Research Paper

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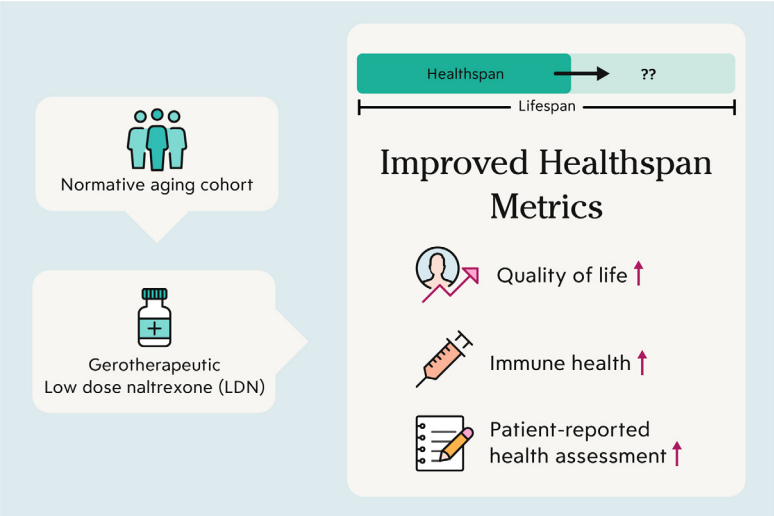

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Research Paper

Low-Dose Naltrexone as a Potential Healthspan-Enhancing Intervention in a Normative Aging Cohort: Changes in Quality of Life and Immune Health Metrics

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Low-dose naltrexone (LDN) has been suggested to target the multiple hallmarks of aging and improve healthspan metrics in humans. However, to date, no studies have evaluated LDNs potential as a gerotherapeutic. We collected real-world data utilizing the short form 36 quality of life (QoL) assay, immune status questionnaire, and a patient-reported health assessment to evaluate the effectiveness of LDN for improving multiple healthspan metrics in a normative aging cohort still within their healthspan. Most participants taking LDN for ≥3 months (69.2%) had a significant improvement in mean QoL scores (29.9%). The largest improvements were observed in the QoL categories of energy and fatigue, physical role limitations, emotional role limitations, social functioning, and pain. Participants also exhibited a significant improvement in mean immune function (24.6%). Data from a patient-reported health assessment at ≥4 weeks (N = 5500) and ≥10 weeks (N = 1450) of LDN use revealed that a majority of participants reported improvements in pain, fatigue, inflammation, and mood. A healthspan-enhancing drug should demonstrate the ability to enhance the health of individuals before significant age-related diseases and disability arise, thereby extending the period of life spent in good health. We found that 45% of responders to LDN had average to above-average baseline QoL scores, which increased to 76.6% of responders after LDN treatment. Furthermore, 23.8% of individuals taking LDN were able to discontinue other medications and 10.5% of participants reported avoiding planned clinical procedures. These data suggest that LDN might play a role in enhancing healthspan, warranting further research into its potential geroprotective effects.

Introduction

Aging can be characterized as a time-dependent functional decline in physical, cognitive, and physiological health. The biological aging process is driven by cellular damage and dysfunction that increases an organism's vulnerability to age-related diseases, frailty, and death[1]. Age-related diseases, such as cardiovascular disease, cancer, and osteoarthritis, are the major causes of morbidity and mortality within the United States[2,3]. Data from the U.S. Centers for Disease Control and Prevention (CDC) suggest that 60% of Americans live with at least one chronic age-related disease[4,5]. Furthermore, data from the Global Burden of Disease Study suggest that the average American spends almost 50% of their lives in less-than-optimal to poor health[6]. These statistics highlight the need for a preventative healthcare approach focusing on proactive health optimization rather than reactive disease management. The field of longevity medicine has adopted this approach to target the aging processes and optimize an individual's healthspan—the period of life lived in good health, free of frailty, and age-related chronic disease—before significant pathology arises[7].

Utilizing a preventative approach to target the root cause of age-related diseases, aging biology, might be the most effective route to reduce their incidence and the health decline that precedes them. Such an approach holds promise to significantly improve the health and quality of life (QoL) of an aging population. One of the most promising areas of translational geroscience is the repurposing of U.S. Food and Drug Administration (FDA)-approved drugs with gerotherapeutic potential[8]. Repurposed gerotherapeutic interventions have been demonstrated to target the fundamental molecular pathways that drive the biology of aging, gathered significant preclinical evidence in enhancing lifespan and healthspan, and have been shown to target multimorbidity and lower the incidence of diseases in large epidemiological studies. These include drugs such as rapamycin, metformin, and sodium-glucose transport protein 2 inhibitors (i.e., canagliflozin), and potentially, low-dose naltrexone (LDN)[9].

Naltrexone is a nonselective opioid antagonist that is FDA approved for the treatment of opioid and alcohol dependence at doses of 50–150 mg/day. It has a high affinity for μ-opioid receptors (MORs), blocks the inhibition of the gamma-aminobutyric acid

receptor, and inhibits dopamine release, which interferes with the pleasurable feeling associated with these dependencies, thus mitigating addictive behaviors[10].

Alternatively, in doses between 0.5 and 9 mg, LDN is known for its immunomodulatory effects and has been suggested to improve health and QoL in individuals with autoimmune conditions, such as fibromyalgia, myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS), multiple sclerosis (MS), and Crohn's disease[11]. Furthermore, evidence is emerging for LDNs efficacy in addressing chronic inflammation and compromised immune function (collectively known as "inflamm-aging") that accompanies a range of age-related chronic diseases, such as osteoarthritis, Parkinson's disease, and cancer[12–14]. Intriguingly, in a previous paper, we demonstrated that the therapeutic role LDN may play as a part of a combinatorial intervention (alongside NAD+) to address long COVID-related persistent fatigue[15].

One of the main mechanisms of action behind LDNs effects on chronic inflammation has been demonstrated to be related to its inhibitory effects on Toll-like receptor 4 (TLR4) on cells of the innate immune system, which mediate a proinflammatory response through, among other factors, the release of cytokines like interleukin-6 (IL-6) and their downstream effects[16–19]. TLR4 is activated by damage-associated molecular patterns, molecules released upon cellular stress or tissue injury, and pathogen-associated molecular patterns, molecules associated with pathogen infection, both of which increase with age, leading to a chronic inflammatory state. By blocking TLR4 and blunting the secretion of proinflammatory cytokines, LDN addresses fundamental imbalances in the age-related immune response that drive the aging process, namely a hyperresponsive immune state[20,21]. By breaking the vicious cycle of low-level sterile chronic inflammation that accompanies (and likely precedes) nearly every chronic disease of aging, LDN holds great potential as a gerotherapeutic candidate[22].

Another mechanism through which LDN exerts its action is by temporarily blocking the opioid growth factor receptor (OGFr) and the MOR[12]. Of note, OGFr expression is highest in monocytes, microglia, and lymphocytes, supporting its efficacy in addressing pathologies that compromise cognitive and immune health[23]. Upon LDN administration, the transient blockade of OGFr results in a compensatory increase in OGFr and OGF expression levels. As LDN blockage wears off in the following hours, the signaling effects of the additional OGF and OGFr are amplified[13]. This subsequently improves the regulation of cell growth, promotes healing, reduces inflammation, and stimulates autophagy[24]. Furthermore, LDN has been shown to increase the production of natural killer T cells that boost the efficiency of the adaptive immune system for clearing cancer cells and fighting infections[10].

