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It is an exciting time to be involved in Anti-Aging Medicine. With your involvement, the Anti-Aging medical specialty continues to expand and become more widely accessible. By attending this event, you are transforming the future of medicine and we salute you for leading the evolution of the healthcare industry.

With warm regards,



Ronald Klatz. MD, DO President, A4M



Robert Goldman, MD, PhD, DO, FAASP Chairman, A4M

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# PROFESSIONAL MEDICAL EDUCATION WORKSHOPS (NON-CME)

# Thursday, May 19<sup>th</sup> - 7:00pm

Telomeres 101: The Ultimate Anti-Aging Scorecard Location: Atlantic Ballroom Presented by: Mark Rosenberg, MD Mark Tager, MD Stephen Matlin, CEO

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Hormonal Imbalances: Diagnostics & Treatment in Clinical Practice Location: Regency Ballroom Presented by: Pamela W. Smith, MD, MPH, MS Sponsored by: GENOV

# Friday, May 20<sup>th</sup> - 6:15pm

Unlock The 7-Figure Secret LIVE! Location: Atlantic Ballroom Presented by: Edward Kondrot, MD

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Highlight of TA-65MD Lengthening Telomeres in Humans Location: Regency Ballroom Presented by: Ron Rothenberg, MD Sponsored by:



How to Successfully Implement Profitable Medical Aesthetic Treatment Location: Diplomat Ballroom Presented by: Manon Pilon Sponsored by:



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# A NATURAL PRODUCT TELOMERASE ACTIVATOR LENGTHENS TELOMERES IN HUMANS: A randomized, double blind and placebo controlled study

Laura Salvador<sup>1</sup>, Gunasekaran Singaravelu<sup>2</sup>, Calvin B. Harley<sup>3</sup>, Peter Flom<sup>4</sup>, Anitha Suram<sup>2</sup> and Joseph M. Raffaele<sup>5</sup> \* \* 1. Anti-aging Group Barcelona, Barcelona, Spain 08017 2. T. A. Sciences Inc., NY 10170 3. Independent consultant, Murphys, CA 95247 4. Peter Flom Consulting, NY 10024 5. PhysioAge Systems, LLC, NY 10019

Due to space constraints, this article may be missing tables that support the research. For in-depth information and additional research, please visit http://www.ncbi.nlm.nih.gov/pubmed/26950204

### Abstract

TA-65 is a dietary supplement, based upon an improved formulation of a small molecule telomerase activator that was discovered in a systematic screening of natural product extracts from traditional Chinese medicines. The current study summarizes the findings on telomere length changes from a randomized, double blind, placebo controlled study of TA-65 over a one year period. The study was conducted on 117 relatively healthy CMV positive subjects aged 53-87 years old. Subjects taking the low dose of TA-65 (250 units) significantly increased telomere length over the 12 months period (530  $\pm$  180 bp; p=0.005) while subjects in the placebo group significantly lost telomere length (290  $\pm$  100 bp; p=0.01). The high dose of TA-65 (1000 units) showed a trend of improvements in telomere length compared to the placebo group, however the improvements did not reach statistical significance. Telomere length changes in the low dose group were similar for both median and 20th percentile telomere lengths. The findings suggest that TA-65 can lengthen telomeres in a statistically and possibly clinically significant manner.

## Introduction

TA-65 was discovered as a chemically defined small molecule activator of telomerase in the year 2000 from an empirical screen of natural product extracts from traditional Chinese medicines.<sup>1, 2</sup> (Patent number: US7846904). Since that time, there have been research and observational studies on TA-65 in humans and animal models supporting improvements in biomarkers of aging including immune, cardiovascular, metabolic, bone, and inflammatory markers, without significant signs of toxicity.<sup>2-4</sup>

The formulation (TA-65MD) is manufactured under the regulations of Current Good Manufacturing Practice (cGMP); it is designated as GRAS (Generally Recognized As Safe) for use in a medical food, and is sold as a dietary supplement by the company TA Sciences.

Interest in TA-65 as a telomerase activator is largely driven by the potential health benefit of telomere maintenance. Without telomerase, telomeres gradually shorten with each cell division due to the "end replication problem", oxidative stress and other natural DNA processing at chromosome ends, ultimately triggering cell senescence, i.e. the loss of cell replication capacity and ensuing tissue degeneration when telomeres become critically short.<sup>5</sup> There is abundant evidence that telomerase activation can help maintain and/or lengthen telomeres, 6-8 and in some cases restore tissue and organ function that has been compromised by critical telomere shortening.9 However, to date, there have been no blinded, placebo controlled human studies of TA-65. This report provides the first evidence from a randomized, double blind, placebo controlled study that dietary supplementation with TA-65 has the ability to lengthen telomeres and potentially improve health outcomes in humans, with no observed safety concerns.

Cytomegalovirus (CMV) infects the majority of the population worldwide asymptomatically. Seventy to eighty percent of individuals by the age of fifty are infected with CMV. CMV has been implicated in decreased T cell immunity, associated immunosenescence, and decrease in the T cell receptor repertoire, causing clonal expansion of senescent CD8+CD28–T cells with a pro-inflammatory profile.<sup>10</sup>

Recent studies also suggest that CMV infections are associated with increased mortality in the elderly and are a potential factor in the development of cardiovascular disease among immunocompromised individuals.<sup>11, 12</sup> Here we investigated whether TA-65 can alleviate telomere attrition in CMV+ subjects, to support our previous observational study finding that TA-65 appears to preferentially lengthen critically short telomeres in CMV+ subjects. The current study is aimed at understanding telomere length changes in CMV+ subjects taking the telomerase activator TA-65 in comparison to the placebo group.

### Materials and Methods Study design

This is a randomized, double blind, placebo controlled, parallel group study with three arms. Subjects were randomized to placebo, low dose, or high dose groups using a random number table. The Principle Investigator and subjects were blinded until the completion of the study. Following initial screening (168 subjects), a total of 117 subjects were recruited and 97 subjects completed the study. Forty-five subjects (45) received TA-65: 23 subjects received one TA-65 capsule (250 units) and three placebo capsules; 22 subjects received four TA-65 capsules, each consisting of 250 units of TA-65 (i.e. 1000 units/4 capsules). Fifty-two (52) subjects received four placebo capsules. The study involved 104-day cycles consisting of 90 days of taking product or placebo, followed by 14 days of abstinence from taking the test materials. The trial was run for a period of one year. The subjects had 6 visits during the study: pre-selection, day 0 (baseline), at 3 months, 6 months, 9 months and 12 months (final visit). The capsules were taken on an empty stomach in the morning. After baseline testing, subjects were given a three months' supply of the pills, which they took home for consumption. After baseline, additional visits to the clinic were scheduled each 3 months until the end of the study. The PI, Dr. Salvador checked to see that all the pills given at the prior visit had been consumed to confirm compliance.

The study was conducted in Barcelona, Spain. All the subjects were from Barcelona except one, who was from Malaga (South of Spain). Inclusion criteria were subjects with IgG antibodies positive for CMV, aged between 53 and 87 years old and who were able to sign informed consent. Exclusion criteria were subjects with active carcinoma, a prior history of cancer, severe infectious diseases (Hepatitis C, Hepatitis V, HIV and syphilis), autoimmune diseases, hormonal therapy, prior intake of TA-65, or nutritional supplements enriched with Omega-3. The male to female ratio was 1.25.



## **Blood collection**

Blood was collected 5 times during the study: at day 0, at 3, 6, 9 and 12 months. Blood was tested for the clinical biomarkers, and an aliquot was used to isolate peripheral blood mononuclear cells (PBMC) for the high-throughput measurement of telomere length by fluorescent in situ hybridization (FISH).

## **Measurement of telomere length**

Median telomere length in PBMC was measured by Life Length (Spain) using the high throughput (HT) Q-FISH technique. This method is based on a quantitative fluorescence in situ hybridization method modified for cells in interphase.<sup>13</sup> In brief, telomeres are hybridized with a fluorescent Peptide Nucleic Acid probe (PNA) that binds to telomeric repeats (sequence: Alexa488-OO-CCCTAACCCTAACCCTAA, Panagene). Images of nuclei and telomeres are captured by a high-content screen system. The intensity of the fluorescent signal from telomeric PNA probes that hybridize to a given telomere is linearly proportional to the length of the telomere. Intensities of fluorescence that are translated to telomere lengths by comparing the obtained intensities of fluorescence versus a standard regression curve built with control cell lines of known telomere length.

On processing day, samples and control cell lines were thawed at 37°C and cell counts and viability were determined. Cells were seeded in clear bottom black-walled 384 well plates at a fix density with five replicates of each PBMC sample and 8 replicates of each control cell line. Cells were fixed with methanol/acetic acid (3/1, vol/vol). Following hybridization in situ with the PNA probe cells were washed and DAPI added for DNA staining. Quantitative image acquisition and analysis were performed on a High Content Screening Opera System (Perkin Elmer), using the Acapella software, Version 1.8 (Perkin Elmer). Images were captured, using a

### **Clinical laboratory assays**

During visits at baseline and at the end of visits at 3, 6, 9 and 12 months after initiation of the test products (placebo or TA-65), vitals were checked and blood was drawn from each subject. Assays for a comprehensive metabolic panel (insulin, glucose, blood urea nitrogen, creatinine, estimated glomerular filtration rate, sodium, potassium, phosphorus, bilirubin, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase); hematology panel (RBC, hemoglobin, hematocrit, complete blood count, white blood cells count, differential leukocytes and platelets); lipid panel (total cholesterol, HDL cholesterol, triglycerides, LDL cholesterol); inflammatory markers (C reactive protein and homocysteine) and immune cells including immunosenescence biomarkers (B lymphocytes, T lymphocytes, Natural Killer cells), were carried out at Labco, Spain. 40x 0.95 NA water immersion objective. UV and 488 nm excitation wavelengths were used to detect the DAPI and A488 signals respectively. The telomere length distribution and median telomere length were calculated with Life Length's proprietary program.

The length of each individual telomere is calculated by interpolation of the corresponding intensity of fluorescence into the regression curve prepared with the controls. A distribution of telomere length is thereafter calculated and the 20th percentile of said distribution is given in representation of the percentage of short telomeres. In order to remove machine variances over time, all samples (baseline, 3 months, 6 months, 9 months, and 12 months) were tested at the same time. The samples were blinded during the analysis.

### Statistical analysis: multilevel model

Since each person was measured multiple times, the errors from a regression model would not be independent, thus violating one of the key assumptions of the model. To deal with this, we used a multilevel model. Because we were interested in nonlinear and possibly non-monotonic relationships between time and median telomere length, we used month as a categorical variable. Alternatives such as spline models were considered and rejected because the number of time-points per subject was relatively few. We used an unstructured covariance matrix based on fit indexes (Akaike Information Criterion). We included time, group and their interaction in the model. The interaction term is most important, since it indicates whether the effect of time on median telomere length was different in the different groups.

## **RESULTS** Median telomere length: baseline characteristics

We used a linear regression model to analyze cross-sectional data of telomere lengths of all 97 subjects at baseline. Telomere length at baseline ranged from 7 to 15 kilo base pairs (kb) for the subjects aged from 53 to 87 years, and was inversely correlated with age (R-Square = 0.056). The cross sectional rate of decline in telomere length for the baseline population was  $50 \pm 21$  bp/year. (To see the distribution of telomere length of the study participants at baseline, visit http://www.ncbi.nlm.nih.gov/pubmed/26950204 and see Figure 1).

