strength improvement, but it will be essential to objectively measure strength in future FST trials.

A/G ratio showed signs of trending toward decrease, which would be indicative of a shift toward healthy fat distribution, away from excessive belly fat and/or visceral fat. FST can positively impact the closely related process of adipose beiging [54], consistent with FST having a net positive effect on metabolic status.

We observed signs of a trend toward slightly increased BMD, consistent with literature describing such a correlation [55]. Bone grows slowly, so it is possible later time points or therapy re-administration could lead to quantifiable BMD improvements even in elderly subjects. Again, there were extraordinary case studies in our trial in which BMD improved rapidly, but these will be reserved for a separate publication. Conversely, FST in mice has been shown to cause tibial fractures [56] and dramatically reduce bone quality [57], but these effects are secondary to extreme skeletal muscle growth [58] that is not seen in humans.

Inflammation

Our secondary goal in terms of efficacy was improvements to markers of inflammation and corresponding reports of subjective improvements in patients with inflammatory conditions, e.g. rheumatoid arthritis. Our results were directionally aligned with our expectations, but, unlike body composition changes, inflammatory markers hsCRP and Hcy only trended toward decrease. We did, however, observe outstanding individual results that may be covered in separate case study publications. Mechanistically, sarcopenia-induced frailty, discussed above, itself might be a rheumatic inflammatory condition [59], strengthening the case for FST action at the intersection of frailty and inflammation.

Inclusion body myositis (IBM) is an inflammatory condition that might be treatable with FST. Inclusion body myositis is the most common inflammatory myopathy in older adults, most commonly affecting men after age 50 [60]. Circulating levels of Activin A in IBM patients are higher than controls, and circulating myostatin levels of IBM patients are higher than those of other neuromuscular disease patient groups [61]. Because of this, FST may be able to address the unique pathology of IBM. Indeed, early results have shown improved ambulation and muscle regeneration as well as decreased fibrosis in IBM patients injected with follistatin gene therapy [62–64].

No decrease in LH or FSH

FST was originally discovered as an FSH-suppressor and was previously called "FSH suppressing protein" [65, 66]. Therefore, we wanted to address to possibility that our therapy could lower FSH. We observed neither FSH nor LH decrease (Fig. 6) in any groups, consistent with the expectation that our FST 344 therapy would improve body composition without negatively impacting these elements of the hypothalamic-pituitary-gonadal (HPG) axis. This is likely due to the FSH-suppressing activity of FST being carried out by an isoform other than FST 315 [67, 68].

Glucometabolic markers

Literature offers mixed evidence about the effects of FST on glucose metabolism [39, 69, 70]. Thus, we were not completely surprised to see signs of small trends consistent with worsened glucose metabolism. The changes we observed are unlikely to be clinically significant but are nonetheless important to note.

Lipids

The main change to subjects' lipid values was a trend toward LDL increase observed in a minority of participants. It is possible that the anabolism we observed in the form of lean mass gain required choles-

terol export from the livers of subjects. The phenomenon of increase was large enough to warrant some caution, but we suggest that diet, exercise, and/or prophylactic medication are potentially sufficient to prevent the observed increase. Additionally, our data beyond three months suggest that the LDL trend may be transient, though these data will be reserved for a separate publication and/or exploration in future trials. HDL and TG both showed signs of trending toward favorable changes, but neither trend was of a large magnitude.

Lowered estimates of epigenetic age

Epigenetic analyses of aging were first developed 10 years ago [71], and they are currently considered premier, if still evolving, biomarkers of individual aging [72]. Loss of epigenetic information is a major cause of mammalian aging [73].

IEA trended toward multi-year decrease, and EEA, considered more meaningful because it takes immune cell counts into consideration [74], decreased by over five years. This was somewhat unexpected and will require further investigation to propose a model of relevant mechanisms. Our current hypothesis is that FST-mediated inhibition of Activin might reverse epigenetic estimates of age by decreasing inflammation.

Telomere length trended toward a modest increase, and DunedinPACE rate of aging was essentially unchanged. It is not obvious why rate of aging would remain constant while epigenetic age decreased. Here also, further investigation will be required to generate mechanistic understanding.

Both epigenetic age and telomere length changes were not only well conserved directionally across groups, but they were also progressively more pronounced in older age brackets. An intuitive hypothesis is that this is due to more room for improvement in older subjects' bodies with more extensive inflammation and more advanced epigenetic information loss. Overall, our findings are consistent with the Information Theory of Aging's suggestion that some secretory factors might be capable of inducing epigenetic rejuvenation [23].