A growing body of evidence supports LDNs potential to address multimorbidity by targeting distinct molecular pathways that converge on immune health and chronic inflammation[11,13,14,25]. This, combined with decades of clinical safety data[11,26–28], lends great promise to efforts determining whether LDN might serve as a gerotherapeutic candidate. However, to date, no such data has been published.

In a previous review, we highlighted the need for more longevity studies evaluating the efficacy of repurposed, FDA-approved interventions in improving healthspan metrics within normative aging human cohorts and emphasized the role of collecting real-world evidence to accelerate their validation as gerotherapeutics[29]. We highlighted QoL as an important healthspan metric, proposed QoL data as a biomarker of aging and endpoint for geroscience clinical trials, and suggested the short form 36 (SF-36) as a well-suited QoL assay for this purpose[29].

In the present exploratory pilot study, we hypothesized that LDN might demonstrate gerotherapeutic effects within a normative aging cohort by improving multiple aspects of health and QoL that decline with age and are critical components of healthspan. To test this hypothesis, we collected real-world data from two large, normative aging cohorts utilizing the SF-36, immune status questionnaire (ISQ), and a participant-reported outcome questionnaire to evaluate the effectiveness of LDN for improving these healthspan metrics and determine whether it may be a good candidate for further assessment as a gerotherapeutic.

Methods

Study design

This was a single-center, retrospective, observational study conducted as a decentralized trial. Participants were located across the United States and participated via a telemedicine platform using the study sponsor's website (www.agelessrx.com).

The study was conducted in accordance with the standards of Good Clinical Practice, as defined by the International Conference on Harmonization and all applicable federal and local regulations. The study protocol was approved by the Institutional Review Board of the Institute of Regenerative and Cellular Medicine (IRCM; approval number IRCM-2022-346).

Participants seeking treatment with LDN via the telemedicine platform were asked to complete a series of questionnaires over several months assessing changes to various healthspan metrics, such as QoL, general physical and mental health, family history, medication use, and immune status. The standard dosing practice protocol for LDN was to start with an oral dosage of 1 capsule (1.5 mg LDN) at bedtime for 10 days, then increase to 2 capsules for the following 10 days, then increase to 3 capsules daily, and if well tolerated, 4.5 mg is used as the final dosage. However, dosing was optimized based on the participant's adverse event (AE) profile and tolerance and subject to change based on the individual.

The study consisted of two different cohorts to evaluate the clinical effectiveness of LDN in improving various healthspan metrics, such as QoL outcomes and immune health. For the LDN QoL cohort, two different endpoints were assessed, the SF-36 survey to obtain QoL data and the ISQ to obtain immune function data, both of which are standardized clinical assessments. For the LDN check-in cohort, more general participant check-in data were collected, evaluating self-reported improvements in healthspan metrics, such as pain, energy, inflammation, and mood. Due to the simplicity and feasibility of the questions administered to the LDN check-in cohort, it was amenable to collect data from a much larger cohort. All assays were optional and electronically administered. Participants were prompted to engage with assessments throughout the trial period through periodic email reminders. Data were stored and analyzed through AgelessRx Health Insurance Portability and Accountability Act of 1996 (HIPAA)-compliant electronic medical record (EMR) database. A study flow chart is depicted in **Supplemental Figure S1**.

Participants

AgelessRx participants were eligible for inclusion in the study if they requested LDN from the AgelessRx Telehealth platform, were deemed to be a good candidate for LDN, and consented to share medical information. For the LDN QoL cohort, inclusion was contingent upon completing the SF-36 QoL questionnaire

and ISQ at baseline and at or after 89 days on-treatment (follow-up assessment). For the LDN check-in cohort, inclusion was contingent upon the completion of at least one participant check-in survey at various intervals after LDN administration (check-in 1 was conducted approximately 28 days after starting LDN administration and check-in 2 was conducted at or after 70 days on-treatment). Participants were excluded from taking LDN if they were "deemed medically unfit" based on the concurrent use of any narcotic medication, pregnancy or breastfeeding, history of psychiatric hospitalization, active cancer or malignancy, under 18 y of age, presence of uncontrolled/unmanaged disease, or history of significant cardiac, renal, or hepatic dysfunction. Participants were included in the study if they indicated having non-age-related syndromes, such as fibromyalgia and ME/CFS as these pathologies do not have a clear association with age and are distinct from age-related disease.

Outcome measures LDN QoL cohort

Primary outcome measure

The QoL data were collected using the standardized self-assessment questionnaire SF-36. The SF-36 is a 36-item survey that evaluates eight health categories, such as physical functioning, pain, energy and fatigue, emotional wellness, general health, social functioning, and role limitations due to physical and emotional health. The SF-36 questionnaire provided each participant with a score for each health category ranging between 0 and 100[30]. Scores in the ranges 50–60 represent an "average score" for adults in the United States, accordingly, those receiving higher or lower scores represent "above-average scores" (associated with improved performance) and "poor scores" (associated with pathology), respectively[31–38]. Overall, SF-36 scores were calculated as the average of the scores of the eight health domains. For the purpose of expanding on the aspects of healthspan evaluated, participants were also asked to complete the ISQ. The ISQ consists of seven, five-point Likert questions that are optimized to obtain self-reported data to evaluate immune health status by assessing general immune health as well as the frequency of symptoms indicative of immune issues over the past three months. These include fever, diarrhea, headache, skin problems, muscle and joint pain, common cold, and cough. The ISQ has been validated as a tool for evaluating immune health in response to interventions within different patient demographics, including students, adults, and individuals with chronic disease[39–41].

For the ease of interpreting ISQ scores alongside the SF-36 and further expanding its role as a multivariate healthspan metric, the ISQ scoring scale was standardized and recalculated relative to the SF-36 scoring system. The ISQ is scored on a scale from 0 (poor immune function) to 10 (excellent immune function) and this was translated to an SF-36 score of 0–100 by increasing each ISQ score by a factor of 10. The ISQ (immune function) score was not included in the calculation for the overall SF-36 score.

Secondary outcome measures

Secondary outcome measures for the LDN QoL cohort included data on LDN dosage and self-reported discontinuation of any medications and/or avoidance of clinical procedures since starting the LDN regimen. Demographic data collected included age and sex.

Outcome measures LDN participant check-in cohort

Primary outcome measures

Participants in the LDN participant check-in cohort completed an electronically administered self-reported check-in survey to collect data on the effectiveness of LDN. Effectiveness was determined as self-reported improvement or deterioration in various healthspan metrics with the following questions: "since starting LDN have you seen improvements in your aches and pain?," "since starting LDN have you seen an improvement in your mood?," "since starting LDN have you seen an improvement in your inflammation?," and "since starting LDN have you seen an improvement in your fatigue?." Participant responses were qualitative in nature with self-reported responses confined to whether these various healthspan metrics "got worse," "no noticeable improvement," "mild improvement," "moderate improvement," and "considerable improvement" since starting LDN or since the last check-in was performed.

Secondary outcome measures

Secondary outcome measures for this cohort included the frequency and type of AEs and the reasons for seeking LDN prescription.

Timepoints

Participants within the LDN QoL cohort were prompted to complete surveys to measure primary and secondary outcome measures before treatment to establish baseline scores and at least 89 days after treatment initiation to track trajectories of change (on-treatment score).

Participants within the LDN participant check-in cohort were prompted to complete surveys at or after 28 days on LDN treatment with a follow-up check-in sent at or after 70 days on LDN treatment, with regular reminders sent until surveys were completed.