# Average change in the median telomere length for TA-65 group and placebo group

Median telomere length (TL) was measured in placebo group, low dose TA-65 (250 units) group and high dose TA-65 (1000 units) group at baseline, 3 months, 6 months, 9 months and 12 months (see Table 1). At baseline, there were no significant differences in TL among the three groups, although the range of lengths was bigger for the placebo group. The telomere lengths shown in Figure 1 are significantly longer than those measured in similar age-range cohorts by qPCR.14 The reason for this is likely due to the fact that FISH-hybridization assays often detect signal from non-canonical telomeres (degenerate telomere sequences found in the sub-telomeric region at chromosome ends). It is also possible that telomere clustering in the hTP-qFISH over-estimates telomere length, or that the methodology for assessing average telomere length is based on TRFs that may contain relatively large sub-telomeric DNA.

# Change in the median telomere length for TA-65 group vs placebo group: multilevel analysis

As discussed in the statistical methodology, to understand the non-monotonic relationships with time and telomere length, a multilevel analysis was run. The effect of greatest interest was the interaction effect between time and group. The main effect of time tests whether the placebo changed over time, while the main effect of group tests whether the groups were different at baseline. While both must be accounted for, our interest is in whether the three groups behaved differently over time and this is tested by the interaction (Group and Time interaction). It is important to distinguish between the raw data (shown in table 1) and the parameter estimates by the multilevel analysis (shown in table 2).

The telomere lengths at baseline among the three groups were not significantly different as estimated by the group effect (Table 2). In the placebo group, there was a decrease in median TL at 9 and 12 months compared with baseline (Table 2 and Figure 2). At 9 months the decrease was  $170 \pm 90$  bp (p= 0.07) and at 12 months the decrease was  $290 \pm 100$  bp (p=0.01). Overall, the placebo group telomere data behaved slightly worse than expected (50-150 bp/year), which may be due to the CMV positive status of the individuals tested. Also it may suggest that this cohort was either not as healthy at baseline as expected, or perhaps had a relatively poor set of lifestyle behaviors.<sup>15</sup>

In the low dose TA-65 (250 unit) group, there was an increase in median TL at 3 months followed by relative stability (Table 2). Compared to the placebo group, the effect of time was significantly different in the TA-65 groups. The effect of low dose TA-65 (250 units) on median TL was significantly higher at 9 months (median TL was 530  $\pm$  170 bp longer, p=0.002), and 12 months (again, median TL was 530  $\pm$  180 bp longer, p= 0.005) and borderline significantly higher at 3 months (median TL was 380  $\pm$  190 bp longer, p=0.05) but not significant at 6 months (see Table 2 and for more information, visit http://www.ncbi.nlm.nih.gov/ pubmed/26950204 and see Figure 2).

The high dose TA-65 (1000 units) showed a trend of improvement in telomere length compared to the placebo group, but the improvements did not reach statistical significance. It is not known why in this study the high dose TA-65 (1000 units) group appeared to change in a random manner. This may have resulted from a compliance issue with subjects who took the higher dose. In future studies, it may be necessary to more tightly monitor compliance over time and increase the number of subjects and doses tested.

#### Table 1: Average of median telomere lengths at 5 visits.

The average telomere length in Placebo, low dose TA-65 (250 units) and high dose TA-65 (1000 units) groups at baseline and at the end of 3, 6, 9 and 12 months in kilo base pairs (kb) with standard deviation (s.d.).

	Average of median telomere length (s.d.) in kb						
Group	Baseline	3 months	6 months	9 months	12 months		
Placebo	11.03 (1.49)	11.00 (1.38)	11.19 (1.28)	10.85 (1.36)	10.74 (1.55)		
TA-65 (250 Units)	10.57 (1.12)	10.92 (1.30)	10.89 (1.30)	10.92 (1.23)	10.81 (1.40)		
TA-65 (1000 Units)	10.44 (1.04)	10.86 (1.40)	10.59 (1.32)	10.61 (1.11)	10.22 (1.19)		

# Table 2: Multilevel model analysis of median telomere lengthchanges compared to baseline.

Placebo, low dose TA-65 (250 units) and high dose TA-65 (1000 units) groups are compared at baseline (0 months), and at the end of 3, 6, 9 and 12 months for median telomere length. The data show change in telomere length in comparison to the reference group(s). Results were adjusted for age and sex.

Effect	Group	Time (months)	Change in TL (kb)	Standard Error	P value
Group effect	Placebo		Reference group		
	TA-65 (250 Units)	At baseline	-0.47	0.32	0.15
	TA-65 (1000 Units)		-0.24	0.33	0.47
Time effect	Placebo	0	Reference group		
		3	-0.02	0.11	0.82
		6	0.16	0.09	0.07
		9	-0.17	0.09	0.07
		12	-0.29	0.10	0.01
Group and time effect	TA-65 (250 Units)	0	Reference group		
		3	0.38	0.19	0.05
		6	0.16	0.16	0.34
		9	0.53	0.17	0.002
		12	0.53	0.18	0.005
	TA-65 (1000 Units)	0	Reference group		
		3	0.25	0.20	0.22
		6	-0.13	0.17	0.46
		9	0.22	0.17	0.20
		12	-0.06	0.19	0.77

# Change in telomere length of the short telomeres (20th percentile) for TA-65 group and placebo group

The shortest quintile of telomere length (< 20th percentile) was measured in the placebo group, low dose TA-65 (250 units) group, and high dose TA-65 (1000 units) group at baseline, and at the end of 3, 6, 9 and 12 months, the average lengths were represented in table 3, which can be viewed at http://www.ncbi.nlm.nih.gov/pubmed/26950204.

In the placebo group, there was a gradual decrease in average shortest quintile telomere length with time, as expected. However,

# Key changes in the safety markers

Statistically significant differences between the baseline and 12 months measurements in the safety markers are shown in Supplementary Table 1 (S1). There were no clinically significant changes in the safety markers during the study as judged by the physician (Joseph M. Raffaele). Immune cell bio markers were unfortunately inappropriately run and hence could not be used. telomere length of the 20th percentile in the low dose TA-65 (250 units) group increased at 3 months and was then relatively stable. In the high dose TA-65 (1000 units) group, there was

# DISCUSSION

In a previous observational study, subjects taking TA-65 along with other supplements showed improvements from baseline in health biomarkers, especially in CMV+ subjects.<sup>1</sup> Since the subjects were blind to their CMV status while taking TA-65, it is unlikely that the positive effects of TA-65 were due to a placebo effect. To confirm that there was in fact no significant placebo effect, the current study was designed to be randomized, double blind and placebo controlled. We tested a cohort of CMV+ subjects for the effect of TA-65 on telomere length. The telomere

lengths were measured

using HT Q-FISH with automation to handle large numbers of human samples and to improve consistency. The cross-sectional analysis of telomere length at baseline indicates a decline of  $50 \pm$ 21 bp per year, which is higher than in some studies, but consistent with other published data.<sup>5, 16, 17</sup> The rate of telomere loss has been reported to be exacerbated in CMV+ individuals18 which may also contribute to the relatively high rate of change in the cross-sectional analysis. The rate of loss reported in this study18 was 94 ± 9 bp per year in CMV + subjects and 77 ± 9 bp per year in CMV– subjects.

In the current study, the placebo group had an average telomere attrition of 290  $\pm$  100 bp/year (p = 0.01) while the low dose TA-65 (250 units) group had net increase of 530  $\pm$  180 bp/year (p = 0.005). Interestingly there were no statistically significant changes in telomere length in the high dose TA-65 (1000 units) group. Loss of 290 bp/year in the placebo group is indeed large, but a large loss is to be expected in a group that is 100% CMV positive and consists of older individuals aged > 60yrs. The accelerated attrition is supported by: (1) CMV infection- which causes significant shortening of telomere length in the age group of >60 years18 and

(2) CMV seropositivity increases the oligoclonal expansion of the immune cells with age.<sup>19</sup> Although variation in the rate of TL loss over time cannot be ruled out, there are limited studies on telomere lengths in CMV subjects.

In the previous observational study, the subjects who took a very low starting dose of 5 to 10 mg/day of unformulated TA-65 (i.e. active ingredient alone) had no significant change in telomere length. In the current study, with an improvement in formulation (TA-65MD) to enhance bioavailability, the TA-65 250 units (with 8 mg of active ingredient) increased telomere length, whereas TA-65 1000 units (with 32 mg of active ingredient) showed no consistent changes in the telomere length. These data raise a possibility that TA-65 may have a bell-shaped dose response curve. Murine cell data suggests that TA-65 results in reduction of cells with short telomeres.<sup>3</sup> It is possible that the high dose TA-65 (1000 units), by increasing the short telomeres lengths, rescued the near senescent cells resulting in a reduction in the median telomere length. A future study has been planned to address the expansion of near-senescent cells with additional TA-65 doses.

Again, the key results are whether the effect of time was different in the different groups. We found similar trends to those for the median. Here, the differences between the low dose TA-65 (250 units) group and the placebo were significant at 3, 9 and 12 months (increment of 200 bp, 260 bp and 270 bp, and the p values are 0.04, 0.001 and 0.002, respectively). Also as with the median length, the effects in the high dose TA-65 (1000 units) group were inconsistent and non-significant (visit http://www.ncbi.nlm.nih.gov/ pubmed/26950204 see Table 4).



Analysis of the 20th percentile group showed trends similar to that of the overall group: telomere length increased in the low dose TA-65 (250 units) group at 12 months (268  $\pm$  85 bp), but there was no consistent change in the high dose TA-65 (1000 units) group. The cause of no significant change over time in the high dose TA-65 group is unknown and unexpected. However, there was a trend in the observational studies<sup>1, 2</sup> that high doses partially reverse some of the positive effects of TA-65.

Overall the most significant finding of this study was that the low dose TA-65 (250 units) increased both median and short telomere lengths in a statistically significant manner which could have clinical significance as well. For example, telomere length has been positively associated with increased regenerative capacity of cells,<sup>20, 21</sup> reduced mortality and disease risks in humans,<sup>22-24</sup> and increased resistance to infection.<sup>25</sup>

Based on the animal studies, the no-observed-adverse-effect level (NOAEL) for oral TA-65 was considered to be greater than 150 mg/kg/ bw/day in male and female rats, equivalent to 10,500 mg/ day in a 70-kg individual, which is orders of magnitude higher than the highest doses seen in human PK studies. Note also that TA-65 is designated as GRAS4 and has been extensively tested for safety in humans. Additionally, no cytotoxicity was identified in in vitro testing of  $1 \mu M$  active ingredient on CD8+ T cells.<sup>20</sup>

The bulk of the evidence suggests that TA-65 lengthens telomeres by increasing telomerase activity. However, the dose response of TA-65 for telomere length could not be accurately ascertained with only

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two doses tested, and with one of the doses showing no significant change over time. For these reasons, a more highly powered study with three or more doses is being planned. In addition, the results from the current study are consistent with the previous observations regarding the lack of any toxicity associated with the intake of TA-65. We did not find any product-related toxicities, as assessed by the biochemical markers of liver, kidney, and metabolic functions.

# To view additional research, please visit http://www.ncbi.nlm.nih.gov/pubmed/26950204



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#### **Author Disclosure Statement**

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# Specialized Pro-resolving Mediators (SPMs): Resolution-based Nutrition

By Jyh-Lurn Chang, PhD, Michael B. Montalto, PhD, and John Troup, PhD Metagenics Medical Affairs Research and Development, Gig Harbor, WA 98332



#### THE PURPOSE OF INFLAMMATION

When microbes enter the tissue or the tissue is injured, the innate immune system mounts a coordinated response designed to remove these foreign organisms or injuries. This protective response is called inflammation.<sup>1</sup> The goal of inflammation is to bring cells and molecules of host defense – including circulating leukocytes (white blood cells) and plasma proteins – to the site of infection or damaged tissue. It initiates a series of events that eventually lead to wound healing and tissue repair.

