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Results of a 5-Year N-of-1 Growth Hormone Releasing Hormone Gene Therapy Experiment

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Abstract

Here presented for the first time are results showing persistence over a 5+ year period in a human who had a hormone gene therapy administered to muscle. This growth hormone releasing hormone (GHRH) therapy was administered in two doses, a year apart, with a mean after the second dose of 195 ng/mL (13 × normal, $\sigma = 143$, $\sigma_M = 34$, max = 495, min = 53). This level of GHRH therapy appears to be safe for the subject, although there were some adverse events. Insulin-like growth factor 1 levels were little affected, nor were the growth hormone test results, showing no indications of acromegaly for the hormone homologue used. Heart rate declined 8 to 13 bpm, persistent over 5 years. Testosterone rose by 52% ($\sigma = 22\%$, $\sigma_M = 6\%$). The high-density lipoprotein/low-density lipoprotein ratio dropped from 3.61 to mean 2.81 ($\sigma = 0.26$, $\sigma_M = 0.057$, max = 3.3, min = 2.5), and triglycerides declined from 196 mg/dL to mean 94.4 mg/dL ($\sigma = 21.9$, $\sigma_M = 5.0$, min = 59, max = 133, min = 59). White blood cell counts increased, however, the baseline was not strong. CD4 and CD8 mean increased by11.7% ($\sigma = 11.6\%$, $\sigma_M = 3.3\%$, max = 30.7%, min = −9.6%) and 12.0% ($\sigma = 10.5\%$, $\sigma_M = 3.0\%$, max = 29.1%, min = −6.7%), respectively. Ancillary observations comprise an early period of euphoria, and a dramatic improvement in visual correction after the first dose, spherical correction from baseline (L/R) −2.25/−2.75 to −0.25/−0.5. Over the next 5 years, correction drifted back to −1.25/−1.75. Horvath PhenoAge was cut 44.1% post-treatment. At completion, epigenetic age was −6 years (−9.3%), and telomere age was +7 months (+0.9%).

Keywords: GHRH, GRF, GHRF, growth hormone releasing hormone, somatocrinin, somatoliberin, somatorelin, self-experimentation, n-of-1

Introduction

This self-experiment by the first author sought to examine the safety and effects of a growth hormone releasing hormone (GHRH) homologue over a period of 5 years in a single individual in good health. GHRH is named for the first function it was found to perform, triggering release of growth hormone (GH). However, GH is suppressed by higher levels of insulin-like growth factor 1 (IGF-1), GH, and glucocorticoids.[1–3]

The GHRH receptor (GHRHR) is expressed in many tissues besides the pituitary, including lymphocytes, uterus, ovary, testis, placenta, cerebral cortex, kidney, prostate, liver, and lung.[4] GHRHR is present as well at relatively high levels in the spleen, thymus,[5] and heart,[6] with therapeutic effects from GHRH administration.[7] GHRH also has therapeutic effects on cognition.[8,9]

These features make GHRH of great interest to increasing health span in older adults. The presence of literature on GHRH gene therapy in animals appeared to show safety[10,11] even with hormone levels 25–50 times the normal maximum.[12] Since gene therapy tends to have an initial spike in expression, followed by a drop to sustained expression, this wide range of tolerance made it reasonable to consider a human self-experiment.

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N-of-1 self-experiment: limitations and value

A limitation is that an N-of-1 experiment lacks statistical power. However, medicine was built on case reports, and because physiology is largely similar, many results tend to be upheld, with exceptions for certain areas such as cancer treatment and for detecting outlier adverse effects. Adverse events and positive findings are subject to confirmation, and we cannot be certain that any specific finding will be repeatable. That said, the authors do not find it is credible to ascribe the effects described to placebo.

Ethics and self-experimentation

Two of the authors examined the ethics and regulation of self-experiments intensively, culminating in a journal publication.[13] There are 14 Nobel Prizes awarded to self-experimenting scientists, with 7 Nobel Prizes in the area of their self-experiment, and no ethical obstructions to self-experimentations. There are 473 documented self-experiments, 48 of them since 1975, with multiples of this number estimated. This study was entirely defined and developed by the first author, who provided training and oversight to the surgeon. Others discussed markers, how to best follow the subject, and effects.

Methods

Protocol

Injection locations were marked, and the largest practical bore (22 gauge) was used to minimize DNA shearing during injection. Smooth injection was performed with the aid of a jig for precision bolus placement in the electrical field between electrodes. The injection needle was fully withdrawn before electroporation (100 V, 5 cycles of 50 ms, 1 second between cycles). Time between withdrawal of the syringe needle and electroporation was kept to the minimum. Two identical injections were done symmetrically, one in each thigh, and marked with a tattoo.

For the first set of injections in 2014, there was no anesthetic, nor site chilling. For the second, anesthetic and icing were performed. Xanax (1 mg) was administered 1 hour before second injections, however, this was unnecessary. Lidocaine (1%) was injected per site, 1 cc intradermal and 2 cc intramuscular, to prevent activation of the muscle. Sites were chilled for 10 minutes with an ice pack. Residue in vials was sent out for confirmation sequencing.

Plasmid

The expressed peptide is a 31 aa sequence: MHVDAIFT NSYRKVLAQLSARKLLQDILNRQ, under control of a myosin promoter instead of a constitutive promoter. The myosin promoter couples expression of the gene product to exercise/repair inoculated muscle. The myosin promoter is also a safeguard against the possibility of cancer. While cancer has not been reported in literature for bare DNA gene therapy, it is a theoretical possibility, dependent on a promoter splicing into the chromosome. Plasmid was preserved by lyophilizing with a 20:1 ratio of pharmaceutical sucrose and storing at −20°C.