Statistical analysis

For the LDN QoL cohort, data collected from questionnaires were deidentified and compared across demographic and clinical characteristics. The mean and standard deviation for each health category, and the overall SF-36 score, were calculated at baseline and after LDN treatment. To assess whether there were significant changes in each item from baseline to treatment follow-up, the Wilcoxon signed-rank test was employed. The statistical analysis was conducted using R (Version 4.0.2), and the significance level was set at a p-value <0.05. Discontinuation of medications or avoidance of clinical procedures was presented as a percentage of all participants or all responders.

The analysis for the LDN participant check-in cohort was a crude analysis consisting of a general evaluation and quantification of the percentage of participants experiencing degeneration or benefits over time in various healthspan metrics after taking LDN. Observed changes after each check-in were quantified as a percentage of all participants who completed each check-in.

Results

Study population

Data for the LDN QoL cohort were collected between August 12, 2021 and February 4, 2023. Participants were included in the analysis if they met the eligibility criteria for LDN prescription and completed both a baseline assessment and a follow-up assessment at least 89 days after the baseline assessment.

For the LDN participant check-in cohort, data were collected between April 7, 2022 and February 9, 2023. Participants were included in the analysis if they met the eligibility criteria for LDN prescription and completed at least the check-in 1 survey

(28 days on LDN treatment) and/or check-in 2 survey data (at least 70 days on LDN treatment).

Demographics LDN QoL cohort

A total of 3500 normative aging individuals between the ages of 19 and 96 y completed the SF-36 survey to evaluate baseline health status. Of the 3500 participants that took the baseline assessment, 665 (19.0%) completed a second assessment ≥3 months (range 89–425 days) after initiation of LDN treatment and were included in our study group to determine the effects of LDN on SF-36 scores. The average age of participants was 54 y, with >85% of participants aged ≥40 y and 32.1% of participants aged >60 y (Table 1). Furthermore, 80.6% of participants were female and 19.4% were male, and 17 sex data points were missing (Table 1). Participants' LDN dose ranged from 0.5 to 9.0 mg/day. As 4.5 mg/day LDN seems to be the most commonly used effective dose based on clinical efficacy data[11,14,42,43], participants typically were advised to start at a 1.5 mg/day dose and gradually increase the dose to 4.5 mg/day (in 1.5 mg increment increases every two weeks) based on tolerability. Most participants in the study (65.6%) were taking a dose of 4.5 mg/day as their maintenance dose and ≤10% of participants taking each of the other doses (Table 1).

Demographics LDN participant check-in cohort

The LDN participant check-in cohort consisted of 12,134 normative aging participants who were prescribed LDN, of whom 9085 (74.8%) were female and 3049 (25.1%) were male. Of these participants, 5500 (45.3%) completed check-in 1 (≥28 days on LDN treatment; range 28–48 days) and 1450 (11.9%) completed check-in 2 (≥70 days on LDN treatment; range 70–205 days) to determine the effects of LDN on various healthspan metrics, such as pain, inflammation, mood, and fatigue.

The reasons for taking LDN were quantified for all 12,134 participants who were prescribed LDN and are listed in **Supplemental Table S1**, stratified by sex. Participants listed as primary reasons for taking LDN: to reduce inflammation (31.4%), reduce aches and pains (24.9%), reduce fatigue (14.6%), encourage weight loss (10.9%), improve mood (6.9%), addiction control (5.4%), and other (nonspecified) reasons (5.8%).

Table 1. Demographics of LDN QoL cohort (N = 665).

Age	n (%)
<40 y	106 (16.2)
41–50 y	183 (28.2)
51–60 y	152 (23.5)
≥61 y	208 (32.1)
Missing	17 (2.6)
Sex	
Male	126 (19.4)
Female	522 (80.6)
Missing	17 (2.6)
LDN dosage	
0.5–1.5 mg/day	22 (3.3)
3.0 or 4.0 mg/day	89 (13.4)
4.5 mg/day	436 (65.6)
5.0 or 6.0 mg/day	30 (4.5)
7.0, 8.0, or 9.0 mg/day	20 (3.0)
Unknown	17 (2.6)

LDN, low-dose naltrexone; QoL, quality of life.

LDN administration for ≥3 months improves mean QoL and immune function

Participants completed a baseline SF-36 QoL and ISQ assessment before taking LDN (baseline score) and a follow-up assessment at least 3 months after starting daily LDN administration (on treatment score). For the 665 participants in the LDN QoL cohort who completed the surveys, the mean total SF-36 score significantly improved from a baseline score of 55.8 (SD 21.3) to an on-treatment score of 64.0 (SD 19.8), representing a 14.7% improvement ($P < 0.001$) (Fig. 1; **Supplemental Table S2**). Previous research has shown that a score of 50 is an average score for the general U.S. population[31,33,35,37,38]; therefore, this significant improvement shifted participants from an average score to an above-average overall SF-36 score.

All individual health categories assessed by the SF-36 significantly improved following LDN administration, with a minimal 10% improvement in scores for six out of eight health domains (Fig. 1; **Supplemental Table S2**). This is a particularly noteworthy improvement as LDN was administered to a normative aging population without unmanaged age-related chronic disease. The largest improvements were observed in the health categories "energy and fatigue" in which participants had a mean baseline score of 32.2 and an on-treatment score of 42.5 (31.9% improvement; $P < 0.001$) and "physical role limitations" in which participants had a mean score of 49.5 at baseline and an on-treatment score of 62.7 (26.9% improvement; $P < 0.001$) (Fig. 1; **Supplemental Table S2**).

As the geroscience field has revealed, interventions that target and preserve immune functionality are promising gerotherapeutic candidates[44–46]. We next evaluated how immune status is influenced in participants taking LDN for ≥3 months.

Immune health is a critical component of healthy aging with a considerable impact on an individual's healthspan[47,48]. Immune dysfunction and chronic inflammation increase with age are primary drivers of age-related adverse outcomes, such as frailty and the chronic diseases of aging (i.e., cardiovascular disease, cancer, type 2 diabetes, and Alzheimer's disease)[22,49]. Therefore, engaging with interventions that preserve immune health and functionality throughout the aging process is a promising gerotherapeutic strategy[44–46].

The ISQ is a well-validated, standardized assessment tool utilized to evaluate the changes in immune health status in normative aging populations over time and response to interventions[39,40]. We recalculated the participant's ISQ score relative to the SF-36 scoring system to be included with the other SF-36 health categories for ease of interpretation. We observed a substantial improvement in the average immune function scores of participants, as the baseline score of 59.3 went up to an on-treatment score of 69.2, representing a 16.7% improvement in immune health score (Fig. 1; **Supplemental Table S2**).

