Submitted to a PubMed-indexed and peer-reviewed journal for publication

A Natural Product Telomerase Activator Lengthens Telomeres in Humans: A Randomized, Double-Blind and Placebo-Controlled Study

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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±180 bp; p=0.005) while subjects in the placebo group significantly lost telomere length (290±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.[1, 2] (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.[2-4]

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.[5] 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.[10]

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 immuno-compromised individuals.[11, 12] 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.[1] 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, at 3 months, 6 months, 9 months and 12 months (final visit). The capsules were taken on an empty stomach in the morning.

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.[13] 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 (see below). 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 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.

Control cell lines and Southern blot

Life Length's control cell lines C0126, C0154, C0106 are immortalized human B cells purchased from the European Collection of Cell Cultures (ECACC). Lymphoblastoid tumoral cell lines REH and RAJI were purchased from ATCC (CRL-8286, CCL-86). Cellular stocks were prepared and kept in liquid nitrogen. Telomere length of the cell lines above were determined by a non-radioactive terminal restriction fragments (TRFs) by southern blot assay following protocol as

described in Kimura et al.[14]

Sample Preparation for HT Q-FISH

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 the density of 30,000 cells per well 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). On the next day, fixed cells were treated with pepsin to digest cytoplasm and nuclei were processed for hybridization in situ with the PNA probe. After a few washing steps adding DAPI for DNA staining, the plate was filled up with mounting medium and kept overnight at 4°C.

HT Microscopy

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 40x 0.95 NA water immersion objective. UV and 488 nm excitation wavelengths were used to detect the DAPI and A488 signals respectively. With constant exposure settings 15 independent images were captured at different positions for each well. After image acquisition, the nuclei image was used to define a region of interest for each cell measuring telomere fluorescence intensity in the A488 image in all of them. Results of intensity for each foci identified were exported from the Acapella software (Perkin Elmer). 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 variability from different operators and machine variances over time, all samples (baseline, 3 months, 6 months, 9 months, and 12 months) were tested at the same time by the same operator. The samples were blinded during the analysis.

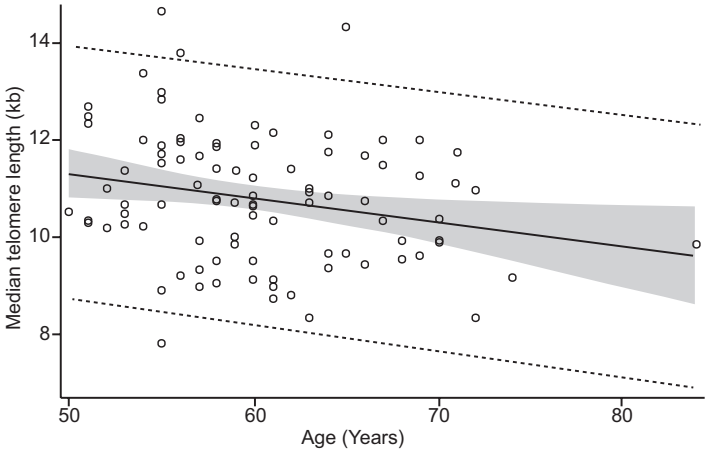

Figure 1: Baseline median telomere length. *Least squares fit method is used to generate the linear regression model. Each bubble indicates a subject. Solid line indicates 95% Confidence Limits and broken line indicates 95% Prediction Limits. R-Square is 0.056. The cross-sectional rate of change by age is -50 ± 21 (SE) bp per year.*

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.

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 ± 21 bp/year. Figure 1 shows the distribution of telomere length of the study participants at baseline.

	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 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 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.[15] 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.

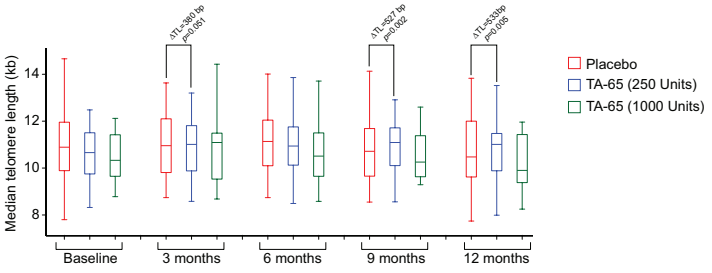

Figure 2: *Change in the median telomere length compared to baseline in placebo group, low dose TA-65 (250 units) group and high dose TA-65 (1000 units) group. Δ TL represents change in telomere length compared to baseline.*

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

Effect	Group	Time (months)	Change in TL (kb)	Standard Error	*P* value
Group effect	Placebo	At baseline		Reference group	
	TA-65 (250 Units)		-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

Table 2: Multilevel model analysis of median telomere length changes 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.