Doses

Bare DNA in phosphate-buffered saline (PBS).

Dose 1: 2.5E-14 mol divided into two injections delivered in 500 μL of PBS. Dose 2: 5.0E-14 mol divided into two injections with the same media.

Assays and markers

A broad-spectrum blood profile monitoring, testosterone, inflammatory markers, acromegaly markers, lipid panel, liver/kidney metabolic panel, and complete blood count (CBC) lymphocyte differential, was used at regular intervals in addition to tests for GHRH (Appendix Table A1).

Quest Diagnostics performed routine blood work with a few exceptions. Preliminary blood work came from a health care provider. The majority of GHRH assays were performed by the laboratory of George Church at Harvard, with the balance at Butterfly Sciences, using a GHRH ELISA inhibition assay [GRF (human) EIA; Cat No. S-1172.0001; Peninsula Laboratories International, San Carlos, CA/BMA Biomedicals, Augst, Switzerland]. The protease inhibitor used in serum for some of the GHRH assays is aprotinin (VWR International, Radnor, PA).

Telomere testing was provided by Cell Science Systems, Deerfield Beach, FL. Epigenetic age testing was provided by EpiAging USA, Brick, NJ.

A certified clinical research professional (CCRP) followed the subject and was kept informed of all the developments. The CCRP and a representative from the Church laboratory observed the second inoculations. The first inoculations were observed by a coauthor. A postdoc from the Church laboratory accompanied the subject to obtain a blood draw and took immediate custody of the sample.

Ethics

As a self-experiment this study was exempt, however, an IRB was obtained, IRCM-2016-093.

Results

Subjective first-person overview

A self-experiment provides for more than an objective observation of the experiment. The subjective experience can also inform us, allowing observations that could be missed. This experiment generated a number of such, and a third person will be dispensed with for this section.

The first inoculation was traumatic, causing strong activation of the quadriceps, and an electrical shock sensation. Inoculation sites on both legs felt ''hit with a hammer.'' Modifications made the second inoculation go smoothly. This is ascribed to two things. Without tetany of muscle cells near the electroporation site, there was no trauma to the muscle from that cause. Also, chilling prevented tissue heating.

I was surprised (because I believed that this dose would be too low to be perceptible) that in the first half-hour, I felt a tingling sensation that I had never experienced. I speculate that this was due to rapid stimulation of gonadotropin release, and the rise in testosterone level fits this idea. The first inoculations were primarily intended as a live test of the protocol. Low dose was serendipitous, as I well may have

canceled the experiment and removed the site if the euphoria had continued to increase.

Over the first several days I felt better and better—my legs and whole body felt lively when going cycling, and I wondered if this could plausibly be a placebo effect. This liveliness then went over the edge into euphoria that was so strong I did not care enough to bother putting my foot down as I fell over on my bicycle due to moving too slowly. I think this suggests that GHRH, through receptors in the central nervous system, has an upregulating effect on a range of neurotransmitter receptors in the receptorome.

A curious effect on muscles occurred in the first week that I suspect is connected to later developments. During arm weight work, a sensation occurred as if miniscule spots at or near the attachments of upper body muscles were popping. This slight stinging sensation was so distributed, and so tiny for each of the countless locations, that it didn't bother me enough to stop. Later, I had old soft tissue injuries recur, a second lumbar disc herniation, then a new shoulder injury. This shoulder injury occurred on a relatively light body weight rep after a maximal weight effort competing with young men in their mid-20s. The injury was not a full tear.

However, there was an unusual event that suggests something more. I had a motorcycle accident at 18, which left me with a gouge in my right kneecap and a lump of collagen/scar $\sim 0.6\,\text{cm}$ thick $\times 1\,\text{cm} \times 2.5\,\text{cm}$. This lump spontaneously came loose and slipped down under my skin. It was absorbed in a 3-week period.

The first hypothesis about these injuries is that higher exercise tolerance drove my body beyond its current limits. The second hypothesis is based on speculating what saturation levels of this hormone might do to a senescent cell. It may be that senescent cells respond and create weaker tissue, or undergo apoptosis in doing so. A third hypothesis is that there may be an expression level of GHRH that corresponds to childhood, perhaps very young childhood, and triggers some neotenous cell growth pattern.

Because of concerns about further soft tissue effects, in July of 2019, I decided on a course of senolytics (dasatinib 400 mg and quercetin 4 g per day for 5 days) repeated 2 months later. Since then, there have been no new events.

A cycling crash (over 20 mph) shortly after the second course of senolytics resulted in a mild concussion, and no other injuries, despite hitting so hard, that immediately afterward I was sure I had multiple broken bones. I have had cycling accidents in the past, breaking both wrists, a collarbone, etc. This crash was like having an accident in my 20s. As I sprinted to avoid a speeding car, I put too much focus on the car that stopped half-way across the intersection with squealing tires, and caught a pedal on the pillar in the middle of the bike path entrance.

The mental effects were pleasant after the first inoculation and largely so after the second. However, the second inoculation also included disturbing effects. My physiological responses to the world around me changed completely. This isn't a mental thing, it's in the body, what the Japanese call the Hara. It became apparent that my identity foundation is integral with this. I talked with a psychiatrist, and did meditative exercises intentionally embracing and accepting who I was now. This was difficult, including what most would call nightmares. My unconscious operated like a child's, piecing metaphors together to understand what I had done. I didn't feel afraid, I felt unmoored, wondering why I felt no fear. This was probably a dose effect.