Responders to LDN

The results highlighted above on improvements across the entire LDN QoL cohort after ≥3 months of LDN treatment take into account responders, participants who exhibit improvements after taking LDN, as well as nonresponders, participants who exhibit no change or a decline in scores after taking LDN. We next

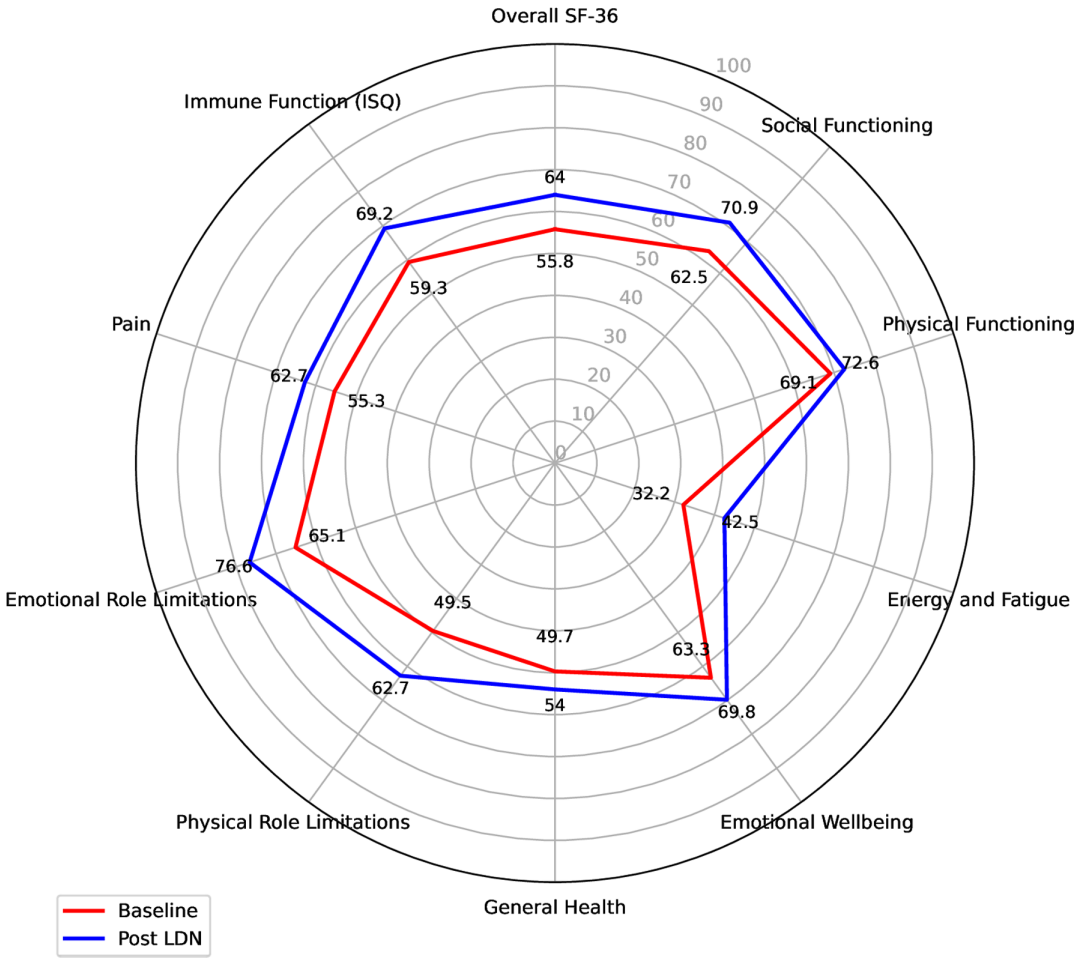

Figure 1. Spyder plot depicting mean baseline (red lines) and on-treatment scores (blue lines) for total SF-36 scores, and each of the health domains of the SF-36 and ISQ scores normalized to the SF-36 scale.

determined the percentage of individuals who could expect a benefit from LDN and the magnitude of benefits for both primary endpoints, by characterizing responders.

Of the 665 participants in the LDN QoL cohort, 69.2% (n = 460) experienced any improvement in their overall SF-36 QoL, exhibiting a significant 29.9% improvement from a mean baseline score of 51.7 to an on-treatment score of 67.2. To verify that most of this improvement was not due to a small subset of outliers, we next evaluated the proportion of responders that exhibited at least a 15% or 20% improvement in SF-36 QoL from baseline. Among the 460 responders, 280 individuals (60.9%) had at least a 20% improvement in their overall SF-36 QoL and 70.7% (n = 325) exhibited at least a 15% improvement, demonstrating that a majority of responders experienced relatively large magnitude improvements in QoL from LDN treatment.

Change in SF-36 health categories in responders

In responders (based on SF-36 scores), at least a 25% improvement in scores was observed for five out of eight health categories, such as social functioning (57.3 vs. 74.8, 30.5% improvement), energy and fatigue (29.2 vs. 45.9, 57.2% improvement), physical role limitations (42.0 vs. 68.6, 63.3% improvement), emotional role limitations (58.1 vs. 80.6, 38.8% improvement), and pain (51.6 vs. 65.6, 27.5% improvement) from baseline to on-treatment, respectively (Fig. 2; **Supplemental Table S3**). Notably, mean on-treatment scores in six out of eight health categories of the SF-36 (excluding "energy and fatigue" and "general health") represented above-average scores, as determined in previous studies[33,38,50]. In four of eight health categories, mean on-treatment scores were above 70 suggesting LDN facilitated improvements toward an optimal state of health. This applied to scores for social functioning (74.8), physical functioning (74.3), emotional well-being (71.8), and emotional role limitations (80.6) (Fig. 2; **Supplemental Table S3**).

Consistent with effects seen across the total LDN QoL cohort, responders based on the SF-36 scores demonstrated significant improvement in immune function score, which improved by 24.6% from a baseline of 56.4 to an on-treatment of 70.3 (Fig. 2; **Supplemental Table S3**).

Sex- and age-specific differences in responders

We compared the 522 female and 126 male participants in the LDN QoL cohort in terms of responders and nonresponders, to assess whether there were sex-specific differences in response to LDN. Almost 70% of both sexes (361 females [69.1%] and 88 males [69.8%]) demonstrated improvements in their on-treatment SF-36 scores relative to baseline (Fig. 3A). When assessing differences between age groups, we found a majority of participants demonstrated improvements in their on-treatment SF-36 scores in all age categories. However, the youngest participants,