A normal, self-limited process; an acute inflammatory response can be divided into an initiation phase and a resolution phase. Normally, an inflammatory response lasts from a few minutes to as long as a few days (Figure 1). The initiation phase begins within seconds to minutes following the presence of harmful stimuli, and neutrophils predominate in the inflamed site during the first 6 to 24 hours. The initiation phase is orchestrated by pro-inflammatory eicosanoids, cytokines, chemokines, and other mediators.<sup>2,3</sup> The subsequent resolution phase lasts up to a few days, with macrophages occupying the inflamed site especially in 24 to 48 hours.<sup>1</sup> Specialized pro-resolving mediators (SPMs) are produced in tissue exudates during the resolution phase and function as "resolution agonists" to accelerate the return to homeostasis.<sup>4,5</sup>

Figure 1. The approximate kinetics of an acute inflammation, from initiation to resolution. Image adapted from Serhan.<sup>6</sup>



Normally, inflammation is a controlled, self-limited, acute response. The ideal outcome is complete resolution and a return to homeostasis the previous normal condition. However, the components of the inflammatory reaction are also capable of injuring the surrounding normal tissues when uncontrolled, inappropriately activated, or left unresolved. In those cases, an acute inflammatory response can lead to persistent chronic inflammation.<sup>1</sup> Unresolved inflammation complicates the management of many conditions such as obesity, metabolic syndrome, cardiovascular disease and diabetes. Improved management of an elevated inflammatory response is an important objective in patient care.

For decades, treatments have focused on blocking the initiation phase of inflammation through pharmacological agents or anti-inflammatory dietary ingredients. While these treatments can act to reduce the degree and intensity of the inflammatory response during the initiation phase, they do not act to resolve inflammation. Inflammation does not just fade away, but is actively resolved through the actions of SPMs.

#### SPMs ORCHESTRATE RESOLUTION ACTIVITIES

Three families of SPMs – resolvins (Rv), protectins (PD), and maresins (MaR) – are derived from EPA and DHA.<sup>7</sup> During inflammation, free EPA and DHA appear rapidly in the exudates from circulation (albumin is likely their main carrier). Membrane-bound EPA and DHA (i.e. in esterified form) can also be released into exudates via PLA<sub>2</sub>. SPMs are then synthesized by leukocytes and macrophages via different enzymatic pathways (Figure 2).<sup>7</sup> SPMs are structurally different from EPA and DHA.

Figure 2. SPMs that are biosynthesized from omega-3 fatty acids EPA and DHA.





#### SPMS ACTIVELY ORCHESTRATE RESOLUTION ACTIVITIES SUCH AS:<sup>8</sup>

• Cessation of neutrophil infiltration

- Decreased productions of pro-inflammatory mediators and increased productions of anti-inflammatory mediators
- •Vascular permeability/edema returning to normal
- Timely apoptosis (programmed cell death) of existing neutrophils
- Active clearance of apoptotic neutrophils and debris by macrophages (efferocytosis)
- Stimulation of macrophages into a pro-resolution phenotype
- •Tissue repair and return to homeostasis

#### SPMs ARE FUNCTIONALLY DIFFERENT FROM OMEGA-3 FATTY ACIDS

EPA and DHA are important nutrients for health; EPA is especially good at lowering serum triglycerides.<sup>9,10</sup> During the initiation phase of inflammation, potent pro-inflammatory mediators such as PGE<sub>2</sub> and LTB<sub>4</sub> are derived from arachidonic acid (AA). EPA and DHA themselves can affect the initiation phase by competing with AA for enzymes, leading to production of metabolites such as PGE<sub>3</sub> and LTB<sub>5</sub> that have lower biological potency than AA metabolites.<sup>11</sup> However, EPA and DHA do not have the pro-resolving properties of SPMs. Furthermore, EPA and DHA are subject to multiple downstream metabolic checkpoints for eliciting their biological effects.<sup>4</sup>

#### POTENTIAL BENEFITS OF SPMS SUPPLEMENTATION

Recent scientific studies have revealed that chronic health issues including peripheral vascular disease and asthma are, in part, influenced by an underdeveloped or poor responding resolution pathway characterized by reduced SPM concentrations.<sup>12,13</sup> This observation in patients suggests additional nutritional support for

a more optimal restoration of the resolution processes. Additional research shows that obesity is associated with reduced SPM concentrations in tissues, and that high sugar, high fat diets down-regulate murine 12/15-LO activity, an enzyme involved in SPM synthesis.<sup>14</sup> Furthermore, individuals with loss-of-function in SNPs in the gene encoding 12/15-LO have been reported to have an increased risk of developing coronary artery disease,<sup>15</sup> a condition promoted by chronic inflammation and impaired resolution within the vascular tissue. Thus, from a therapeutic standpoint, direct treatment with SPMs represents a targeted approach in promoting inflammation resolution.<sup>4</sup>

#### SELECTION OF A STANDARDIZED AND ACTIVE SPM FRACTION FROM SELECTED OIL SOURCES

Prior to understanding the pro-resolving capabilities of a SPM supplement in clinical studies, selection of SPM supplements based on standardization of concentration and activity is important. The effective use of SPM supplements requires a standardization of key actives as well as availability of full resolving activity from the product.

## SETTING THE STANDARD FOR RESOLUTION NUTRITION

The clinical use of SPMs to manage inflammation is supported by the following key points:

- 1. SPMs are detected in human plasma and tissues at pictogram (pg) and nanogram (ng) levels<sup>12,13,16</sup>
- 2. Recent clinical studies have demonstrated that supplementation with SPM products standardized to 17-HDHA and 18-HEPE show measurable functional responses (i.e. macrophage phagocytic activity) important for inflammation resolution (unpublished data)
- 3. Inflammation is an underlying pathogenic process and there is a clear clinical need to optimize inflammation resolution in many chronic disease states
- 4. SPMs can be built into clinical practice guidelines for the patient management and resolution of the inflammatory response




#### **SUMMARY**

When inflammation is not resolved and becomes chronic, it is understood that cellular responses leading to resolution have failed. In these situations, and when there are many proinflammatory mediators resulting in chronic inflammation, the production of SPMs leading to resolution is compromised and inefficient. Recent studies discovered that some health conditions lead to deficiencies of SPMs. For example, in the experimental setting of obesity, the formation of SPMs is severely deregulated, <sup>4,12</sup> and the enzyme that inactivates SPMs is markedly up-regulated.<sup>17</sup> Although EPA and DHA are the substrates of SPMs, the biological effects of EPA and DHA are subject to multiple downstream metabolic checkpoints (e.g., receptors and enzymatic activities). Thus, researchers have suggested that direct supplementation of SPMs may represent a more targeted, effective approach than supplementing the parent omega-3 fatty acids, especially when chronic inflammation is already present.<sup>4</sup>



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# THE CLINICAL VALUE OF TELOMERE TESTING

Mark J. Tager, MD

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Since the award of the 2009 Nobel Prize for the discovery of telomerase, the field of telomere biology has grown dramatically. At last count there were more than 20,000 articles in Pub Med, with cancer, stem cells, cardiovascular disease, diabetes, CNS and pulmonary conditions comprising the majority.

Clinicians today are increasingly becoming interested in incorporating telomere testing into their practices. This is driven in large part by two converging forces: a growing understanding of the role telomeres play in personalized medicine; and increasing consumer demand for integrative treatment that can slow the aging process. The development of reliable commercial testing now allows for the measurement of individual telomere lengths and quantification of the number of critically short telomeres, both key determinants in overall cellular health. As telomere science moves from the lab to the clinic, measurement of this novel biomarker holds promise for improving early disease identification, risk stratification, treatment algorithms, and health behavior change.

#### **Telomere Biology**

The ends of linear chromosomes are capped by telomeres repeating sections of non-coding DNA TTAGGG units that protect the important areas from degradation. Telomeres shorten after each chromosome replication because the machinery of DNA replication cannot copy the last bit of the lagging strand. When the telomeres become critically short, this triggers a process of replicative senescence whereby further cellular division is normally halted. This avoids the risk of genetic mutations from chromosomal fusions due to "uncapped" chromosomes. In special cells such as stem cells, germ cells and cancer cells, an enzyme called telomerase is highly active.

Telomerase is a unique ribonucleoprotein complex consisting of an RNA template (TERC) and a reverse transcriptase catalytic subunit (TERT) that works along with a protein complex called shelterins to lengthen the telomeres. Telomerase is also highly expressed in most human cancer cells. These telomerase-positive cells do not experience telomere shortening with increased cell division, making them immortal.

Depending on cell type, the Hayflick limit establishes the absolute number of cellular divisions in the range of 50-70 times. This governs the upper end of potential cell longevity. With aging, the progressive loss of telomeres continues over the years.

To put the numbers in perspective, a fetus in utero may have telomeres with around 15,000 to 20,000 base pairs or 15 to 20



kilobases. By birth this number may be reduced to 10 to 15 kilobases. Over the span of a lifetime, telomeres shorten heterogeneously at rates that vary from 100 to several hundred base pairs per year.

The vast body of telomere research has provided strong evidence associating short telomeres with a variety of disease states.<sup>2</sup> Critically short telomeres contribute to cardiovascular disease<sup>3</sup>, Type 2 diabetes<sup>4</sup>, cancer<sup>5</sup>, osteoporosis<sup>6</sup>, osteoarthritis<sup>7</sup>, pulmonary fibrosis<sup>8</sup>, major depressive disorders<sup>9</sup> and CNS diseases.<sup>10</sup> Telomere length is increasing being recognized as an independent variable in predicting disease.

A study of more than 43,000 subjects reported in the British Medical Journal concluded that telomere length is an independent risk factor for cardiovascular disease.<sup>11</sup> A large meta-analysis showed significant association between shortened telomere length and Type 2 diabetes.<sup>12</sup> Other studies are showing that the finding of short telomeres may be associated with the aggressiveness of certain cancers.<sup>13,14</sup>

#### **An Anti-Aging Scorecard**

Telomere length provides a marker of the proliferative history of somatic cells, and as such allows for the determination of biological, as opposed to chronological age. Maintaining telomere length involves a number of coordinated activities including the enzyme telomerase and the associated effects of environmental and lifestyle factors. Accelerated telomere attrition is affected by negative health habits such as smoking<sup>15</sup>, obesity<sup>16</sup>, excessive alcohol consumption <sup>17</sup>, stress and social deprivation<sup>18</sup>, sedentary habits<sup>19</sup> and poor diet<sup>20</sup>. Research is also showing that positive health practices can impact the rate of telomere shortening<sup>21</sup>. Among these positive activities are a Mediterranean diet<sup>22</sup>, certain vitamins and supplements including Omega-3 fatty acids<sup>23</sup>, exercise<sup>24</sup>, and mindfulness.<sup>25</sup>

#### Mark J. Tager, MD | THE CLINICAL VALUE OF TELOMERE TESTING

The ability to monitor telomere length, and more importantly, to assess short telomeres in individual cells can be an important tool in clinical practice. The discovery that short telomeres in the somatic cells of mice could be increased by the activation of telomerase holds promise that telomerase enhancing therapies may be able to expand the human lifespan.<sup>26,27</sup>

#### An Overview of Telomere Testing

The most commonly accepted technique for measuring telomere lengths has been assessing circulating peripheral white blood cells, including both granulocytes and lymphocytes. Although different tissues age at different rates, and have their own distinct telomere lengths, overall tissue loss has been well correlated with peripheral white blood cell measurements.<sup>28</sup> This correlation can be seen, for example, in the relationship between leukocyte telomere lengths and carotid atherosclerosis in hypertensive patients.<sup>29</sup>

There are three currently available commercial technologies that measure leukocyte blood telomeres. There are pros and cons to each of the current tests on the market. (There are no salivary tests or home diagnostics that are cleared by the FDA at this time, nor does it appear that such testing is on the horizon.)