An effect I didn't expect and still remains is that I feel I felt rejuvenated after doing leg work. This isn't a minor effect. I consistently go in tired and by the end of my workout feel like doing it again. This begins to dissipate 2–3 hours later, and I suspect it is a direct effect of GHRH production triggered by use of the affected muscles. This also signals that upregulation of the myosin gene happens within 20–30 minutes of heavy exercise stimulation.

Sleep improved dramatically, becoming like a teens sleep for a couple of months after first inoculation. This faded, in part, probably from stress, but overall sleep improved. Since 2 months after second inoculation I wake up so hungry I cannot sleep. Eating a sizeable (800–1000 calories) meal before bed can sometimes get me through 6 hours. However, I should note that my normal exercise schedule is 6–7 days a week, 1–2.5 hours per weekday session and up to 5 hours on weekends.

The GHRH graph shows impressive expression for more than 5 years, the first finding of such long-term expression. After 5 and ½ years, this level of long-term expression does appear to be reasonably safe.

Baseline physical condition

Baseline physical condition for the subject was generally good, with old injuries and a few years of nagging joint pains. The subject was fairly athletic for most of his life, engaging in running, hiking, swimming, bicycling, high intensity interval training, yoga, and gymnastics. At the time of the first inoculation, the subject would cycle for an hour 2 days per week, lift weights 3 days per week for 2–3 hours, and cycle 30–60 miles 1 day per week with a friend. Weight was 74.5 kg, height 1.753 m, body mass index 25, with 16% body fat.

The most significant preexisting injury was an L4-L5 disc herniation that required a laminectomy microdiscectomy in 2001 (14 years prior) after paralysis developed. The prognosis was that continuation of degenerative disc disease would probably affect other discs. In addition, both shoulders and both knees had been injured in gymnastics and running. The left elbow had dislocated 180°, a tear had occurred in the upper midback, and miscellaneous sprains to shoulders and ankles. The right shoulder was broken into five major pieces in a cycling accident followed a year later by surgery to regain full mobility. Other minor injuries such as broken wrists had also occurred in the previous 20.

Events: adverse, favorable, and neutral

Of the three grade 3 events out of the 25 events in this study, 2 are injuries that are possibly related to the therapy, the other is unrelated. There are three possibly related grade 2 events, and the other four events are related to the therapy. Of the grade 1 events, 4 are possibly related, and 7 related to the treatments (Fig. 1, Table 1).

Positive observations

Horvath PhenoAge, telomere, and epigenetic age. The Horvath PhenoAge equation was scaled to synchronize to baseline as a measure of aging.[14] This was done as a more conservative way to interpret results. The scaled results (Fig. 2) suggest a mean decrease of 28.6 years (–44.1%)

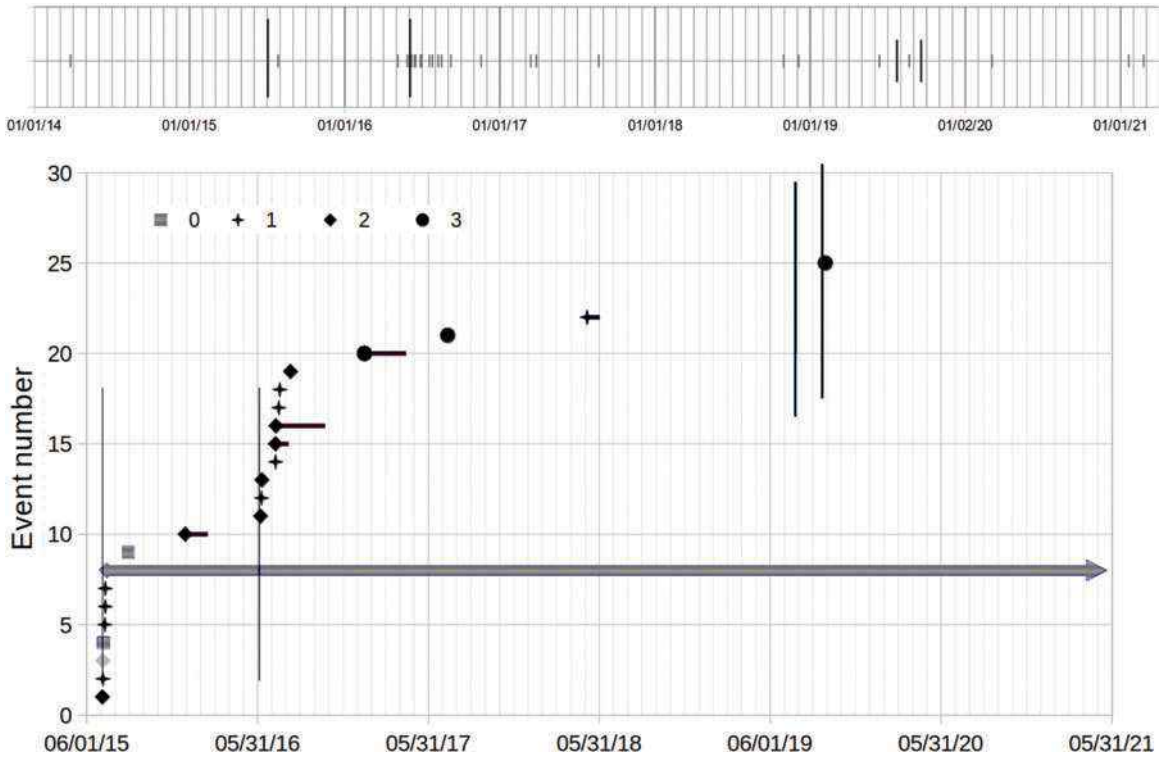

FIG. 1. (Upper) Time line—Tall black bars are gene therapy inoculations (July 5, 2015, and June 4, 2016), short gray bars are blood draws. Midsize black bars are senolytics (July 25, 2019, and September 20, 2019). (Lower) Adverse, favorable and neutral events by date. Grade 0 is favorable/neutral. Grade 1—mild, grade 2—moderate, grade 3—temporarily disabling. Gray-horizontal stripe = favorable, light gray = neutral, black = adverse. Horizontal lines indicate ongoing event. Black vertical bars are inoculation dates, and blue vertical bars are senolytic dates. One grade 2 event began as adverse concern, but resolved as favorable. Color images are available online.