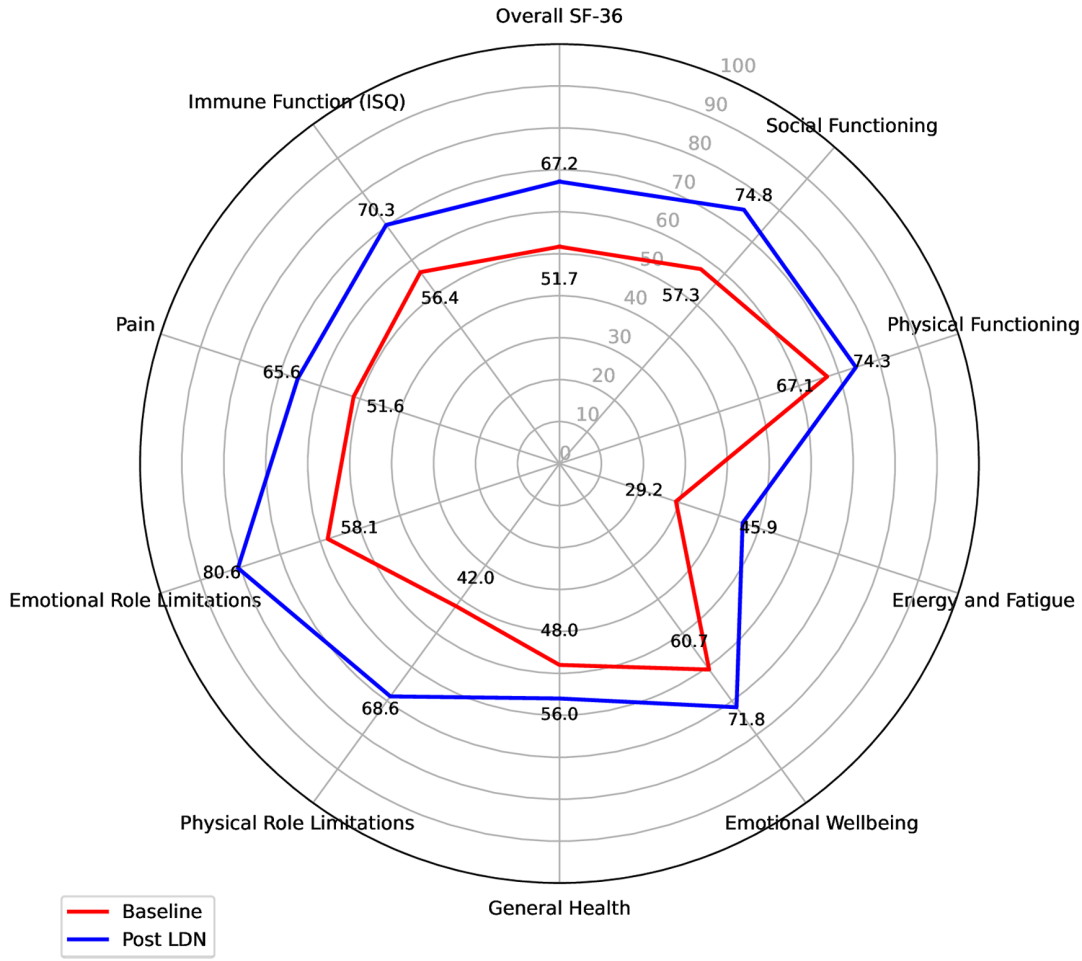

Figure 2. Spyder plot depicting responder mean baseline (red lines) and on-treatment scores (blue lines) for total SF-36 scores, and each of the health domains of the SF-36 and ISQ scores normalized to the SF-36 scale. Responders were defined as those participants with any change in total SF-36 scores between baseline and on-treatment assessment.

those younger than 40 y, had the highest percentage of responders (80.0%), and the oldest participants, over the age of 61 y, had the lowest percentage of responders (65.4%) (**Fig. 3B**). Notably, there was nearly double the number of participants in the 61+ age subgroup compared with the younger than 40 subgroup.

Changes in overall SF-36 in individuals with self-reported syndromes

One of the main hypotheses we tested in this study was whether LDN might demonstrate gerotherapeutic effects by improving healthspan metrics within a cohort not afflicted by chronic age-related disease. Because the LDN QoL cohort included some individuals afflicted with various syndromes, we postulated that this subgroup of participants could represent a disproportionate percentage of the improvement observed in the SF-36 score in the total normative aging cohort.

We stratified participants based on the self-reported presence of syndromes (e.g., ME/CFS, fibromyalgia, and MS). This group represented 23.9% of the participants. Next, we evaluated changes in total SF-36 scores between those who self-reported a syndrome compared with those who did not. In the syndrome subgroup, the mean baseline SF-36 score was 44.4 and the mean on-treatment SF-36 score improved to 55.6, with a mean difference of 11.2. In contrast, those who did not indicate having a syndrome had a mean baseline SF-36 score of 59.4 and mean on-treatment SF-36 score of 66.6, with a mean difference of 7.2. This translates to a four-point mean difference in improvement in SF-36 score between the participants who reported having a syndrome compared with those who did not. Furthermore, as 69.2% of participants were responders to LDN and only 23.9% of the total cohort indicated having a syndrome, improvements in SF-36 detected in this study are not likely solely driven by the subgroup with syndromes.

LDN as a healthspan-enhancing candidate: LDN improves the QoL scores of participants who are already performing well at baseline

Given the observations that the majority (69.2%) of normative aging individuals are responsive to LDN and demonstrate significant improvements in multiple domains of health and QoL, with the youngest age demographic having the highest percentage of responders, we rationalized that LDN may serve as a healthspan-enhancing intervention. By its very definition, a drug that enhances healthspan should improve or optimize the health of individuals who are already generally healthy in contrast to only being effective in those with a poor health status[29,51]. The SF-36 uses a norm-based scoring scale where scores of 50 are considered as average, scores of 0 are considered as poorest, and scores of 100 represent perfect health[33,38,50]. To validate the potential of LDN

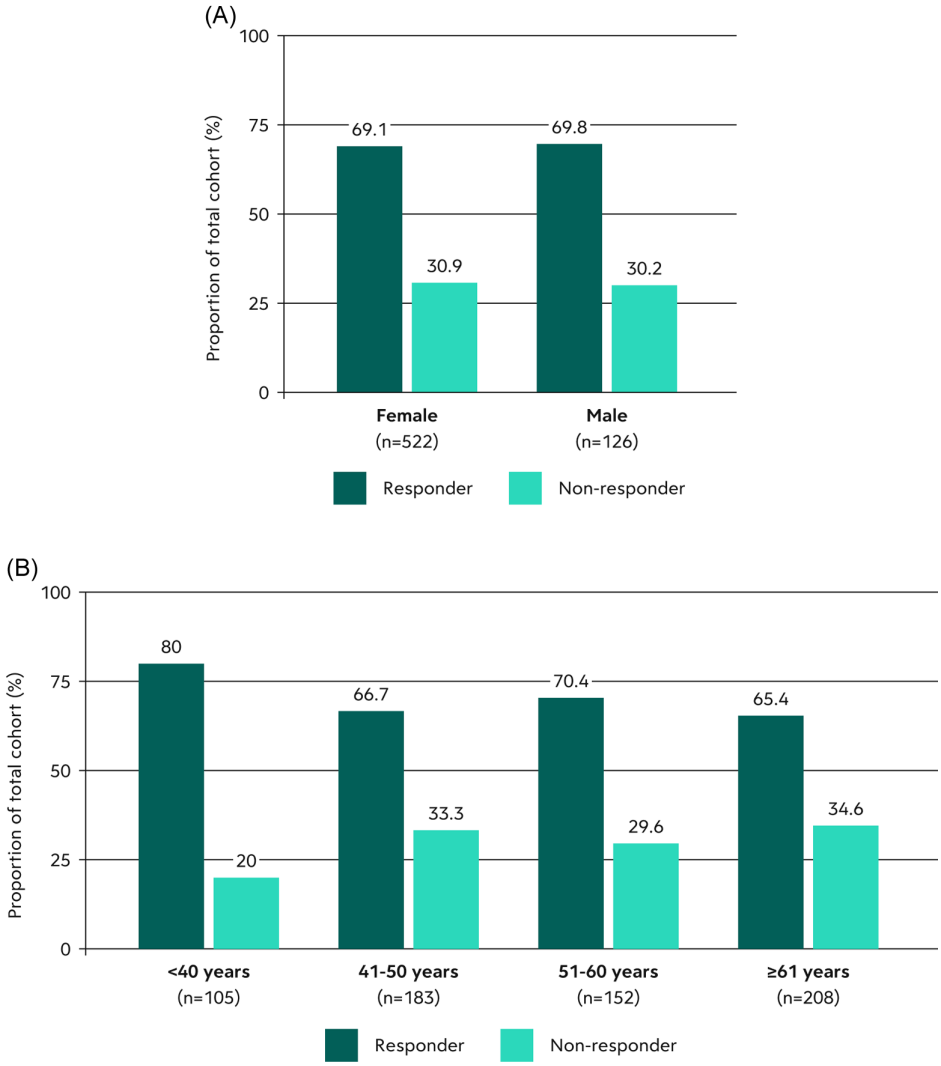

Figure 3. Characteristics of responders: **(A)** male and female responders and **(B)** responders and nonresponders in each age group. Responders were defined as those participants with any change in total SF-36 scores between baseline and on-treatment assessment.

as a healthspan-enhancing gerotherapeutic candidate, we determined whether LDN improved the SF-36 scores of participants who were already performing well based on the overall SF-36 score, as defined by a baseline score greater than 55.