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 ± 90 bp (p= 0.07) and at 12 months the decrease was 290 ± 100 bp (p=0.01). Overall, the placebo group telomere data behaved slightly worse than expected (50-150 bp/year), which may suggest that this cohort was either not as healthy at baseline as expected, or perhaps had a relatively poor set of lifestyle behaviors.[16]

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 ± 170 bp longer, p=0.002), and 12 months (again, median TL was 530 ±180 bp longer, p= 0.005) and borderline significantly higher at 3 months (median TL was 380 ± 190 bp longer, p=0.05) but not significant at 6 months (Table 2 and 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 monitor compliance over time and increase the number of subjects and doses tested.

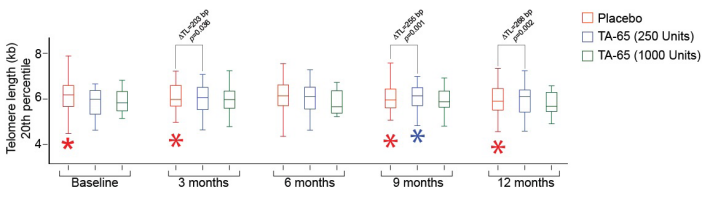

Figure 3
*Change in the 20th percentile telomere length compared to baseline in placebo group, low dose TA-65 (250 units) group and high dose TA-65 (1000 units) group. Δ TL represents change in telomere length compared to baseline. * indicate outliers.*

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.

	Average of 20th percentile telomere length (s.d) in kb				
Group	***Baseline***	***3 months***	***6 months***	***9 months***	***12 months***
Placebo	6.14 (0.71)	6.08 (0.64)	6.17 (0.62)	6.01 (0.66)	5.97 (0.68)
TA-65 (250 Units)	5.84 (0.68)	5.98 (0.78)	5.98 (0.81)	5.96 (0.72)	5.93 (0.49)
TA-65 (1000 Units)	5.93 (0.49)	6.01 (0.64)	5.83 (0.52)	5.92 (0.52)	5.77 (0.52)

Table 3: Average of 20th percentile telomere lengths.
The average 20th percentile telomere length in Placebo, low dose TA-65 (250 units) and high dose TA-65 (1000 units) group at baseline and at the end of 3, 6, 9 and 12 months in kilo base pairs (kb) with standard deviation (s.d.).

In the placebo group, there was a gradual decrease in average shortest quintile telomere length with time, as expected (Table 3). However, 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 no consistent change in the 20th percentile telomere length (see Table 3).

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 (see Table 4).

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.

Effect	Group	Time (months)	Change in TL (kb)	Standard Error	*P* value
Group effect	Placebo	At baseline		Reference group	
	TA-65 (250 Units)		-0.30	0.17	0.07
	TA-65 (1000 Units)		-0.20	0.17	0.25
Time effect	Placebo	0		Reference group	
		3	-0.06	0.05	0.22
		6	0.03	0.05	0.54
		9	-0.13	0.04	0.0026
		12	-0.17	0.05	0.0005
Group and time effect	TA-65 (250 Units)	0		Reference group	
		3	0.20	0.10	0.0358
		6	0.11	0.08	0.1763
		9	0.26	0.07	0.0009
		12	0.27	0.09	0.0023
	TA-65 (1000 Units)	0		Reference group	
		3	0.10	0.10	0.30
		6	-0.14	0.09	0.11
		9	0.10	0.08	0.21
		12	-0.01	0.09	0.93

Table 4: Multilevel model analysis of short telomere length changes 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 20th percentile telomere length. The estimate represents the change in telomere length in comparison to the reference group(s). Results were adjusted for age and sex.

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.1 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 ± 21 bp per year, which is higher than in some studies, but consistent with other published data.[5, 17, 18] The rate of telomere loss has been reported to be exacerbated in CMV+ individuals[19] 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 ±100 bp/year ($p = 0.01$) while the low dose TA-65 (250 units) group had net increase of 530 ± 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. In the previous observational study,[1] 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.[3] 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. Also, as compliance was not monitored in this study, it is possible that compliance declined in the high dose group.

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 ± 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[1, 2] 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,[20, 21] reduced mortality and disease risks in humans,[22-24] and increased resistance to infection.[25]

The dose response of TA-65 for telomere length could not be accurately ascertained with only 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.

Acknowledgement

We thank Life Length (Spain) for the measurement of telomere lengths.

Author Disclosure Statement

Laura Salvador, as clinical investigator of the study was supported by funding from T.A. Sciences Inc. Gunasekaran Singaravelu and Anitha Suram are employees of T.A. Sciences Inc. Calvin Harley, Peter Flom and Joseph Raffaele consult for TA Sciences Inc.