from baseline for this therapy. The drop at the end is probably due to lifting dietary change restrictions. Unscaled Horvath PhenoAge baseline was 26.9 years; post-treatment mean = 15.0 years ($\sigma = 0.86$, $\sigma_M = 0.23$).

At the end of the study, telomere age (Cell Science Systems, Deerfield Beach, FL) was 65, well within one standard deviation of normal. Epigenetic age (EpiAging USA, Brick, NJ) was tested at 58. Calendar age was 64.

Euphoria. The subject reported being euphoric after the first inoculation, a feeling of ''more intense reality'' with joyful/blissful body feelings. One adviser to the study was

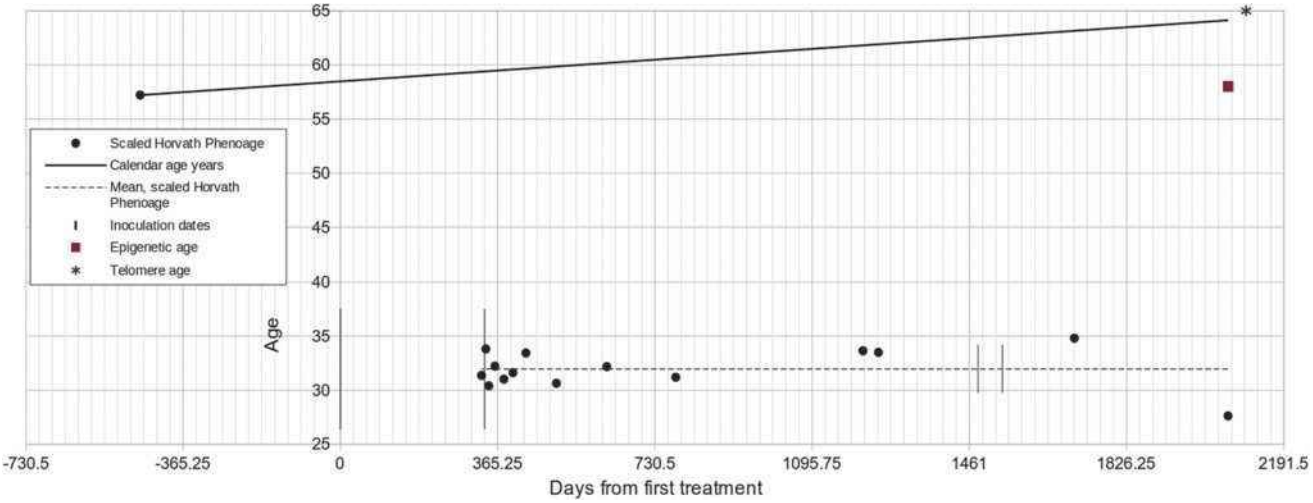

FIG. 2. Age measures—Solid line shows predicted PhenoAge of subject for duration of the study. Black dots show scaled PhenoAge-calculated results. Dashed line is mean post-treatment scaled PhenoAge 32 years ($\sigma = 1.84$, $\sigma_M = 0.49$). Tall vertical bars are gene therapy inoculations. Short vertical bars are senolytics. Color images are available online.

quite concerned, being of the opinion that euphoria was probably signaling pathology, and so, it was logged as a mild grade 1 adverse event. The subject did not think it was pathological, nor adverse.

The second inoculation did not result in a repeat of euphoria. However, 2 days before the second treatment, a severe emotional stressor began that continued for a year. This suggests that the euphoria may be situational and required relaxed circumstances to appear.

Faster healing. Subject reported faster healing of sports injuries. A chronic shoulder injury resolved almost entirely after the first inoculation. When the subject incurred a new injury to this same shoulder at the end of December of 2015, he reported that he was doing full-weight pull-ups 4 weeks later, although the shoulder still had some pain for a few more weeks. Mid-August of 2016, an upper back injury occurred from weighted dips with 100 lbs (45.45 kg), double his usual. A previous injury to the same spot that occurred 5 years prior required almost a year to heal. This injury resolved within a month.

Apparent resolution of arthritic ankle and multiple locations of tendonitis. Low-grade arthritic left ankle pain of several years standing could flare and interfered intermittently with hiking resolved rapidly after the 2015 first treatment. Subject reported sporadic stinging pain in that location starting at the end of the second week after the 2016 second treatment, which resolved within the first month. Subject reported resolution of chronic tendonitis in multiple locations after the 2015 inoculation.

Testosterone up, IGF levels virtually unchanged. Testosterone level is plotted against the 25th to 75th percentile (dashed line) of nonobese men (374–605)[14] using a rate of decline of 1.6% per year[15] (Fig. 3). Baseline testosterone rose from 484 to a high of 846, and settled into the 600–800 range. Total testosterone normal range is 250 to 1100 pg/mL. Some aging physicians consider 500 pg/mL to be the proper normal level, and some target the 700 to 900 ng/mL range.