We found that nearly half (45%, n = 207) of participants who were responsive to LDN had an overall baseline SF-36 score of > 55 and their mean change in score from baseline to on-treatment was 9.95 points (SD: 7.84). This change in scores suggests that LDN can not only enhance the QoL of participants with poor baseline scores but also for those with average scores. Furthermore, although 253/460 (55%) responders had a baseline SF-36 score of <55, 340 participants (76.6%) increased their scores on-treatment to a score of >55. These data suggest that in a majority of responders, regardless of their baseline score, LDN improves QoL scores, resulting in scores in the healthy range (average to above-average SF-36 score).

A subpopulation of participants can discontinue medications and avoid clinical procedures after at least 3 months of LDN administration

Healthspan is the period of life that is spent free of disease, frailty, and major age-related disabilities and limitations[7,29,52]. One characteristic of declining healthspan is an increase in the utilization of medications (polypharmacy) and the need for clinical procedures (e.g., hip surgery, knee replacement, and neurostimulator implants). Gerotherapeutic interventions that improve healthspan should prevent or delay the onset of various diseases and dysfunctions resulting in a reduction in the use of medications or the need for clinical procedures[8,53]. Therefore, we tracked the (self-reported) use of medications and clinical procedures to evaluate the changes in participants' health status (and corresponding healthspan) over time.

Within the limited follow-up time after starting LDN administration, 10.5% of participants were able to avoid anticipated/planned clinical procedures. Avoided clinical procedures included neurostimulator implants, bladder cancer removal, thyroidectomy, hysterectomy, cholecystectomy, ankle surgery, rotator cuff surgery, knee replacement, lipedema surgery, and hip surgery. Furthermore, 23.8% of participants in the LDN QoL cohort reported being able to discontinue medications. Medications discontinued mostly included drugs that act on the immune and central nervous system and included anti-inflammatory drugs, pain medication, antidepressants, anxiolytics, migraine medications, epilepsy medications, diabetes

medications, cough suppressants, antibiotics, sleep aids, muscle relaxants, antirheumatics, hypothyroid medications, blood pressure medications, and drugs for blood clot preventions. The most common medications discontinued were antiinflammatories (for pain, allergies, gastrointestinal issues, neurological disorders, and joint inflammation) and pain medications. Interestingly, there were individuals not classified as responders based on improvements in total SF-36 score who were able to discontinue medications and procedures. Furthermore, several individuals indicated being able to discontinue multiple medications, supporting LDNs role in mitigating polypharmacy—a hallmark of the aging population within the United States[53–55]. These results further suggest that LDN may be effective at combating declines in healthspan over time.

LDN check-in cohort

One of the major strengths of utilizing the SF-36 as an endpoint and healthspan metric is that it serves as a standardized, quantitative assessment of the impact of a gerotherapeutic on QoL and healthspan. The SF-36 has been validated as a robust assay for evaluating the effectiveness of interventions in modifying QoL parameters associated with disease progression and age-related decline in normative aging cohorts[29]. However, the SF-36 takes 10–15 min to complete[56], which may have impacted the number of participants in our study, as among several thousands of participants prescribed LDN within our telemedicine platform, a limited proportion was willing to complete the surveys and participate in the LDN QoL cohort study (N = 665). To get a broader understanding of the effects and responsiveness to LDN in a larger cohort, we analyzed data from our LDN patient EMR database (LDN check-in cohort), in which we collected data based on a single question evaluating whether participants experienced benefits in inflammation, energy, pain, and mood (among other health parameters not evaluated in this study) as well as the incidence of AEs. Data from this LDN check-in cohort represent a qualitative, descriptive assessment of LDN efficacy and, due to the ease of completing the assessment, we were able to collect data from thousands of participants (check-in 1: 5500 and check-in 2: 1450).

LDN administration facilitates self-reported improvements in multiple healthspan metrics in the majority of participants in a large normative aging cohort

At the first check-in (at least 28 days after initiation of LDN treatment), participants were asked if they perceived improvements in healthspan metrics since starting LDN and at least 60% indicated that they experienced improvements (mild, moderate, or considerable improvements) across the healthspan metrics surveyed (aches and pain: 69%, fatigue: 60%, inflammation: 66%, and mood: 65%) (**Fig. 4A**). This effect was even more

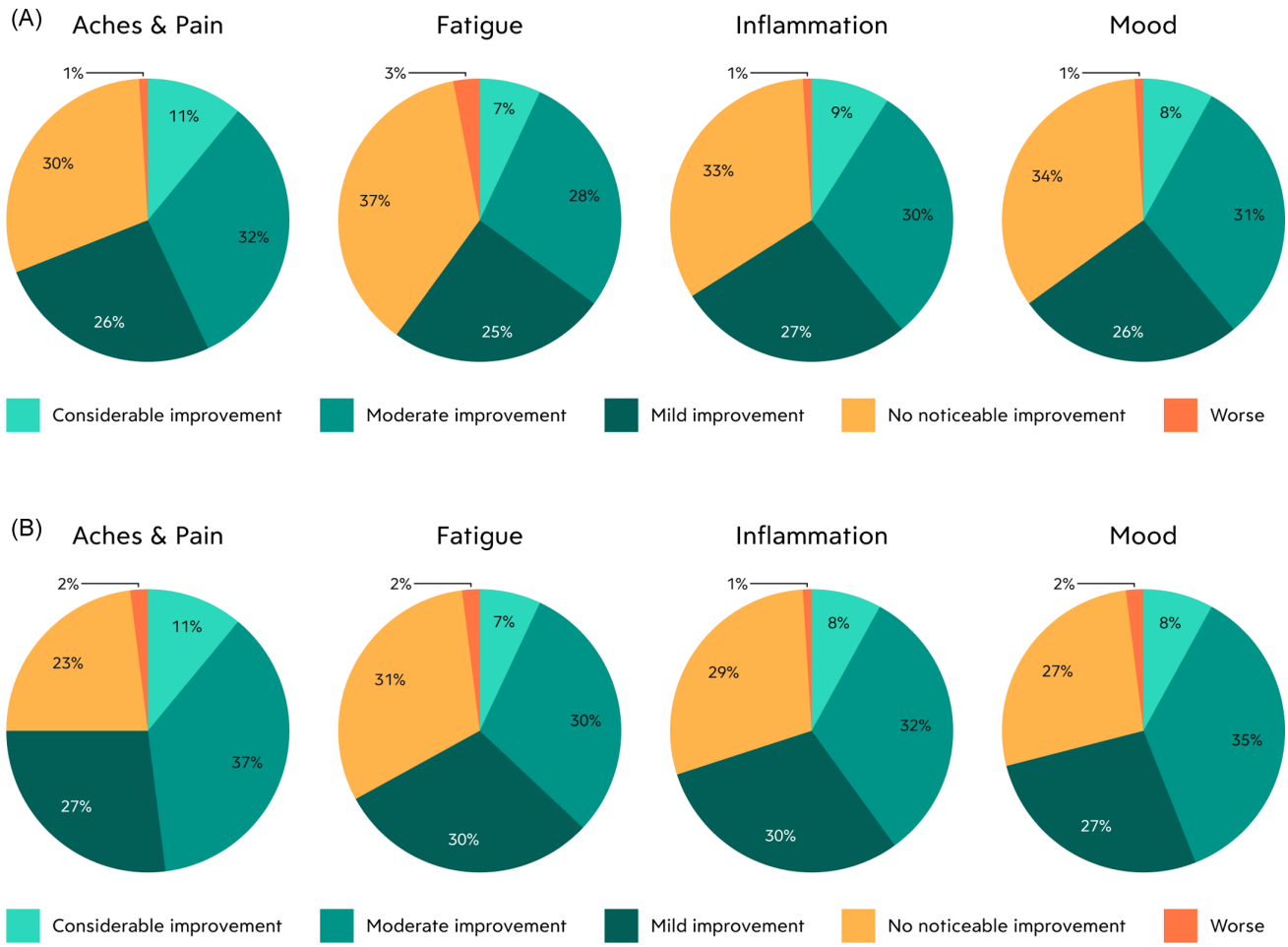

Figure 4. Self-reported effects on healthspan metrics such as aches and pain, fatigue, inflammation, and mood for participants in the check-in cohort: **(A)** check-in 1 and **(B)** check-in 2.