Q-PCR is based on the DNA amplification signal of single copy genes. This methodology is relatively inexpensive, easily scalable, and has been used in many of the larger epidemiological studies. A significant database exists to compare results and derive statistical validation. The test generates a two-page report plotting the average telomere length on a normative graph. One page of patient recommendations follow.

Compared to other modalities, PCR tends to be less accurate and reproducible. The methodology is not directly quantifiable and values are extrapolated from amplification cycles. The largest negative to the use of PCR is that it only provides information as an average of telomere lengths reported as telomere score and does not provide information on the length of the shortest telomeres, the measurement of which is most important clinically. While PCR results can be compared against a large database, biologic age cannot be assessed or inferred from this method.

The flow-FISH (fluorescence in situ hybridization) method uses a FACS (fluorescence activated cell sorter) to analyze telomere length in a cell population after hybridization with a fluorescence telomere probe. The methodology is more quantitative and reproducible than QPCR. The test has been employed in genetic counseling. Flow-FISH has been in use long enough to generate a good data base to compare results.

Because the test is more labor and time intensive, it is the most expensive methodology of the three. The flow-FISH assay provides accurate and reproducible measurements of mean telomere length from defined blood cell subsets of separate granulocytes and lymphocytes, together with reference values from a set of healthy individuals (currently, according to the company ~400 between a few months and 100 years of age). Similar to Q-PCR, there is no information provided on individual telomere lengths and the analysis of critically short telomeres. Results are reported as a one-

page comparison of granulocyte and lymphocyte telomere lengths compared to the 50% norm and also displayed as a graph.



Figure 1: Photomicrograph showing leukocytes (blue dots) and telomeres (pink spots). A higher intensity of fluorescence in the red dots indicates greater telomere length and a lower percentage of short telomeres. (Source: Life Length, patient report)

Telomere Analysis Technology<sup>\*</sup> (TAT<sup>\*</sup>) based on HT Q-FISH is derived from quantitative fluorescence in situ hybridization augmented with digital confocal microscopy to access large populations of individual cells (Figure 1). Approximately 100,000 individual telomeres are measured in each blood sample. The technology was initially developed at the Spanish National Cancer Research.<sup>30</sup> The accuracy and reproducibility of TAT is superior to flow-FISH with coefficients of variation below 5%. The database of test results has expanded, given that this technology has become the scientific standard in Europe. TAT with confocal microscopy is the only technology that can measure individual chromosomes, assess the presence of critically short telomeres and determine biological age. In addition, test pricing approximates the cost for Q-PCR.

A detailed six-page patient report provides both median and average telomere lengths as well as detailing the numbers and percent of critically short telomeres as represented by the lowest 20th percentile (Figure 2).



Figure 2: Histogram showing the 20% percentile of critically short telomeres (in red), Median Telomere Length (MTL) and Average Telomere Length (ATL) Source: Life Length patient report



#### New Tests on the Telomere Horizon

Telomapping (US Patent No 8,084,203 B2) has recently been developed to determine the telomere lengths on chromosomes from tissue sections. Fixed tissue sections are used to determine the degree of short telomeres within specific cell types in the tissue. The special topology of the sample is maintained. This methodology has great applicability for the detection of precancerous lesions, as well as aiding in staging the aggressiveness of cancer, and providing feedback on treatment prognosis. Telomapping is time intensive and the process is not yet scaled for high throughput.

Q-TRAP stands for quantitative Telomeric Repeat Amplification Protocol and is a highly sensitive measurement of telomerase activity in biological samples. The ability to accurately measure telomerase is taking on increased importance for pharmaceutical testing of anti-cancer agents. In addition to assessing cancer activity, telomerase measurement may be able to help substantiate anti-aging effects from lifestyle and nutraceutical interventions.

Reliable telomere tests are now available commercially. Currently, there is no reimbursement for these tests, so patients must pay out of pocket. Given the cost it is important that the test provide the most clinically relevant data: the number of individual cells bearing critically short telomeres.

Regardless of the test you employ, it is important to remember that white blood cells vary with the patient's health status. Testing a patient with an acute or unstable condition may affect both the numbers of cells and their telomere length.

#### Bringing Telomere Testing Into Your Practice

Increasingly telomere health is becoming an important component of personalized medicine, where it plays a role in the spectrum of diagnosis, prognosis and treatment modification. Telomere syndromes, most notably dyskeratosis congenita, are caused by abnormally short telomeres.<sup>31</sup>,<sup>32</sup> There is promising research showing that telomere length may be a diagnostic biomarker for another orphan disease, Idiopathic Pulmonary Fibrosis<sup>33</sup>, which currently is challenging to diagnose clinically. Telomere length may also be a risk factor for early onset of ovarian cancer.<sup>34</sup>

Perhaps the greatest value of telomere testing is in obtaining serial measurements by incorporating it as part of an annual health status assessment. It is like a report card, letting both the physician and the patient know how well the total intervention and program

is working. This requires two pieces of data: 1) the number and percent of critically short telomeres and, 2) the rate of telomere attrition. Annual snapshots may also serve as powerful lifestyle behavior change motivators or as tools to enhance treatment adherence.

#### Summary

The ever-increasing body of telomere-based research provides deeper understanding of the critical role telomere attrition plays in the aging process. More specifically, the ability to measure critically short telomeres in individual cells provides the key determinant of cellular health and senescence. As we seek to limit cellular damage through environmental, lifestyle and supplementary modifications, we now have a method of assessing long-term progress. Assessing telomere length can also aid in the detection and prognosis of certain diseases. New tests for telomerase and tissues appear promising. As the data grow and the link between telomere length to disease and prevention becomes more established, so too will telomere testing become the standard of care for anti-aging.

Disclosure: Dr. Tager is a consultant for Life Length.

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12:15 PM	Special Presentation – <b>Dr. Jim LaValle</b> Listen to and engage with one of the most-renowned experts in metabolic medicine.
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#### **ORIGINAL RESEARCH**

# Novel Testing Enhances Irritable Bowel Syndrome Medical Management: The IMMINENT Study

Kelly Parsons, PhD; Julius Goepp, MD; Bryan Dechairo, PhD; Elizabeth Fowler, PhD; Nathan Markward, PhD; Patrick Hanaway, MD; Teresa McBride, ND; Darryl Landis, MD

Due to space constraints, this article may be missing tables that support the research. For in-depth information and additional research, please visit https://www.gdx.net/clinicians/medical-education/articles/imminent-study

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### Abstract

**PRIMARY STUDY OBJECTIVE:** To evaluate the economic utility of a fecal biomarker panel structured to suggest alternative, treatable diagnoses in patients with symptoms of irritable bowel syndrome (IBS) by quantifying, comparing, and contrasting health service costs between tested and non-tested patients.

**STUDY DESIGN:** Retrospective, matched cohort study comparing direct medical costs for IBS patients undergoing fecal biomarker testing with those of matched control subjects.

**METHODS:** We examined de-identified medical and pharmacy claims of a large American pharmacy benefit manager to identify plan members who underwent panel testing, were eligible for covered benefits for at least 180 days prior to the test date, and had data available for 30, 90, and 365 days after that date. We used propensity score matching to develop population-based control cohorts for each tested cohort, comprised of records

with IBS- related diagnoses but for which panel testing was not performed. Primary outcome measures were diagnostic and medical services costs as determined from claims data.

**RESULTS:** Two hundred nine records from tested subjects met inclusion criteria. The only significant baseline differences between groups were laboratory costs, which were significantly higher in each tested cohort. At each follow-up time point, total medical and gastrointestinal procedural costs were significantly higher in non-tested cohorts. Within tested cohorts, costs declined significantly from baseline, while costs rose significantly in non-tested control cohorts; these differences were also significant between groups at each time point.

**CONCLUSIONS:** Structured fecal biomarker panel testing was associated with significantly lower medical and gastrointestinal procedural costs in this study of patients with IBS symptoms.

### Background

Irritable bowel syndrome (IBS), a functional gastrointestinal (GI) disorder with unknown and probably multiple causes, is highly prevalent and costly. Ten to 20% of Americans suffer from IBS, with those in their prime years of productivity and employment being disproportionately affected.<sup>1-3</sup> The cumulative financial impact of IBS is greater than that of many other chronic illnesses, including asthma and migraine, and comparable to that of hypertension and congestive heart failure.<sup>4</sup>

The annual cost of IBS in the United States is estimated to be more than US \$20 billion. In 2005, a study of one Fortune 100 company revealed that IBS direct costs to the employer were 1.5 times higher in affected employees (\$6364) than those accrued by a matched sample of controls (\$4245).<sup>4,6</sup> This resulted in an estimated \$1.9 million in costs borne by that employer alone. Furthermore, 43% more claims per beneficiary are filed with health payers on behalf of IBS patients, a positive difference that climbs to 180% for prescription claims.<sup>4,7</sup>

The bulk of the direct cost burden of IBS is related to excessive prescription of diagnostic procedures that (1) are administered in an unstructured, serial fashion over the course of many months or years and (2) arise from the concerns of clinicians and patients who wish to rule out every credible competing diagnosis.<sup>8,9</sup> IBS patients undergo significantly more diagnostic testing than matched controls, with odds ratios for common and expensive studies such as endoscopy and radiological imaging tests ranging from 2.5 to 5.7. As many as 50% of patients being evaluated for IBS will undergo colonoscopy<sup>10</sup>; 25% of all colonoscopies performed in the United States are for evaluation of IBS symptoms.<sup>10,11</sup>

Despite such aggressive testing, the overwhelming majority of these procedures show normal findings in patients being assessed for IBS. Among the group of diagnoses that are typically being considered during a clinical evaluation, only maldigestion of lactose occurs at a frequency greater than 5%. Additionally, organic pathologic conditions, such as colorectal cancer and inflammatory bowel disease (IBD), occur at levels of less than 1%, and at equal or lower frequencies than they do in the general population.<sup>12,13</sup> Even in patients with "alarm features," for whom more invasive testing is currently recommended, organic disease was identified in only 3% of patients with suspected IBS in a study of 575 subjects; 1% had gastrointestinal cancer, 1.2% had IBD, and 0.7% had malabsorption.<sup>14</sup>

By contrast, a growing body of evidence suggests that, rather than being a single diagnostic entity, IBS instead represents an "umbrella" diagnosis comprised of different, often treatable conditions.<sup>15</sup> Habba et al demonstrated that 98% of patients had a final diagnosis that differed from IBS, and 68% of studied patients had treatable bile acid abnormalities or related conditions.<sup>15</sup> Furthermore, 98% of the latter group showed a favorable response to therapy, a figure vastly higher than that generally accepted for symptomatic response in IBS in general.<sup>15</sup>

Others have shown a meaningful prevalence of exocrine pancreatic insufficiency (6.1%) in subjects who fulfilled concurrent Rome criteria for IBS, using fecal pancreatic elastase levels as a diagnostic tool.<sup>16-19</sup> Similarly, fecal calprotectin levels have been demonstrated to effectively differentiate IBS from IBD,<sup>20-23</sup> and, when used as an alternative, noninvasive diagnostic testing may reduce the demand for colonoscopies and associated costs by as much as 50%, with the attendant realization of substantial cost savings.<sup>24</sup>

A computer-simulated economic analysis undertaken by the National Health Service in the United Kingdom showed that the use of fecal calprotectin was less costly and more diagnostically discriminative than routine blood tests — erythrocyte sedimentation rate (ESR), C reactive protein (CRP), serological markers, other neutrophil product markers, labeled white cell tests, and M2-pyruvate kinase that are currently employed to categorize the inflammatory profiles of IBD and IBS.<sup>25</sup> Use of calprotectin testing resulted in fewer unnecessary endoscopies and an increase in the number of patients who were correctly diagnosed.