IGF-1 (not shown) had a minor bump after inoculation to 130–189 ng/mL (mean 152 ng/mL, $\sigma = 20.1$, $\sigma_M = 6$ ng/mL). Peak of 189 ng/mL occurred on March 15, 2017, and the final three test results were 141, 124, and 134 ng/mL. All IGF-1 test results are well within the 84 to 257 ng/mL normal range for the subject's age.[16] Thus, acromegaly does not appear problematic for this GHRH homologue. Three blood tests for GH were conducted on day 0 (baseline), day 13, and day 27, consisting of a fasting dose of 75 g of glucose, followed 90 minutes later by the blood draw. All three showed GH levels of 0.1 ng/mL or less. No further GH tests were conducted.

Lipid profile improved. After the 2015 first treatment, the low-density lipoprotein (LDL)/high-density lipoprotein (HDL) ratio baseline (3.61) dropped into optimum range, remaining there. Post-treatment, the mean LDL/HDL ratio = 2.81, $\sigma = 0.26$, $\sigma_M = 0.057$. Cholesterol normal range is 125 to 200 mg/dL. From 200 and 239 mg/dL is considered borderline high, with 240 mg/dL as the high boundary. A probably more accurate measure is the LDL/HDL ratio[17] (Fig. 4).

- Subject's triglycerides began in the borderline high range (196 mg/dL), dropping to low or median normal. Post-treatment mean triglycerides = 94.37, $\sigma = 21.89$, $\sigma_M = 5.02$.
- Healthy median HDL = 50 mg/dL, high HDL ≥60 mg/dL, low HDL ≤40 mg/dL. The entire range of HDL for the subject from 64 to 88 mg/dL is in the highest part of the standard range. Post-treatment mean HDL = 74.43, $\sigma = 7.37$, $\sigma_M = 1.69$.
- Healthy LDL has no lower limit. The LDL optimum range varies based on whether there is diabetes or heart disease. In the absence of both, LDL of 100 to 129 mg/dL is considered normal, and LDL ≥159 exceeds top of range. The subject's LDL baseline was 139, and has varied within a range from 99 to 137 since. After the first inoculation, 9 out of the 11 samples were ≤129. Two were in the borderline high range, however, high HDL is considered protective. Post-treatment mean LDL = 112.53, $\sigma = 11.51$, $\sigma_M = 2.64$.

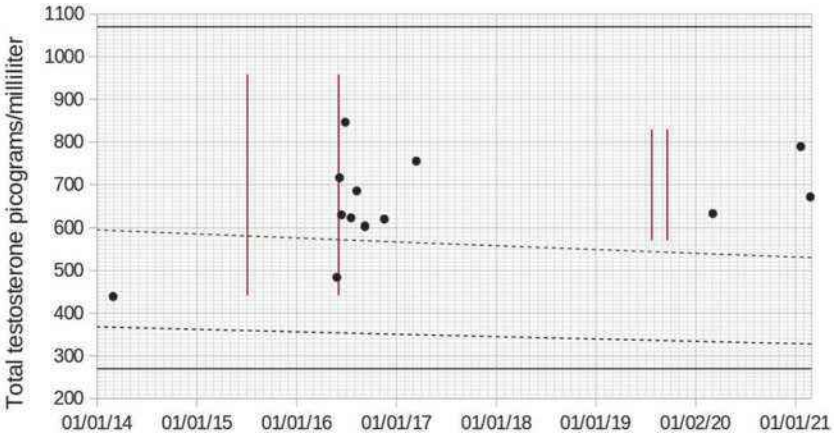

FIG. 3. Total testosterone. Tall vertical bars are gene therapy inoculation dates. Short vertical bars are senolytics. Upper and lower solid horizontal lines are total normal range. Dashed lines are 25th and 75th percentiles for subject age (*i.e.*, mean ± 25%). Free testosterone had a similar graph (not shown). Mean post-treatment 671 pg/mL, $\sigma = 96$, $\sigma M = 28$. A rise of 53%, $\sigma = 22\%$, $\sigma M = 6\%$. Color images are available online.

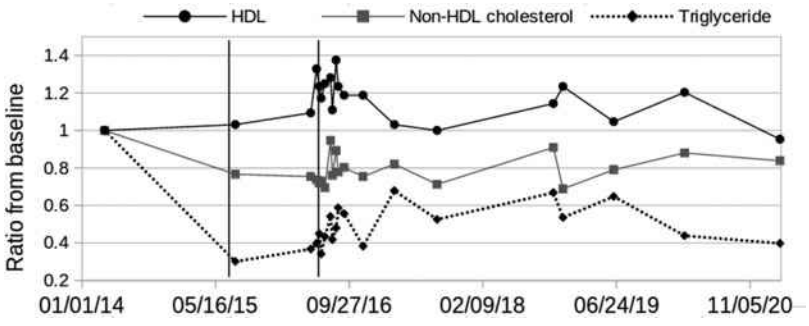

FIG. 4. Lipid profile—Ratios to baseline and values. Efforts were made not to change dietary or exercise patterns throughout the 5-year test. Mostly this was managed, however, the postinoculation sweet tooth period was not possible to completely resist. From the time of the second inoculation, more lipid raising foods (gelato, cheese, eggs) were consumed.

Pulse rate dropped by 8 to 13 bpm. Figure 5 shows pulse as low as 45 bpm in the morning, although most are above 50 bpm. The subject had no history of resting pulse below 60 as an adult.