pronounced at the second check-in (at least 70 days after initiation of LDN treatment) where participants were asked if they perceived continued improvements as well as improvements relative to the past 4 weeks as approximately 70% of the study population responded that healthspan metrics surveyed improved (pain: 75%, fatigue: 67%, inflammation: 70%, and mood: 70%) (**Fig. 4B**). On average, fewer than 2% of participants at either check-in responded that the various healthspan metrics surveyed had declined since taking LDN (**Fig. 4A,B**).

We next characterized the relative magnitude of improvement following LDN administration and found that a significant subpopulation of the LDN check-in cohort experienced moderate to considerable improvements across all health domains at the first check-in (aches and pain: 43%, fatigue: 35%, inflammation: 39%, and mood: 39%; **Fig. 4A**) and this improvement persisted or increased upon the second check-in (aches and pain: 48%, fatigue: 37%, inflammation: 40%, and mood: 43%; **Fig. 4B**).

LDN AEs

We also asked the check-in cohort to self-report AEs related to LDN (**Table 2**). Out of the 5995 respondents, 2763 (46.1%) experienced any AE. AEs were typically mild, resolved without treatment within the first couple of weeks of taking LDN, or could be addressed by lowering the dosage of LDN. The most common AEs were sleep disturbances, such as insomnia, restless sleep, and vivid dreams (36.8%); fatigue (25.0%); and headache (15.4%) as self-reported at the first check-in. At the second check-in, fewer AEs were reported (1014 vs. 4,737), which similarly consisted of fatigue (30.9%), sleep disturbances (37.4%), and headache (13.3%) as the most common AEs reported.

Discussion

Geroscience research has evolved rapidly in the past two decades, gathering evidence for promising gerotherapeutic candidates that prolong lifespan and healthspan in animal models, leading to the deceleration and possible reversion of age-related physiological decline[57]. However, translating these results in human studies comes with many challenges, including regulatory hurdles, costs, and study time. This is especially true for studies aimed at demonstrating amelioration or prevention of age-related adverse outcomes (including frailty and disease) in normative aging cohorts. The collection of real-world data, including self-reported assessments of health, daily life activities, mood, and functionality, can help accelerate validation of gerotherapeutics that show potential to enhance healthspan[29,58].

Therefore, we conducted a decentralized study to collect real-world data on the effects of LDN on two healthspan metrics, QoL and immune health (using the SF-36 and ISQ), in a large normative aging cohort. We observed statistically significant improvements in mean scores for both the SF-36 QoL and ISQ surveys after 89 days (and up to 425 days) of treatment with LDN. The largest magnitudes of improvements were detected in immune health (as evaluated by the ISQ), and the SF-36 categories energy and fatigue, and physical role limitations, aspects of age-related decline that severely compromise health and QoL in the elderly.

In this cohort, 69.2% of participants were considered as responders to LDN based on improvements in their overall SF-36 score during the follow-up period, suggesting LDN might have broad effectiveness as a QoL-enhancing gerotherapeutic candidate. One hallmark of a healthspan-enhancing intervention is that it optimizes health in those who are not yet compromised or with significant pathology[29,51,58]. Intriguingly, even though nearly half of responders were already performing average to above average at baseline, LDN improved scores in the majority of participants, supporting LDNs potential as a healthspan-enhancing drug. Furthermore, LDN treatment transitioned many individuals with below average scores to average/above-average scores (scores of 50 and above) and 76.6% of responders to LDN increased their overall SF-36 score to over 55, suggesting meaningful improvements in health as it is compared with the average scores in healthy populations. However, normative data utilized for scaling and interpreting SF-36 scores are based on data from the U.S. general population from the 1990s and early 2000s and these scores may have generally changed over time[33].

One of the most important aspects of our study design is that it evaluated the effectiveness of LDN within a normative aging cohort. Limited data are available on the efficacy of LDN in improving longevity and healthspan in a normative aging population, as it has mostly been prescribed and studied within pathological contexts, such as individuals with MS, ME/CFS, Crohn's disease, and fibromyalgia, diseases that have a high incidence in younger demographics and often have genetic or acute environmental triggers[11,59,60]. Because participants were excluded from our study if they had an uncontrolled or unmanaged age-related disease, presumably most individuals taking LDN were still within their healthspan[51,61]. The most common reasons for buying LDN, for both men and women, were to reduce perceived inflammation and aches and pains. Although we did include individuals with syndromes, they made up 23.9% of the total cohort and there was only a small difference in improvement between those who indicated having a syndrome and those who did not, suggesting similar effectiveness across health demographics.

We supported the quantitative data from our LDN QoL cohort with a survey evaluating participant-reported outcomes for a more descriptive assessment of LDNs effectiveness within a much broader population and observed improvements in healthspan metrics, such as pain, inflammation, mood, and fatigue. Given the lack of a comparable control group, the improvements in healthspan metrics demonstrated in both study groups could be attributed partially to the placebo effect or natural improvements in health over time. Furthermore, the study was conducted in individuals actively engaging with a longevity telemedicine platform, which could introduce selection bias for health-conscious individuals who are more likely to engage with other interventions (lifestyle or otherwise) over the study period that may have

Table 2. Self-reported adverse events in the LDN check-in cohort.

	Check-In 1	Check-In 2
Adverse event	n (%)	n (%)
Fatigue	1,187 (25.0)	314 (30.9)
Headache	734 (15.0)	135 (13.3)
Insomnia	731 (15.4)	153 (15.0)
Vivid dreams	646 (13.6)	139 (13.7)
Upset stomach	527 (11.1)	74 (7.2)
Restless sleep	371 (7.8)	79 (7.7)
Increased anxiety/jittery	329 (6.9)	58 (5.7)
Other	212 (4.4)	62 (6.1)

LDN, low-dose naltrexone.

influenced outcomes. However, given that the average age of participants was above 50, some individuals were followed up for a timeframe of 6.5 months, and most were taking LDN to address persistent concerns, our data suggest that at least a considerable subgroup of participants may benefit from LDN treatment, and outcomes are not wholly attributable to placebo effect. Randomized controlled trials will be needed to robustly validate the observations from this study.

Declines and imbalances in immune functioning are one of the major focuses of the geroscience field as it is recognized as one of the major drivers of biological aging. Indeed, "inflamm-aging" has been coined as a term to indicate the low-level, sterile inflammation that accompanies the aging process and is associated with the occurrence of nearly every chronic age-related disease[22,47,62]. Increased senescent cell burden, the accumulation of advanced glycation end products (AGEs), toxins, pathogen exposure, mitochondrial dysfunction, and other age-related changes and stressors drive inflamm-aging[22,47,62]. Engaging with interventions that preserve immune health and functionality throughout the aging process is, therefore, a promising gerotherapeutic strategy.