Many other underlying and readily treatable causes of IBS symptoms exist. These include celiac disease/gluten sensitivity, intestinal parasites and protozoans, and intestinal dysbiosis.<sup>5,17-19,22,23,26-28</sup> Emerging evidence suggests thatthere may exist a colonic microbiome pattern unique to IBS patients<sup>29-31</sup>; the advent of 16S ribosomal DNA polymerase chain reaction amplification may allow rapid detection of such patterns within the gut microbiome.<sup>32-38</sup>

We recently completed a retrospective review of 2256 records from patients who underwent simultaneous, parallel testing for a group of fecal biomarkers relevant to disorders that may produce IBS symptomatology, with treatable diagnoses suggested in 82.8% of cases.<sup>39</sup>

The combination of awareness of the multifaceted nature of IBS and availability of low-cost fecal biomarker testing means that clinicians now have the ability to rapidly screen for, and in many cases identify specific, treatable diagnoses that produce the symptom constellation of IBS, while excluding dangerous conditions (such as IBD) with acceptable diagnostic accuracy.

The accurate evaluation of a broad array of GI functional biomarkers might also provide much-needed comfort to patients and clinicians alike and support implementation of symptom-based, psychosocially sensitive interventions with greater confidence. With concrete, objective laboratory information in hand that excludes significant inflammatory pathophysiology and guides a targeted treatment regimen leading to quicker improvement in patient symptoms, clinicians might be expected to order fewer expensive, invasive tests in attempts to rule out potentially significant alternative disease states. As a result, payers might in turn realize substantial cost savings.

We hypothesized that a structured, parallel, fecal biomarker panel would reduce total and GI-related diagnostic testing costs compared to the routine approach to diagnosis and managing IBS.

To test this hypothesis, we designed a retrospective cohort study to compare healthcare utilization and costs in patients whose clinicians made use of one such fecal biomarker panel (Genova Diagnostics, Asheville, North Carolina, http://www.gdx.net; detailed in Table 1), and matched controls, who received standard evaluation for IBS. The study, part of a series of investigations into the use of fecal biomarker testing in IBS, was named IMMINENT (Improved Medical Management of IBS Needs Enhancement by Novel Testing) in recognition of the needs of clinicians to find better ways to understand the biology of their patients who present with symptoms consistent with IBS.

Performance characteristics of these biomarkers for diagnoses that may present as IBS have been published elsewhere for pancreatic elastase,<sup>54-57</sup> calprotectin,<sup>58-60</sup> eosinophil protein X,<sup>61</sup>

Clostridium difficile,<sup>62,63</sup> parasitology exam,<sup>64</sup> with sensitivities and specificities for such diagnoses ranging from 83% to 96% and specificities in the range of 82% to 96%. The precise relationship of gut microbiota patterns to human health and disease is not yet sufficiently clear to provide specific performance characteristics.

### Methods

#### **OBJECTIVES:**

The objective of the project was to evaluate the utility of the fecal biomarker panel in a clinical setting by quantifying, comparing, and contrasting health ser- vice and pharmacy costs incurred by panel-tested and non-tested IBS patients.

#### DESIGN

We chose a retrospective, matched cohort design to compare the direct medical costs incurred by IBS patients tested with the fecal biomarker panel with those of matched control subjects.

#### SETTING

We examined the medical and pharmacy claims of a large American managed pharmacy benefit manager patient database (Medco Health Solutions, now part of Express Scripts, St. Louis, Missouri).

#### ETHICS CONSIDERATIONS

Because this study used only de-identified records of claims data, no protected health information could be linked to individual patients. Consent for use of medical and pharmacy claims data for research purposes was obtained by participating insurance carriers. For these reasons, institutional review board approval was not deemed necessary.

#### PATIENT POPULATION

#### Case Cohorts

Medical and pharmacy claims of plan members were searched to identify a cohort of patients who had been tested with the fecal biomarker panel by Genova Diagnostics, and who had one or more IBS-related diagnoses (Table 2). Because of major administrative changes at the participating institutions, actual percentage breakdowns for each ICD-9 code are not available. In a related study of a similar population,39 ICD-9 codes 789 (abdominal pain), 564.1 (IBS), and 797.1 (diarrhea) accounted for more than three-guarters of all records.

Records were eligible for inclusion in the study (1) if the patient had been continuously eligible to receive benefits for at least 180 days preceding and 30, 90, or 365 days following the fecal biomarker panel test date and (2) if each member's sponsoring client had approved the use of medical and pharmacy claims for research purposes. For this study, all data were de-identified prior to analysis, and no protected health information was recorded.

This selection process resulted in identification of three longitudinally nested cohorts (Table 3). The M30 cohort (209 patients) consisted of patients with records available at 30 days after the fecal biomarker panel test date; the M90 (203 patients) consisted of members of the M30 cohort for whom data were available at 90 days after the test date; and the M365 (132 patients) consisted of M90 patients for whom data were available at 365 days after the test date.

#### **Control Cohorts**

A population-based control cohort of patients with IBS-related diagnoses (Table 2) was created for each tested cohort. Each control cohort was created from a randomly selected pre-match pool of non-tested members who submitted a claim for one of the IBS-related diagnoses during the 30 days before or after each tested subject's test date. Similar inclusion criteria were then applied. To read the rest of this article please visit https://www.gdx.net/clinicians/medical-education/articles/imminent-study 
 Table 1 Selected Components of the Fecal Biomarker Panel

Selected Biomarkers	Description
Pancreatic elastase	Pancreatic elastase-1 (PE1) is a proteolytic enzyme secreted by the exocrine cells of the pancreas. Fecal PE1 testing provides a convenient, noninvasive, and reliable method of evaluating exocrine pancreatic function, well before steatorrhea occurs. <sup>18,19,40,41</sup>
Calprotectin	Calprotectin is a 36 kDa protein highly expressed in neutrophils, where it comprises up to 60% of the cytosol content. As a surrogate marker for intestinal neutrophil activity, fecal calprotectin levels >50 microg/g are considered a reliable indicator of neutrophil-mediated inflammation in the intestinal mucosa. <sup>42,43</sup>
Eosinophil protein X (EPX)	EPX is a cationic protein found in eosinophils. Upon degranulation, these proteins are released, mediating the eosinophilic immune response. <sup>44-46</sup>
Clostridium difficile	Once thought to be associated nearly exclusively with exposure to antibiotics, bowel infection with Clostridium difficile (C diff) is now recognized as being increasingly common in those without known antibiotic exposure (as many as 45.7% of people with culture-proven C diff infection had no antibiotic exposure in the past 90 days). <sup>47,48</sup>
Parasitology exam (microscopy and enzyme immunoassay)	A variety of protozoan parasitic infestations can produce symptoms of chronic diarrhea, bloating, and ab dominal pain that can overlap with those of IBS; all of these organisms are also capable of causing post-infectious IBS. <sup>49,50</sup>
Gut microbiota	Beneficial flora controls potentially pathogenic organisms, influences nutrient production, removes toxins from the gut and stimulates the intestinal immune system (GALT). <sup>28,51-53</sup>

Table 2 Diad	anostic Codes	for IBS-related	Diagnoses
	gnostie coues	Tor ibb related	Diagnoses

ICD-9 Code	Diagnosis
564.0	Constipation, unspecified
564.01	Slow-transit constipation
564.1	Irritable Bowel Syndrome
564.9	Functional intestinal disorder, unspecified
579.9	Unspecified intestinal malabsorption
787.91	Diarrhea
789	Abdominal pain
789.06	Abdominal pain, epigastric
789.07	Abdominal pain, generalized
536.8	Dyspepsia and other specified disorders of function of stomach
536.9	Unspecified functional disorder of stomach
558.9	Other and unspecified noninfectious gastroenteritis and colitis
787.3	Flatulence, eructation, and gas pain

#### Table 3 Summary of Selection Process

Records with fecal biomarker panel	37 945
Records matched to Express Scripts database	6892
Records eligible for study	1656
Records including pharmacy data	1112
Records with data for 30 days after index date (M30)	209
Records with data for 90 days after index date M90)	203
Records with data for 365 days after index date (M365)	132

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Author Affiliations Express Scripts, St Louis, Missouri (Drs Parsons, Dechairo, and Markward); Lupine Creative Consulting (Dr Goepp); Genova Diagnostics, Asheville, North Carolina (Drs Fowler, McBride, and Landis); Institute for Functional Medicine, Gig Harbor, Washington (Dr Hanaway).

Correspondence Elizabeth Fowler, PhD efowler@gdx.net

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# **A Comparison of Telomere Lengths Derived from Buccal** and Whole Blood Samples via qPCR.

#### **INTRODUCTION**

Whenever cell division occurs for any purpose (growth, wound healing, repair or replacement, etc.), the telomeres on each arm of each chromosome of each daughter cell shorten. Once an individual is fully grown, the rate of telomere loss can in this sense be considered a marker for an individual's level of cellular turnover, wear and tear. Once an individual cell's telomeres reach a certain critical shortness (3-5 kilobases) the cell goes into a state of senescence and is no longer able to divide. As people age, this process appears to impact rapidly dividing cell systems (e.g., digestive systems, skin, vascular tissues, etc.), both limiting the availability of cellular repair, as well as acting as a break on uncontrolled cellular proliferation. Because of this critical function, telomeres play a fascinating role in commonly associated diseases of aging.

Telomere shortening has been implicated as a mechanism explaining variations in life expectancy and a variety of aging-related diseases.Inadditiontothetelomeropathathies and progeroid disorders associated with defects in telomere length or repair, there is a vast body of peer reviewed scientific publication papers showing a reproducible and significant association between short telomere length and increased disease risk for a wide variety of common diseases

#### Jonathan Stein, PhD

(Table 1). It's worth emphasizing that most of these studies use peripheral whole blood to determine average telomere length.

Short telomeres are associated with reduced health, but whether this association is causal, due to reverse causation, or due to the influence of confounding factors is largely unknown (Bojesen, 2013). However, the accumulation of senescent cells is associated with decreased lifespan in animal models, and the reduced availability of cells for repair or replacement has been one proposed explanation (Baker, 2016).

Further, accumulated evidence places oxidative stress as an accelerator of telomere attrition during cell replication (von Zglinicki 2002). Several studies denote inflammation and oxidative stress as the major contributors to pathophysiology of aging and age-related cardiovascular disease (Libby 2002; Stocker & Keaney 2004). In the Cardiovascular Health Study (CHS), in the face of subclinical and clinical cardiovascular disease and Telomere Length, the authors demonstrated negative correlation of IL-6 and C-reactive proteins with Telomere Length, highlighting the role of inflammation in Telomere Length regulation (Fitzpatrick et al., 2007). O'Donovan et al. reported of increased systemic inflammation with shorter Leukocyte Telomere Length in the Health, Aging and Body Composition

Study (Health ABC) cohort (O'Donovan et al., 2011).

While increased risk is no guarantee of disease manifestation, it's often useful for an individual to know if they are at increased risk of a disease in order to modify their lifestyle or seek treatment to minimize that risk. As such, testing may be deemed clinically useful for individuals concerned about their risk for these aforementioned conditions.

Several independent longitudinal population studies have shown that telomeres shorten with age, and that the average size of telomeres and rate of attrition varies between individuals (Berenson et. al., 2008, Demerath et. al., 2010, Whooley et. al., 2010). Many studies have also reproducibly reported the correlation between shortened telomeres, specific disease states, morbidity and mortality.

Mechanistically, Telomere Length reflects the cumulative burden of oxidative stress and repeated cell replication, and it has been hypothesized that cumulative oxidative stress may underlie the link between telomeres and aging-related disease in humans (Serra et al., 2003). Further, reduced average telomere length is associated with increased risk for a wide variety of common diseases, and it may be clinically useful to identify patients at increased disease risk in order to determine the necessity of clinical or lifestyle interventions.