Blood pressure stable, may have slightly declined. Systolic: AM mean = 114, $\sigma = 7.47$, $\sigma_M = 0.66$, max = 129, min = 86; PM mean 116, $\sigma = 8.27$, $\sigma_M = 0.74$ max = 141, min = 96. Diastolic: AM mean = 68, $\sigma = 4.19$, $\sigma_M = 0.37$, max = 86, min = 54; PM mean = 66, $\sigma = 5.44$, $\sigma_M = 0.49$, max = 81, min = 48. Subject reported instances of orthostatic hypotension.

White blood cell counts. White blood cells (WBC, not shown) were monitored as an indicator of possible thymic/immune system function improvement, due to thymic GHRHR. There may be an effect within the high degree of fluctuation versus baseline, and the baseline was weak. WBC mean rose 20.2% ($\sigma = 10.1\%$, $\sigma_M = 2.8\%$, max = 37.1%). CD4's mean rose 11.7% ($\sigma = 11.6\%$, $\sigma_M = 3.3\%$, max = 30.7%) and CD8's 12.0% ($\sigma = 10.5\%$, $\sigma_M = 3.0\%$, max = 29.1%).

Visual correction changed. Serendipitously, new prescription was acquired shortly before the first inoculation. Within a week of treatment, it was impossible to read a street name sign at 25 feet. A second eye examination within 3 weeks of the first showed remarkable improvement (Fig. 6).

GHRH levels increased with good persistence. GHRH levels are dramatic with better than expected persistence (Fig. 7). Normal human range is 7–15 ng/mL. Natural GHRH has a half-life in the bloodstream of roughly 12 minutes. However, the synthetic construct probably has a half-life of hours. Thus, the synthetic homologue overwhelms the normal version. Because the promoter is tied to activation of myosin synthesis, more fluctuation was expected than is visible here. Animal studies have shown a tolerance of similar magnitude over normal with GHRH levels as high as 500 ng/mL.[12]

Discussion

Sans anesthetic, electroporation elicited the remark, ''On a POW that would be a war crime.'' Available literature greatly understated sensations. With lidocaine, there was no discomfort.

Whether this experiment will lead to greater longevity is unknown. GHRH supplementation could lower life span by possibly pushing cells to turn over faster, hitting their

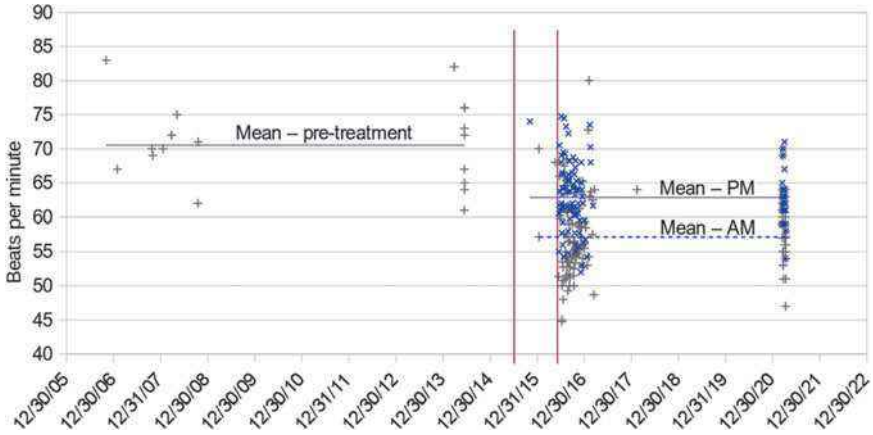

FIG. 5. Resting pulse rate. Vertical bars are inoculation dates. x symbol is PM pulse. + symbol is AM pulse. Mean pretreatment = 70.5, $\sigma = 6.18$, $\sigma_M = 1.50$. Post-treatment AM mean = 57.27, $\sigma = 5.52$, $\sigma_M = 0.48$. Post-treatment PM mean = 62.87, $\sigma = 4.74$, $\sigma_M = 0.43$. Color images are available online.