FST and longevity-optimized mTOR signaling

Additionally, FST might be an mTOR sensitizing/potentiating agent. FST inactivates ActRIIB, and ActRIIB inactivation can induce muscle hypertrophy to a similar degree in control and rapamycin-treated animals [75]. FST might uniquely potentiate mTOR signaling by preventing SMAD3 phosphorylation, leaving more SMAD3 available for mTOR [76]. mTOR inhibition has mostly prolongevity effects [77, 78], but it can negatively impact aging-induced sarcopenia and resulting frailty [79, 80]. If FST renders mTOR more sensitive to anabolic signals, it could hypothetically allow more effective acute bursts of anabolism in the context of general mTOR suppression. Such a paradigm is consistent with our results demonstrating fat-free mass gain with simultaneous epigenetic age estimate decrease.

Future studies

Subsequent FST trials should focus on multiple dosages, a longer study window of 12-24 months, strength testing in addition to body composition measurement, potential benefits in patients with inflammatory conditions like rheumatoid arthritis and/or inclusion body myositis, careful examination of glucose and lipid metabolic changes, and mechanistic investigation of epigenetic rejuvenation. Beyond FST, our results establish a potential path for human trials utilizing the same delivery system with other candidate genes.

If such additional plasmid gene therapies are safely and effectively developed, administration would likely take place yearly. A shortcoming of this system is that it is currently limited to secretory proteins, but this limitation is something we seek to eventually overcome. The future of gene therapy likely involves utilizing all delivery modes according to their complementary strengths and weaknesses to optimize overall therapeutic safety and efficacy.

ABBREVIATIONS	
ActRIIB	Activin receptor type II B
A/G	Android/Gynoid fat ratio
BF%	Body fat %
BMD	Bone mineral density
Cas9	CRISPR-associated protein 9
CRISPR	Clustered regularly interspaced short palindromic repeats
DEXA	Dual energy x-ray absorptiometry
DP	Dunedin PACE of aging calculated from the epigenome
EEA	Extrinsic epigenetic age
ELISA	Enzyme linked immunosorbent assay
FFM	Fat-free mass
FSH	Follicle stimulating hormone
FST	Follistatin
HbA1C	Hemoglobin A1C
Hcy	Homocysteine
HDL	High density lipoprotein
HPG	Hypothalmic-pituitary-gonadal
HsCRP	High sensitivity C-reactive protein
IEA	Intrinsic epigenetic age
LDL	Low density lipoprotein
LH	Luteinizing hormone
mTOR	Mechanistic target of rapamycin
PEI	Polyethyleneimine
SMAD	Small (worm) + Mothers against Decapentaplegic (fly)
TG	Triglycerides
TGFβ	Transforming growth factor beta

Tables

GROUP	n	AGE		
		MEDIAN	MIN.	MAX
All	43	46	23	88
Male	29	42	23	88
Female	14	57	27	86

Table 1: Number of subjects and sex and age statistics.

Value	Mean change (Δ) from baseline	Median Δ	Min Δ	Max Δ	Unit	SE	p-value
FST	**+15.44**	**+12.10**	**+1.67**	**+59.51**	**ng/ml**	**2.13**	**0.001*** **
FFM	**+1.96**	**+1.40**	**-1.29**	**+12.15**	**lbs.**	**0.6**	**0.001*** **
BF%	**-0.87**	**-0.01**	**-3.80**	**+1.50**	**%**	**0.31**	**0.010***
A:G	-0.015	-0.011	-0.155	+0.150	-	0.015	0.32
BMD	+0.0047	+0.0038	-0.0495	+0.0735	g/cm^2	0.0047	0.092
hsCRP	-0.3	+0.0	-6.6	+2.9	mg/L	0.4	0.463
Hcy	-2.5	+0.3	-71.7	+5.7	μmol/L	3.3	0.145
FSH	+2.0	+0.7	-14.2	+27.7	mIU/mL	1.6	0.270
LH	+0.9	+0.7	-16.2	+10.6	mIU/mL	1.0	**0.035***
Glucose	+2	+1.75	-17	+28	mg/dL	2	0.487
HbA1c	+0.1	+0.1	-0.3	+0.4	%	0.05	0.228
Insulin	+1.5	+1.6	-9	+20.1	μIL/ml	1.1	0.191
Leptin	+0.4	+0.2	-2.4	+3.3	ng/ml	0.3	0.139
TC	+5	+2	-28	+55	mg/dL	6	0.378
LDL	+8	+2	-21	+53	mg/dL	5	0.223
HDL	+2	+1	-11	+32	mg/dL	2	0.509
TG	-4	+1	-100	+36	mg/dL	7	0.962
IEA	-3.22	-1.33	-21.08	+18.35	years	1.88	0.102
EEA	**-7.10**	**-5.90**	**-27.91**	**+10.19**	**years**	**2.05**	**0.004** **
Tel	+0.05	+0.04	-0.27	+0.36	kB	0.03	0.184
DP	+0.007	+0.040	-0.39	+0.1	yrs/yr	0.024	0.229

Table 2: Results.

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