#### **TELOMERE TESTING**

At present, there are several different methods routinely used to determine telomere length for research purposes (FISH, qPCR, Southern blots) (Blasco, 2012). Although most testing laboratories (and the majority of research studies) use



whole blood as the testing material, we were interested in evaluating buccal swabs as potential tissue for testing for a variety of reasons (ease of collection and transport, etc.). Studies have compared telomere lengths in vascular tissue, whole blood and muscle, and shown that all of these different tissues provide similar results (Wilson et. al., 2008), suggesting that the rate of telomere loss and repair might be similar across other tissue types.

#### **Materials and Methods**

Briefly, whole blood and buccal swabs were obtained from 38 in-house volunteers and DNA extraction and amplification was performed via routine methods. Sample DNA was initially extracted from the buffy coat of 10 ml of whole blood or buccal cells obtained from a cheek swab.

Purification of DNA was performed in five steps: 1) Centrifugation to collect the cells, 2) Analysis of the cells and precipitation of protein, followed by centrifugation to pellet the protein, 3) precipitation of the DNA with isopropanol followed by centrifugation to pellet the DNA, 4) washing the DNA with 70% ethanol, and 5) addition of rehydration solution to rehydrate the DNA.

High throughput qPCR analysis was then performed to determine the individual sample's telomere length. Telomere length was determined analyzing 10 ng of genomic DNA via high throughput qPCR assay. The assay determines a relative telomere length by measuring the factor by which the sample differs from a reference DNA sample in its ratio of telomere repeat copy number to singe gene copy number (36B4 gene). This ratio (T/S ratio) is thought to be proportional to the average telomere length.

Oligonucleotide primer sets for amplification were as follows:

-36B4 primers (specific for region of 36B4 gene on chromosome 12, which encodes acidic ribosomal phosphoprotein P0.):

-36B4d: 5'-CCC ATT CTA TCA TCA ACG GGT ACA A-3'

-36B4u2: 5'-AAT TTG TCA CAG TCA GGC CCA-3'

-Telomere primers:

-tel1b: 5'-CGG TTT GTT TGG GTT TGG GTT TGG GTT TGG GTT TGG GTT-3' -tel2b: 5'-GGC TTG CCT TAC CCT TAC CCT TAC CCT TAC CCT TAC CCT-3'

During the PCR amplification, the genetic area of interest is copied many times, and the accumulation of the genetic material is monitored during the reaction using fluorescent detection of a DNA binding dye (in this case SYBR Green I). A sample is considered positive once it crosses a detection threshold called the Crossing Point (Cp). Within an experimentally determined dynamic range, the Cp is proportional to the amount of input DNA so that plotting Cp versus log (input DNA amount) yields a linear relationship. Using this information, a standard curve can be generated allowing quantitation of unknown samples. Comparing the amount of fluorescent signal generated from a telomere versus that from an amplification of a single-copy genetic sequence (here the acidic ribosomal phosphoprotein P0, 36B4) provided information about the relative amount of telomere sequence per chromosome. A standard curve in duplicate is run with each sample set. In addition, at least one 'no template' (negative) control and two positive controls of two different known telomere lengths (high and low) were included with each run. A set of at least two dilutions of the DNA used for the standard curve were run as positive control. T/S ratio was calculated using a standard curve and served as a surrogate value for relative TL.

#### Results

Telomere average length results were obtained from buccal cells and whole blood sampes from 38 in-house volunteers can be seen in Table 2 (Sample Results Comparison). On average, the average telomere sizes of buccal swabs and whole blood derived cells tend to show large within patient differences. Two samples, LaJ-36 and SG-16 reproducibly gave large comparative size differences between buccal swabs and whole blood results, 133% and 103% respectively, and were excluded from further analysis.

Excluding the two outliers, the average difference between buccal cell and whole blood derived telomere results obtained from the same patient was 21%, with an average CV of 63%. By comparison, the average difference between whole blood samples obtained from the same patient (as determined by a number of different methods) runs 4-8%. As seen in Figure 1., even after excluding the two outliers, the overall correlation between buccal swabs and whole blood is poor (R2 = 0.11).

#### Discussion

The results obtained show that overall, telomere lengths derived from buccal cells and leukocytes derived from whole blood differ significantly. This suggests that within the same individual, different tissue systems may be subject to different types of wear and tear, resulting in different turnover rates. Although this conclusion may seem obvious, it has important consequences when it comes to getting a patient's telomeres tested. Average telomere length derived from whole blood cells is associated with a wide variety of common clinical conditions (Table 1).

However, if telomere lengths derived from buccal cells do not correlate with whole blood telomere lengths, it's possible that buccal cells will not correlate with these diseases, and as such may lack clinical significance. Supporting this idea, one study comparing leukocytes and buccal cell derived average telomere lengths and ischemic heart failure (IHF), the telomere lengths in IHF patients were found to be significantly shorter than healthy controls in leukocytes (p=0.002), but not buccal cells (p=0.19) (Wong et al., 2011). In a modest study of patients with bone marrow failure due to dyskeratosis congenita, Diamond-Blackfan anemia, Fanconi anemia, or Shwachman-Diamond syndrome), the median qPCR telomere length was longer in buccal cells than in blood (overall T/S ratio= 1.16 vs. 1.05, p=0.001, 0.006, respectively) (Gadalla, 2010).

Although there are reports that telomere lengths derived from buccal cells are foreshortened due to childhood traumas (Drury, 2014) and may also be associated with Alzheimers disease (Thomas, 2008), we suspect that the immune system activation that occurs due to infection or inflammation (and consequently reduces telomere length), may cause the telomere lengths obtained from whole blood to be more relevant than buccal cells, and thus be more clinically relevant as a biomarker for common diseases.

Based on these findings, we continue to offer telomere testing using whole blood and advise against using buccal cells to determine telomere length as a marker for overall morbidity and mortality until additional clinical studies validate the utility of this application.

#### <u>Table 1</u> <u>Telomeres, Telomeropathies and</u> <u>Common Diseases</u>

Atherosclerosis: Telomere Length is inversely associated with arterial calcification and is highly correlated to arterial age rather than chronological age, supporting the notion of telomere being a yardstick for biological aging (Mainous & Diaz 2010). Endothelial cells at the atherosclerotic plaque have been shown to have shorter telomeres compared to endothelial cells from subjects without CAD (Ogami et al., 2004), and an association between shortened telomeres in white blood cells and patients with atheroscerosis has also been reported (Goodall et. al., 2001).

**Cancer:** Meta-analysis of 27 different studies evaluating associations between telomere length and cancer in 12,450 patients yielded a pooled OR of 1.96 (95% CI: 1.37-2.81, P = 0.0001) for the association of short TL (telomere length) and cancer. (Savage et. al., 2011. The Association of Telomere Length and

Cancer: a Meta-analysis. Cancer Epidemiol Biomarkers Prev June 2011 20; 1238). 47,102 general population participants from the Copenhagen City Heart Study and the Copenhagen General Population Study showed an association with shorter telomeres and reduced survival after cancer was diagnosed (Bojesen, 2013).

**Hypertension and Cardiovascular Disease:** Though hypertension is more prevalent among elderly, a relationship between hypertension and Telomere Length has also been reported in the young; Jeanclos and colleagues (Jeanclos et al., 2000) has shown that Telomere Length is shorter among younger patients with hypertension compared to healthy subjects at the same age. In addition to hypertension, age-associated Cardio Vascular Diseases (CVDs) include atherosclerosis, coronary artery disease, myocardial infarction (MI), and heart failure.

Telomere Length has been established as independent risk predictor for myocardial infarction and stroke (Parks et al., 2011). Studies have reported that shortened Telomeres correspond with a threefold increased risk of myocardial infarction and stroke in some individuals compared to healthy controls (Samani 2003, Aviv et al., 2007). Numerous studies support this finding. Studies (Benetos et al., 2004; Brouilette et al., 2007; Fitzpatrick et al., 2007) have shown that high rates of telomere attrition are associated with elevated CVD risk. Welleit et al. demonstrated higher rate of telomere shortening among people with CVD compared to people without CVD (Willeit et al., 2010).

**<u>CHD</u> and Inflammation:** Samani et al. showed that severe coronary heart disease (CHD) is associated with shorter Telomere Length (Samani et al., 2001). Since then, numerous studies have shown that shortened Telomere Length is linked to severity of CVD, (Hoffmann & Spyridopoulos 2011, et al.)

**Telomeropathies:** Disease manifestations for people with shortened telomeres include premature hair greying, aplastic anemia, immunodeficiency and opportunistic infections, entercolitis, emphysema and idiopathic pulmonary fibrosis, cryptogenic liver fibrosis–cirrhosis, osteoporosis, stem cell failure, and some cancers. (Blackburn, Nature Reviews Genetics 13, Oct 2012).



#### Table 2. Sample Results Comparison (TL = Telomere Length, L = Length)

Sample Results Comparison					
sample	blood TL	buccal TL	% differenc	e L difference	Age
MB 01	9.22	8.76	5.12%	0.46	21
JM 02	9.88	6.27	44.66%	3.61	37
KB 03	10.21	7.91	25.39%	2.30	61
LG 04	8.10	9.14	12.09%	-1.04	41
JG 05	9.89	8.10	20.00%	1.80	33
MiB 06	10.48	9.61	8.65%	0.87	22
JC 07	9.48	8.96	5.62%	0.52	25
KrB 08	10.66	8.05	27.86%	2.61	19
RK 09	8.64	6.53	27.80%	2.11	42
TR 10	10.23	9.13	11.36%	1.10	26
BoW 11 (K59335)	5.92	8.87	39.89%	-2.95	63
IE 12 (K59616)	7.82	9.26	16.87%	-1.44	44
NR 13 (K59612)	6.84	8.75	24.50%	-1.91	34
HW 14	6.27	7.47	17.40%	-1.20	35
TS 15	9.10	8.39	8.17%	0.71	26
OS 17	8.29	8.42	1.57%	-0.13	52
RP 18	6.54	4.94	27.93%	1.60	53
MC 19	8.91	5.71	43.67%	3.19	57
BeW 20	7.50	9.02	18.48%	-1.53	51
DL 21	6.51	7.81	18.26%	-1.31	51
KH 22	8.70	6.17	34.09%	2.54	31
SR 23	7.33	7.92	7.78%	-0.59	58
BA 24	6.73	6.01	11.23%	0.72	64
JuT 25	8.52	8.88	4.14%	-0.36	35
MJ 26	6.69	4.85	32.00%	1.85	41
AO 27	7.33	8.72	17.38%	-1.40	34
LB-28 (K59959)	6.50	7.69	16.77%	-1.19	39
PN-29	6.15	5.61	9.19%	0.54	55
RF-30	5.72	3.85	39.13%	1.87	54
RuS-31	6.25	8.53	30.89%	-2.28	33
KrS-32	7.11	4.90	36.71%	2.21	33
MM-33	9.03	8.79	2.68%	0.24	20
DA-34	7.68	8.74	12.98%	-1.07	26
KC-65	9.30	5.79	46.47%	3.51	33
TB-37	8.43	10.98	26.28%	-2.55	40
TiP-38	7.05	4.73	39.39%	2.32	24
TaW-39	7.62	7.19	5.79%	0.43	35
LaJ-36	6.48	32.21	133.04%	-25.73	38
SG-16	8.28	26.03	103.49%	-17.75	38
		avg	26.02%	-0.70	
		sd	0.26	5.33	
		CV	98.02%		
Excluding 2 outli	ers	avg	21.03%	0.44	
		sd	0.13	1.80	
		CV	63.10%		



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Author Biography: Jonathan Stein, PhD is the director of Science and Research at Spectracell Laboratories, Inc. Any questions regarding this material can be directed to jstein@spectracell.com Office: 713-621-3101

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#### ΒY

Richard F. Walker, PhD, RPh and Rakesh Saini, PhD

> Recognizing their anabolic potential, growth hormone (hGH) secretagogues are particularly attractive therapeutic candidates for opposing age/diseaserelated muscle loss and sarcopenia. One such molecule is growth hormone releasing peptide-2 (GHRP-2; DAIaDβNaIAIaTrpDPheLysNH2) an analog of the gastrointestinal peptide, ghrelin1 that displays comparable activity in vivo <sup>2</sup>. GHRP-2 is orally bioavailable <sup>3,4</sup> making it potentially valuable for use in "medical foods" to treat sarcopenia, a major threat to

health and vitality during aging <sup>5</sup>. Medical foods are enteral formulations for dietary management of disease(s)/condition(s) that have nutritional needs which cannot be met by normal diet. Their regulatory category was created by the 1988 Orphan Drug Act Amendments and are subject to FDA general food and safety labeling requirements<sup>6</sup>.