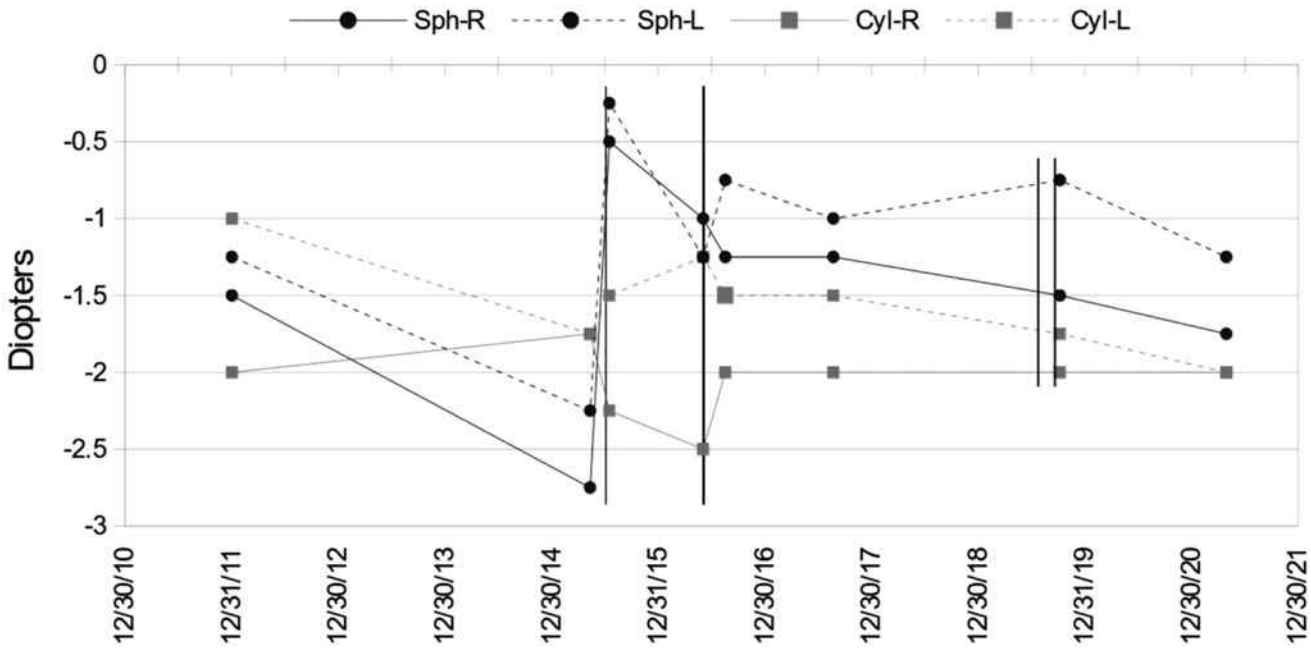

FIG. 6. Eyesight correction—Subject is nearsighted. Spherical (Sph) correction is the nearsighted or farsighted correction for the whole lens of the eye. A correction of zero is 20/20 vision. Cylindrical (Cyl) correction is for astigmatism and has an angle. Astigmatism angle differences (not shown) were within the range of measurement error.

Hayflick limit faster. The jury is out on this, and likely to remain so for this subject, who is unlikely to refrain from further experiments.

Blood draws became problematic due to the number of vials (7) per draw and frequency. Phlebotomists held needles in their hands while switching between the seven vials required, and were unable to avoid significant vein damage. Butterfly needles (supplying the kit) were more tolerable. However, using butterfly needles, if the vials were not switched quickly enough, the line stopped flowing.

Telomere results at the end of the study period were completely normal, unlike PhenoAge or epigenetic age. This suggests that an increase in maximum life span is unlikely, and compression of aging symptoms toward end of life is likely, as seen with senolytics. The epigenetic age change is relatively minor, a 6-year difference. However, the Horvath PhenoAge measure gave startling results.

The subject was already an outlier on the Horvath PhenoAge[18] scale, and there was discussion with the author of the PhenoAge article culminating in errata in the published

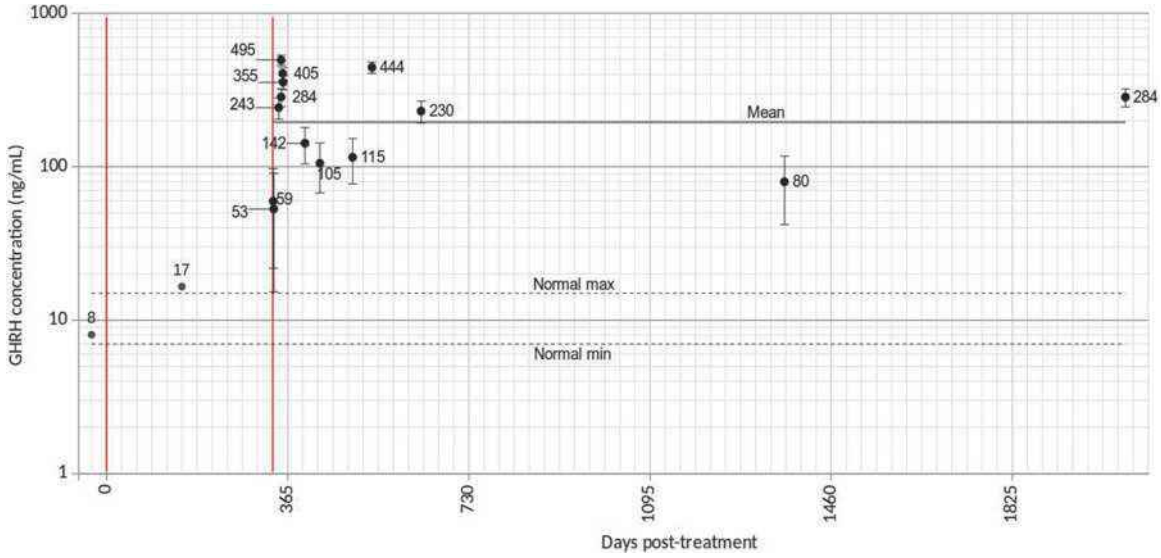

FIG. 7. GHRH levels ng/mL. Normal range (dashed lines) is 7 to 15 ng/mL. Vertical red bars are treatment dates. Mean of GHRH readings after second inoculation, 195 ($\sigma = 143$, $\sigma_M = 34$) is shown as gray line. The error bars show $\sigma_M = 34$ for the second set of readings on a log scale. GHRH, growth hormone releasing hormone. Color images are available online.