LDN has been suggested to mitigate inflamm-aging through multiple routes. The binding of LDN to OGFr suppresses nuclear factor kappa-light-chain-enhancer of activated B cells (NF-KB), activator protein 1 (AP-1), and interleukin-1 receptor associated kinase 3 (IRAK3), all of which are shown to regulate the chronic inflammatory response[11,18,63]. In addition, the binding of naltrexone to TLR4 inhibits inflammation by blocking IL-6 release as well as blocking the binding of TLR4 to other proinflammatory factors that drive chronic inflammation, such as glycated low-density lipoprotein and AGEs[16,17,19,21]. IL-6 is a major signaling factor in the senescence-associated secretory profile implicated in driving senescence-related pathologies[16–21]. Furthermore, LDN has been shown to target known pathways that remedy chronic inflammation, among many other hallmarks of aging, including mammalian target of rapamycin inhibition and sirtuin-1 (SIRT-1) activation[18,64,65]. Finally, the capacity of LDN to modestly increase endogenous opioids has been demonstrated to have anti-inflammatory effects and suggests that it has the potential to reduce stress through the promotion of a greater sense of well-being[66,67]. Alleviating psychological stress has also been shown to reduce levels of systemic inflammation in the body[68].

Our data suggest LDN results in self-reported improvements in immune health as measured by the ISQ as well as reduced inflammation as self-reported in our check-in cohort. This dataset needs to be followed up by measuring changes in the biomarkers of immune health and functionality, such as levels of proinflammatory cytokines (e.g., IL-6), acute inflammatory proteins (C-reactive protein), and emerging markers of chronic inflammation[69,70]. Another limitation is that data were collected during the COVID-19 pandemic and included periods in which participants' behavior may have differed, affecting the risk of common colds and other infections, while the risk of COVID-19 itself may have impacted immune health as well.

Evaluating improvements in healthspan is challenging because it ideally requires longitudinal trials demonstrating delayed incidence of disease. A more tractable way to evaluate the effects of a gerotherapeutic in improving healthspan is through tracking medication use and the need for clinical procedures/surgeries. A hallmark effect of a successful gerotherapeutic is the ability to cut down on polypharmacy and avoid invasive surgical procedures[8,53]. In this study, 23.9% of participants reported to have reduced their medication use and 10.5% reported they were able to avoid planned medical procedures. This is particularly noteworthy as the default case in conventional medicine is to continue to add more medications and clinical procedures as an individual ages[71,72]. These results are limited due to a lack of a control group, its self-reported nature, and some of these reductions in interventions or medication use may also have been due to natural decrease in symptoms and factors unrelated to LDN, such as other interventions not reported to us during the study period.

Another characteristic of gerotherapeutics is that they work to remedy the hallmarks of aging in biologically older organisms, regardless of chronological age. In doing so, these therapeutics could work as well in chronologically younger individuals undergoing premature aging (e.g., with autoimmune conditions) as in older individuals[58,73]. We found LDN to be broadly effective across both sex and age, with the youngest demographic having the greatest proportion of responders, further supporting its validity as a gerotherapeutic.

Self-reported AEs were generally mild and previously noted for LDN in various patient populations[11,14,28]. Furthermore, most of these AEs generally occur within the first few weeks can be minimized by starting at a lower dose and/or resolve on their own[11,14,28]. The retention of participants across the study period suggests that many of the AEs were mild and transient in nature. Furthermore, in the check-in cohort, very few participants indicated their condition got worse over time. However, we acknowledge that our cohort reduced in size over the study period, with a lower number of participants filling out the later surveys. This may be due to participants stopping the use of LDN due to AEs, lack of efficacy, or cost. This may have introduced attrition bias in that those who perceived a benefit from LDN use may have been more motivated to fill out our surveys.

Although our study is the first to be conducted in assessing the efficacy of LDN for improving healthspan metrics in a normative aging cohort, the study design comes with some limitations. One of the major limitations of this methodology of data collection is that it includes self-reported data that rely on subjective assessments and is vulnerable to the placebo effect. We rationalized that, considering the strengths and limitations of each dataset, integrating insights from the LDN QoL cohort and the LDN check-in cohort would provide a more comprehensive and rigorous assessment of LDNs potential as a geroprotective candidate for improving healthspan metrics within a larger cohort of individuals. Future studies should include the measurement of objective biomarkers alongside self-assessment assays, such as proinflammatory factors, and measures of physiological fitness through clinical assays evaluating factors such as VO_2 max and muscular health as well as digital wearables that evaluate factors such as sleep quality and metrics of cardiovascular health. In addition, the SF-36 asks questions like "did you accomplish less than you would have liked to," "how often you felt full of pep," "how often did you feel tired," and "how often were you limited in normal work or activities." These questions have a large subjective component and are relative to an individual's health sometime in the past. Furthermore, since the framing of the questions were based on the Likert scale, participants are subject to "extreme response bias," which predisposes individuals to choosing the lowest or highest responses available. Although these types of questions represent challenges when aiming to determine an "absolute" quantification of healthspan, we argue that the very nature of healthspan has a large subjective/relative aspect to it. Furthermore, the utility of the SF-36 is limited when considered at a single, isolated

time point, but its validity increases when more data points are collected over time in longitudinal trials, particularly as it pertains to the clinical efficacy of a gerotherapeutic candidate on QoL. A challenge with performing clinical studies assessing repurposed drugs for off-label use is that the medication is available outside of the trial setting, which can result in accrual challenges as there is limited benefit for participants, and they might not be willing to "risk" ending up taking a placebo[15].

In summary, given emerging evidence on LDNs effects on the hallmarks of aging, data suggesting its broad potential to address multimorbidity, efficacy at low doses, its well-validated safety profile in humans, and our data suggesting improvements in multiple healthspan metrics across a broad normative aging cohort, we propose LDN as a promising gerotherapeutic candidate that may significantly improve and/or optimize healthspan metrics in normative aging cohorts. As such, LDN is a prime candidate for further validation of longevity-promoting effects in preclinical studies and human trials (particularly randomized controlled trials). Future research should be directed at gaining a more nuanced understanding of factors influencing response to LDN to tailor treatment regimens effectively, potentially through evaluating personalized response to dose/regimen and individual health demographics, lifestyle habits, genetics, and underlying physiological and/or pathological traits. For example, it is possible that individuals with certain genetic polymorphisms in endorphin signaling, poor dietary habits, low-grade sterile inflammation (as reflected by an array of proinflammatory factors such as IL-6 and TNFα) or increased senescence burden are predisposed to responding to LDN in a certain way. Longer term follow-up of patients taking LDN (with age-matched controls) is required to evaluate the associations between improvements in the healthspan metrics under study and outcomes that end healthspan, such as age-related chronic disease and frailty. As our study participants are also our telemedicine patients, we plan to follow up longitudinally to track age-related adverse outcomes and their association with healthspan metrics, including an expanded array of biomarkers of aging and immune health to provide deeper insights into the mechanisms through which LDN exerts its effects on the aging process.

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Author Contributions

SZ and AI designed and conceptualized this study; AN, AI, BV, and SZ developed and executed methodology; GH, AN, BV, and SZ conducted experiments; GH and MW analyzed data; GH and MW wrote the initial draft of this article; GH, AN, MW, AI, BV, and SZ edited and revised the article; AI and SZ supervised the project; and AI and SZ obtained funding support.

Conflicts of Interest Statement

G.H., A.N., A.I., B.V., and S.Z. are employees of AgelessRx and report equity or stocks of AgelessRx. Contribution M.W. was funded by AgelessRx.

Supplementary Materials

Supplemental information can be found here: Supplementary.

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