Extensive review of the endocrine and toxicology literature failed to identify any report(s) of general or reproductive toxicity nor of carcinogenicity for GHRP-2 in animals or humans. In fact, many peer-reviewed publications make anecdotal comment that the peptide is safe for human use and without side effects even when administered at relatively high daily doses for a year or longer<sup>4</sup>. Furthermore, a literature search of ghrelin side effects failed to reveal any pathophysiological effects that might result from the influence of GHRP-2 on ghrelin target sites including the stomach, GI tract, pancreas, heart and brain. Nonetheless, despite the general consensus that GHRP-2 is safe for human use, little formal information on toxicity of the peptide is available. Accordingly, we commissioned a contract research organization (CRO) to perform a repeated oral dosing study to gain some information about its in vivo toxicity profile.

# Safety of Growth Hormone Releasing Peptide-2

### (GHRP-2) FOR USE IN MEDICAL FOOD FORMULATIONS

Due to space constraints, this article may be missing tables that support the research. For in-depth information and additional research, please visit www.sarcotropin.medaus.com/article

abstract

Growth hormone releasing peptide-2 (GHRP-2; DAIaDβNaIAIaTrpDPheLysNH2), an analog of ghrelin, is a pituitary secretagogue that stimulates production and release of human growth hormone (hGH). In its three decades-long clinical history, GHRP-2 has never been reported to cause adverse side effects. Because of its anabolic potential, oral bioavailability and apparent lack of toxicity, the peptide may be effective in "medical food" formulations intended to oppose sarcopenia, i.e., atrophy of muscle that leads to frailty, loss of independence, quality of life and increased diseases of aging. The purpose of the current

study was to evaluate GHRP-2 safety in a 90 day toxicology study. The results of all tests were negative. A human, No Observable Adverse Event Level (NOAEL) based upon rat data was 8 mg/kg, well above commonly used human doses of 0.02 to 0.07 mg/kg. Thus, these findings and lack of toxicity reports in the literature support the view that GHRP-2 is safe for human consumption in "medical foods" indicated for treating sarcopenia.

methods

Testing was performed according to the Globally Harmonized System (GHS)7 using ten week old Wistar rats. GHRP-2 was administered for ninety consecutive days to identify possible health hazards resulting from repeated dosing and also to define a No Observed Adverse Effect Level (NOAEL) for establishing safety levels in humans. Sixty male and 60 female rats were randomly assigned to ten groups each containing 6

males and 6 females. Groups 1 - 4 were given 0, 5, 15, and 50 mg/kg GHRP-2/day, respectively, and observed twice daily for 90 consecutive days. Groups 5 and 6 received 0 and 50 mg/kg/day for 90 days and observed an additional 28 days to determine whether toxic effects associated with treatment if any, are permanent or transient and capable of recovery/ reversal. Groups 7 - 10 received 0, 5, 15 and 50 mg/kg/day, respectively, for toxicokinetic analysis after 90 days.

Each rat was weighed one day before initiating treatment (day 0) and at weekly intervals throughout the study period. The last body weight was recorded one day prior to blood collection (on day 90 for all treated dose groups, on day 118 for reversal dose groups, and on day 89 for all toxicokinetic dose groups before starting their overnight fast).

Food consumption was recorded for the treated and reversal groups by offering a fixed amount of feed each week after which the amount remaining was collected, weighed and consumption calculated.

Blood and urine were collected at the end of the study for hematological and metabolic analysis. Blood for toxicokinetic evaluation was collected under CO2 anesthesia on days 1 and 90, pre- and post- dosing from overnight fasted rats.

Specific tissues/structures were collected for histopathological examination from control and high dose treated groups (1 and 4) and both reversal groups (5 and 6).

Statistical analyses of the data were performed using Bartlett's test of homogeneity of variances, Welch's one way analysis of variance (ANOVA) and Dunnett's test of multiple comparisons using SAS software, version 9.1.3. All analyses and comparisons were considered significant at the 95% confidence limit. During repeated dosing with GHRP-2, changes in body weight were not significantly different in any treatment group relative to controls, indicating that normal growth was unaffected. Mean starting body weights  $\pm$  S.E.M. for males ranged between 123.17 $\pm$  6.20 and 126.33 $\pm$  7.97 grams, and between 404.83  $\pm$  7.40 and 421.83  $\pm$  12.0 grams after 90 days on study. These changes represented 223.0, 236.6, 227.4, and 236.8 percent increases in body weight for groups 1 - 4, respectively. For females, the starting and ending mean body weights were 130.83  $\pm$  4.16 and 131.83  $\pm$  4.49 versus 254.67  $\pm$  8.23 and 274.0  $\pm$  9.36, representing increases of 101.4, 105.8, 94.6 and 109.2 percent, respectively.

Soft stool was occasionally observed in some rats from groups 1, 2, 4 and 5 during treatment. Since GHRP-2 like ghrelin increases gastric emptying, this effect may have contributed to the transient softening of stool in a few animals. During the course of clinical observation no unusual change in any clinical parameter was noted, and animals from all groups survived the full duration of study.

Toxicokinetic measures on day 1 and day 90 of the study are presented in Table 1. There were no major profile changes in the treated groups when compared with gender matched, vehicle controls.



There were no statistically significant changes for the majority of hematological parameters measured. Hematological data from treated and reverse dosing groups are presented Tables 2a and 2b, respectively. Statistical significance (p<0.05) was reached in a few instances including  $\downarrow$ MCV ( $\bigcirc$  - group 2 vs group 1) and  $\uparrow$ neutrophils and  $\downarrow$ platelets ( $\bigcirc$  – group 2 vs group 1),  $\uparrow$ reticulocytes ( $\bigcirc$  – group 3 vs group 1),  $\uparrow$ MCH and MCHC with  $\downarrow$ platelets ( $\bigcirc$  – group 4 vs group 1),  $\uparrow$ RBC ( $\bigcirc$  - 6 vs group 5). Although significant, all values were within the normal range of laboratory control data. Hence, they are considered to be incidental changes/ normal biological variations and not related to treatment.

There were no statistically significant changes for the majority of biochemical parameters measured in treated and reverse dosing groups compared with their controls (Tables 3 a and 3b). As with the hematological measures, statistical significance was reached in a few instances including:  $\uparrow$  triglyceride and  $\downarrow$  phosphorous ( $\bigcirc$  - group 2 and 3 vs group 1),  $\uparrow$  globulin and  $\downarrow$  calcium ( $\bigcirc$  - group 4 vs group 1),  $\uparrow$  sodium and  $\downarrow$  calcium ( $\bigcirc$  - group 5),  $\uparrow$  cholesterol ( $\bigcirc$  - group 3 vs group 1),  $\uparrow$  sodium and chloride ( $\bigcirc$  - groups 3, 4 and 6 vs group 1). However, all values were within the normal range of laboratory control data and thus, considered to be incidental changes or normal biological variations and not related to treatment.

Urinalysis data are presented in Table 4. Values in treated rats were found to be comparable to those in controls, except for two statistically significant differences including  $\uparrow$  specific gravity and  $\downarrow$  pH ( $\bigcirc$  - group 3 and 6, vs group 1 and 5,

respectively. The differences were marginal and did not fit any pattern, thus are considered to have occurred by chance or due to biological variation and not toxicity.

Except for ↑adrenal gland weight (♂ - group 6 vs group 5) organ weights were not significantly different between groups. The difference was within normal laboratory control values and not considered to be due to treatment. Organ weights are presented in Table 5.

Necropsy of rats from all groups did not reveal any external pathological findings. Internal examination of the various groups revealed a few gross lesions that were sporadic and observed in all groups. The lack of pattern indicated spontaneous genesis and an absence of any treatment related effects.

Microscopic examination of tissues collected from treatment groups 1 and 4 and reversal dose groups 5 and 6 as well as tissues with gross lesions from groups 2 and 3 revealed various pathological changes in some organs (Table 6).

As with gross lesions found at necropsy, the lack of pattern of microscopic changes indicated spontaneous genesis and an absence of any treatment related effects. Thus, oral administration of GHRP-2 for 90 consecutive days at doses up to and including 50 mg/kg was not associated with adverse effects in any study parameter and thereby defines the No Observed Adverse Effect Level (NOAEL) as 50 mg/kg.





discussion

GHRP-2 has been used clinically for three decades based upon its ability to impart physical and functional benefits including but not limited to improved body composition, sleep, immune function, cardioprotection, etc.<sup>2</sup> without any evidence of it causing adverse events. For the most part, lack of toxicity has been reported anecdotally in studies performed for therapeutic purposes including muscle loss. Sarcopenia is a serious and global threat to health and quality of life in aging populations. Characteristic of sarcopenia is spontaneous and progressive muscle weakness with actual loss of lean body mass that contributes significantly to frailty, loss of functional mobility and independence as well as increased risk for development of intrinsic disease<sup>8,9</sup>. In

fact, erosion of skeletal muscle mass occurs in as much as 30% of the population beyond the age of 60 years. While sarcopenia is known to result from multiple factors, including age-related GH insufficiency, there are currently no commercially available products that provide a means to replace anabolic hormones as a means of therapy. However, the link between sarcopenia, disability and pathology among elderly men and women highlights the importance of developing all effective and safe means including FDA designated "medical foods"<sup>6</sup> that are indicated to oppose its progression during aging.

Since GHRP-2 is known to stimulate production and release of hGH by the pituitary, it might also be included in future formulations of food supplements such as Abbott Laboratory's "Ensure® Muscle Health" that currently contains the anti-catabolic metabolite of leucine, calcium ß-hydroxy-ß-methylbutyrate. Thus, GHRP-2 has the potential to complement resistance exercise, amino acids and protein supplements as another beneficial factor for opposing sarcopenia and cachexia.

The results of toxicity studies reported herein were negative in every case, which is consistent with the absence of reports of adverse events for GHRP-2 in the peer-reviewed literature. In fact, acute or chronic toxicity could not be demonstrated at even the extremely high doses of 2000 mg/kg or at 50 mg/ kg (NOAEL), respectively. When converting Animal Dose in mg/kg to Human Equivalent Dose (HED), regulatory guidelines recommend that when HED is based upon rat NOAELS, either divide the animal dose by 6.2 or multiply it by 0.16<sup>10</sup>. Based upon this recommendation, a NOAEL for human clinical application of GHRP-2 would be approximately 8 mg/kg. Heretofore, the highest therapeutic dose administered orally to human beings for as long as one year was 0.9 mg/kg twice daily (or 1.8 mg/kg/day)<sup>4</sup>. It has been administered intranasally for twice as long without any evidence of side effects. While effective in stimulating hGH for realization of its intended clinical benefits, the risk of dose-related adverse events occurring at the therapeutic doses was non-existent. Furthermore, doses as low as 4 mg orally or 1 mg subcutaneously per day which are currently being used to sustain lean body mass during aging are equivalent (based upon a 60 kg human being) to only 0.07 or 0.02 mg/kg/day, respectively. Thus, the absence of publications reporting side effects in human subjects receiving GHRP-2 is not surprising.