Table 1. Events: Four Grade 0, Two Favorable/Two Neutral; Eleven Grade 1, Mild; Seven Grade 2, Moderate; Three Grade 3, Disabling for More than a Day

Event	*Grade*	*Category*	*Type*	*Description*	*Start*	*Days*	*Comment*
1	2	Adverse	Related	Electrical activation	July 5, 2015	0	Muscle fibers triggered. Large movement. High discomfort.
2	1	Adverse	Related	Discomfort	July 6, 2015	5	Tender/sore injection sites. ''Hit with a hammer.''
3	1	Neutral	Related	Sleepiness	July 6, 2015	5	Fell asleep quite a few times. Needed 10+ hours per day.
4	1	Favorable	Possible	Right ankle no pain	July 7, 2015	0	Chronic right ankle joint pain was gone.
5	1	Adverse	Related	Twitching and nightmares	July 10, 2015	0	Woke up from nightmares about electroshock with both sites twitching.
6	1	Adverse	Related	Fell over	July 11, 2015	0	Fell over cycling due to feeling so euphoric.
7	1	Adverse	Related	Euphoria	July 11, 2015	6	Sometimes blissfully disinterested in anything.
8	1	Favorable	Related	Better with exercise	July 14, 15	0	First mention of exercise-related good feeling increase.
9	1	Favorable	Related	Old inflammation resolving	August 29, 2015	0	Could sleep on my side for first time in years. Sore joint areas resolving.
10	2	Adverse	Possible	Right shoulder pop	December 29, 2015	48	Popped shoulder. Dislocated and snapped back.
11	2	Adverse	Related	Blood draw trauma	June 7, 2016	7	Too many blood draws, veins torn up both arms.
12	1	Adverse	Related	Highly emotional	June 9, 2016	2	Highly emotional about things, and higher intensity to sights and sounds.
13	2	Adverse	Possible	Polyuria/hyponatremia	June 10, 2016	1	Polyuria/hyponatremia cured by salt. Overexercised in high temperature
14	1	Adverse	Possible	Arthralgia	July 9, 2016	15	All over joint discomfort.
15	2	Adverse	Related	Nightmares	July 9, 2016	28	Nightmares and intense dreams until resolution: I am a different person.
16	2	Adverse	Related	Identity/acceptance crisis	July 10, 2016	105	Feeling so different that I worry about losing who I am
17	1	Adverse	Possible	PVC while hiking	July 16, 2016	0	Strong PVC while hiking (talked with doctor).
18	1	Adverse	Possible	PVC while cycling	July 18, 2016	0	Strong PVC while cycling at high effort (talked with doctor).
19	2	Adverse	Possible	Back muscle pull	August 10, 2016	22	Pulled muscle in my back at old injury site. Magnetic resonance imaging showed no disc/connective injury.
20	3	Adverse	Possible	L3/L4 disc protrusion	January 15, 2017	89	Mind-breaking pain. New disc herniation. Carried full refrigerator 2 days before
21	3	Adverse	Possible	Left shoulder	July 12, 2017	24	Partial tear of muscle attachment.
22	1	Favorable	Possible	Right knee old scar came off	May 6, 2018	27	Lump of collagen scar detached under the skin. Absorbed in 30 days.
23	0	Favorable	Unrelated	First Dasitinib+Quercetin	July 25, 2019	5	Senolytic course.
24	0	Favorable	Unrelated	First Dasitinib+Quercetin	September 20, 2019	5	Senolytic course.
25	2	Adverse	Unrelated	Cycling accident	September 27, 2019	0	Cycling accident at over 20 Mph. Hit very hard. Mild concussion. Treated with montelukast.

PVC, preventricular contraction.

equation. The corrected Horvath equation put the subject's baseline PhenoAge at 26.91 years, final PhenoAge of 12.99 years, and mean post-treatment PhenoAge of 15.03. For this reason, PhenoAge was used as a scaling factor rather than directly.

Authors' Contributions

G.C. provided testing of GHRH, critique, and discussion. K.B. was the attending physician and provided monitoring and comments. B.P.H. was the primary author and initiated, designed, and directed the project.

Author Disclosure Statement

The first author conceived this gene therapy as a potential product that would be part of an HIV/AIDS rescue system and a possible health span product; the second and third authors have no competing interests.

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(Appendix follows →)

Appendix

Appendix Table A1. Blood Work Profile

Testosterone	Lymphocyte differential blood panel
Total testosterone, ng/dL	White blood cells, k/mL
Free testosterone, pg/mL	Absolute lymphocytes
	CD4%
Inflammatory markers	CD4 cells/μL
Interleukin-6, pg/mL	CD8%
C-reactive protein, mg/L	CD8 cells/μL
	Absolute neutrophils
Acromegaly tests	Absolute monocytes
Insulin-like growth factor-1, ng/mL	Absolute basophils
Growth hormone, 75 g glucose +90 minutes ng/mL[a]	Absolute eosinophils
Glucose, fasting	CBC with differential, platelets
	RBC, M/mL
Lipid panel	Hemoglobin, g/dL
Cholesterol, mg/dL	Hematocrit
Triglyceride, mg/dL	MCV, fL
High-density lipoprotein, mg/dL	RDW, RBC %
Low-density lipoprotein—calculated, mg/dL	Mean platelet volume, fL
Prostate-specific antigen[b]	Platelets, k/mL
Liver/metabolic panel	Neutrophils, %
Alanine aminotransferase	Lymphocytes, %
Aspartate aminotransferase, U/L	Monocytes, %
Bilirubin, mg/dL	Eosinophils, %
Alkaline phosphatase	Basophils, %
Creatinine, mg/dL	Neutrophils, k/mL
Blood urea nitrogen, mg/dL	Na, mmol/L
Estimated glomerular filtration rate, mL/minutes/1.73 m^2	K, mmol/L
CO_2, mEq/L	

All other tests were part of the full profile.
[a]Not part of the general profile. This test was conducted 3 times on a separate day from the other blood tests.
[b]Not part of the general profile. Conducted one time, at the request of the IRB panel. Test was negative.
CBC, complete blood count; MCV, mean corpuscular volume; RBC, red blood cells; RDW, red blood cell distribution width.