In conclusion, the findings of this study, taken in conjunction with a history of three decades use in human subjects without a single report of toxicity in the peer-reviewed literature, support the view that GHRP-2 is safe and effective when administered orally as a component of "medical foods" or nutritional supplements to treat age-related sarcopenia.

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# **IONODERME INFUSION SYSTEM-AFFINITY**

A novel system for direct Intrafollicular infusion of liquid solutions containing active ingredients.

#### Marina Landau,<sup>1</sup> M.D., Michael Tavger, PhD

1- Dermatology, Wolfson Medical Center, Holon, Israel

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#### **INTRAFOLLICULAR INFUSION**

Skin provides the largest interface between the human body and the external environment and at the same time serves as an effective barrier. It prevents excessive loss of water from the body and likewise blocks entry of most topically applied substances, except for ones which are lipid-soluble and of low molecular weight. Under physiological conditions the intact stratum corneum significantly impedes penetration of large hydrophilic, charged molecules and particulate substances through the skin.

A number of strategies are currently employed to overcome the barrier function of the skin to enhance penetration of therapeutic compounds. Substance delivery through the hair follicles has gained a lot of interest in the field of skin penetration research as the follicles represent an important pathway for topically applied substances. They function as a reservoir and also as portals of entry to viable skin. In addition, they represent invaginations of the epidermis, which significantly increase the skin surface area available for penetration. It has been shown experimentally that when follicular openings are selectively blocked during intrafollicular infusion, a subsequent decrease of concentration of the active compounds is measured in the skin. Calculation of total ducts inner surface is difficult, since it varies with age, sex, locale, etc., but it is estimated as being ten times greater than the total size of the epidermis. Therefore, the presence of large numbers of hair follicles significantly increases the penetration rates of topically applied compounds. [1-3]

Hair follicles also serve as a reservoir, in which active substances are protected from shedding, and are retained for prolonged time periods, enhancing their penetration to and through the skin cells. Furthermore, due to the follicular ducts' orientation down through the skin, the pores can serve as a channel leading to the depth of the dermis. Deposition of active compounds in the depth of the follicle may target specific cell groups there, such as follicular stem cells, important for skin rebuilding and regeneration. [4]

Following the understanding of the possible advantages of transfollicular penetration, different therapeutic approaches have been developed over the last several years. One of them is direct intrafollicular infusion of liquid solutions containing active ingredients. [5]

To achieve intrafollicular infusion, the system must fulfill the following requirements:

1. The liquid delivery source has to be directly attached to the follicular orifice. If not, due to duct resistance, liquid flow will flood the skin surface instead of being delivered into the duct.

2. The diameter of the flow source apparatus has to be smaller than that of the duct orifice. If not, the flow impact will deform the surrounding tissue to create a "check valve" having a closing effect on the duct orifice. 3. To create a short-term linkage between the duct pores and supplying nozzles to fill up the follicular reservoir, constant motion of the liquid delivery hand piece over the skin is required.

#### **IONODERME INFUSION SYSTEM-AFFINITY**

The Ionoderme Infusion System-Affinity was recently developed to accomplish intrafollicular delivery of active substances. The "heart" of the system consists of plastic tips, having two micro-nozzles each of  $50\mu$ m diameter, attached to the rotating delivery apparatus. As a result, this novel design produces rotating micro-jets of solution (Fig. 1).

Installed on the ergonomic hand piece, the tip comes in direct contact with the skin surface. Then, a negative pressure is created linking the skin surface with the rotating nozzle openings. At this moment, solution begins to flow through the nozzles under moderate pressure. While the operator moves the hand piece slowly over the treatment area, each time a contact occurs between an open pore orifice and a micro-nozzle emitting a micro-jet of solution, follicular space is filled with the solution. (Fig.2). Negative pressure (vacuum) helps to support skin and tip contact during treatment. It also recovers wasted solution, keeping the treatment area dry.

The infused solution is now "locked" in the follicular duct, walled by only two layers of epithelial cells in the depth of the dermis, with a larger potential absorption area and longer contact time. This magnifies the biological activity of the active ingredients in the solution.

The procedure is easily carried out, with the only skill needed by the operator being the ability to keep gentle contact between the treatment tip and the skin surface. Hard pressure will deform the surrounding skin and duct structure and may completely close it.



Fig. 1: Rotating micro-jets of solution are delivered through two micro-nozzles, each of 50µm diameter



Fig. 2: Each time a contact occurs between the pore orifice and the jet nozzle, follicular space is filled with solution

Active ingredients, including bleaching substances, antioxidants, vitamins and any other compounds targeting deeper layers of the skin, but so far blocked by the natural biological defense systems, can be delivered more effectively by the Ionoderme Infusion System-Affinity. Not only is penetration of these ingredients more effective through the follicular walls, but the longer contact time between the solution in the follicular reservoir and the skin cells, increases the partition coefficient of absorption (similar to the patch technique).

Other groups of therapeutic substances that can be delivered by Ionoderme Infusion System-Affinity include peeling agents such

#### as alpha or beta hydroxy acids, trichloroacetic acid, and more. Because of the more efficient absorption, lower concentrations of the peeling agents produce more significant effects. In the case of peeling substances, while lower parts of the follicular duct are "attacked", the upper parts remain relatively spared. In addition, the focal mode of penetration, centered exclusively on the follicles, creates fractional damage to the skin with faster regeneration.

To accommodate various treatment targets and treatment zones, Ionoderme Infusion System-Affinity's working parameters can be changed to regulate the amount of infused solution and the depth of its delivery.

#### **TREATMENT PROTOCOL**

Treatment protocol includes a series of 4-6 weekly or bi-weekly treatments. In the maintenance phase, treatments can be spaced to accommodate the patient's wishes. While during the treatment light discomfort can be sensed (due to motion of the hand piece under negative pressure), the post-procedural course is eventless.



Fig.3: 46-year-old patient with acne scars after 2 sessions of lonoderme Infusion System-Affinity treatment

The results are usually already visible just a few days after the first procedure. Because the intrafollicular effect is three-dimensional, the changes to the quality of the skin take place throughout its thickness, producing the aesthetic result (Fig.3-4).

Due to the high safety of the system, Ionoderme Infusion System-Affinity provides an unmatched opportunity to treat non-facial skin, such as the neck, décolleté and all other folliclebearing areas.



Fig. 4: 42-year-old patient with melasma after one treatment session with the lonoderme Infusion System-Affinity.

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# Diagnoptics





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### **ROUND TABLE**:

**Dr. Mark Rosenberg**, M.D., President of The Institute for Healthy Aging

Dr. Mark Tager, M.D., CEO, ChangeWell

Stephen Matlin, CEO, Life Length



Dr. Mark Rosenberg

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# May 19<sup>th</sup> at 7:00 p.m.

# Spanish Style Cocktail Hour with Tapas & Sangria

Join us as we naturally combine the Mediterranean diet with the latest in telomere education to help you promote healthy lifestyles. (The Mediterranean diet has been shown to lengthen telomeres.) This is a great opportunity to deepen your understanding of how telomere testing can be incorporated into your clinical practice. Telomere testing provides the ultimate scorecard to assess how your treatment plans

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### What you will learn:

- To establish the risk for age related diseases with only one test
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- How to work with valuable information about your patients' aging process
- How to take your practice to the next level and further distinguish your services by including innovative testing

Please RSVP to yfernandez@lifelength.com to allow an appropiate head count for Tapas & Sangría.







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#### Medisca • Booth #420

661 Route 3, Ste. C, Plattsburgh, NY 12901 USA Ph: 813-513-3054 • Fax: 855-850-5855 Email: customerservice us@medisca.com • Website: www.medisca.com MEDISCA is an industry leader in the repackaging and distribution of pharmaceutical compounding supplies. For over 25 years, MEDISCA has provided the compounding community with the highest quality compounding supplies, solutions, and fine chemicals.

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#### Metabolic Code Enterprises • Booth #317

9428 Kenwood Rd., Cincinnati, OH 45242 USA Ph: 513-608-7883

Email: fgeer@metaboliccode.com • Website: www.metaboliccode.com

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1801 N. Military Trail, Ste. 200, Boca Raton, FL 33431 USA

Ph: 561-910-4960

Email: info@mmimedicine.com • Website: www.mmimedicine.com

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Email: • Website: www.metagenics.com

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Email: info@nova-cutis.com • Website: www.nova-cutis.com

NovaCutis, Inc. was founded and headquartered in Miami, Florida, due to its strategic location. Since Miami is considered to be a multi-cultural city as well as a major hub to Central, Latin and North America, it was the perfect choice of location. Many years of experience in the Aesthetic field, medical devices, Dermatology and Plastic Surgery makes us an ideal partner to market and distribute your products.

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Ph: 410-776-4000 • Fax: 410-776-4009 Email: contactus@nutramaxlabs.com • Website: www.nutramaxlabs.com Nutramax Laboratories Consumer Care, Inc. researches, develops, manufactures and markets products that improve the quality of life for people and their pets. We manufacture safe and effective products using high-quality, researched ingredients, and follow quality manufacturing standards. Cosamin® Joint Health Supplements are the #1 Researched Glucosamine/Chondroitin Brand. Cosamin has been recommended by healthcare professionals for over 20 years.

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### PCCA • Booth #601

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### Physician Compounding Alliance • Booth #616

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## PRECISION ANALYTICAL INC.

### Precision Analytical • Booth #801

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FOR LIFE BALANCE



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# 2016 EDUCATIONAL SCHEDULE

## JUNE 16-18

Chicago Marriott Downtown Chicago, IL

- > A4M IV Therapy Symposium (June 17-18)
- > MMI Module V: Nutrition & Exercise

### **JUNE 23-25**

San Diego Marriott Marquis San Diego, CA

- > A4M BHRT Symposium
- > A4M Hormone Protocols Workshop (June 24-25)
- > MMI Module XIV(B): Weight Management

### AUGUST 11-13

Venetian/Palazzo Resor Las Vegas, NV

- > MMI Module IV: Gastroenterology
- > MMI Module VI: Toxicology & Detoxification
- > MMI Module XX (A): Triad
- > MMI Module XXIV: Compounding





### **SEPTEMBER 21-24**

Hyatt Regency Dallas Dallas, TX

- > ABAARM/ ABAAHP Exams
- > A4M BHRT Symposium (Sept 21-24)
- > A4M Symposium A New Prescription for Pharmacy Practice (Sept 24)
- > MMI Module III: Neurology (Sept 22-24)
- > MMI Module VII: Autoimmune Disease & Inflammation (Sept 22-24)
- > MMI Module XVI (A): Cardiovascular [Sept 22-24]
- > MMI Stem Cell Module III (Sept 22-24)

### **SEPTEMBER 30 - OCTOBER 1**

Capitol Hilton Washington, DC

> A4M Immune Symposium

### **DECEMBER 8-11**

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- > 24<sup>th</sup> Annual World Congress (Dec 9-11)
- > ABAARM/ABAAHP Exams (Dec 8-11)
- > MMI Module I: Endocrinology (Dec 9-11)
- > MMI Module VIII: Clinical Practice Protocols (Dec 9-11)
- > MMI Module XIV(C): Weight Management (Dec 9-11)
- > MMI Stem Cell Module IV (Dec 9-11)



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