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Supplementary table 1

	Placebo			TA-65 (250 Units)			TA-65 (1000 Units)		
Clinical Parameters (reference range)	**Baseline (s.d.)**	**12 months (s.d.)**	***p*-value**	**Baseline (s.d.)**	**12 months (s.d.)**	***p*-value**	**Baseline (s.d.)**	**12 months (s.d.)**	***p*-value**
Gamma glutamyl transferase (<40 U/L)	29.79 (22.32)	31.69 (25.09)	0.52	24.21 (13.96)	25.22 (12.01)	0.88	32.07 (20.37)	28.82 (13.96)	0.48
Alkaline phosphatase (30-120 U/L)	**68.84 (18.53)**	**75.54 (22.96)**	**0.002**	74.03 (17.52)	74.47 (14.72)	0.92	73.52 (15.94)	75.45 (14.98)	0.11
Sodium (135-152 mmol/L)	141.05 (2.71)	141.00 (1.94)	0.77	140.29 (2.94)	140.26 (2.05)	0.92	**141.81 (2.82)**	**140.45 (2.42)**	**0.03**
Potassium (3.5 – 5.2 mmol/L)	**4.11 (0.32)**	**4.21 (0.29)**	**0.01**	4.17 (0.34)	4.18 (0.39)	0.92	4.31 (0.42)	4.32 (0.32)	0.68
Total bilirubin (0.3 – 1.2 mg/dL)	0.62 (0.27)	0.64 (0.31)	0.63	0.58 (0.22)	0.62 (0.17)	0.30	0.60 (0.24)	0.65 (0.24)	0.59
Total cholesterol (<200 mg/dL)	219.04 (34.98)	209.95 (31.66)	0.15	216.74 (41.57)	224.83 (45.14)	0.98	228.48 (45.27)	221.36 (43.42)	0.30
HDL cholesterol (Male: >40mg/dL; Female:>50mg/dL)	68.11 (15.73)	67.16 (14.38)	0.72	68.19 (9.85)	70.78 (12.39)	0.46	69.39 (17.50)	69.86 (16.87)	0.69
LDL cholesterol (<100 mg/dL)	128.98 (28.42)	119.71 (26.94)	0.05	129.63 (37.69)	134.28 (45.16)	0.89	136.13 (37.43)	130.00 (40.03)	0.41
Triglycerides (<150 mg/dL)	109.87 (52.51)	114.97 (62.84)	0.30	94.52 (48.61)	99.17 (43.09)	0.91	115.13 (38.71)	107.07 (39.97)	0.47
C reactive protein (<6 mg/L)	1.61 (1.58)	2.42 (3.64)	0.11	2.37 (2.51)	2.30 (2.41)	0.57	2.21 (2.21)	2.58 (2.19)	0.88
Insulin (5-25 µ5-25 n	11.01 (3.89)	11.63 (4.65)	0.39	9.67 (2.87)	8.91 (2.27)	0.29	11.14 (3.91)	10.32 (3.42)	0.25
Homocysteine (5-15 µmol/L)	10.29 (3.21)	10.31 (3.13)	0.97	10.41 (3.66)	11.12 (4.05)	0.69	11.28 (3.57)	10.97 (2.32)	0.42
Glucose (65-110 mg/dL)	92.86 (9.41)	93.79 (12.93)	0.88	91.14 (6.71)	90.17 (3.93)	0.16	95.78 (12.69)	96.59 (16.40)	0.54
Urea nitrogen (0-50 mg/dL)	36.18 (8.42)	34.10 (8.78)	0.11	36.21 (6.38)	37.83 (9.34)	0.51	38.44 (9.09)	38.82 (8.48)	0.88
Creatinine (<1.3 mg/dL)	**0.95 (0.14)**	**0.92 (0.14)**	**<0.001**	**0.93 (0.15)**	**0.91 (0.15)**	**0.03**	**0.98 (0.16)**	**0.95 (0.16)**	**0.02**
Uric acid (2-6.5 mg/dL)	4.70 (1.25)	4.96 (1.27)	0.21	4.66 (1.18)	5.22 (1.59)	0.06	**4.84 (1.29)**	**5.58 (1.41)**	**<0.001**
Alanine aminotransferase (<40 U/L)	21.12 (8.19)	21.46 (10.41)	0.79	19.18 (8.33)	18.70 (8.04)	0.92	29.41 (35.14)	27.50 (23.40)	0.39
Aspartate aminotransferase (<40 U/L)	22.66 (5.33)	22.56 (5.12)	0.94	21.43 (5.11)	21.39 (3.76)	0.95	25.26 (11.35)	25.18 (8.10)	0.97
Systolic BP (<120 mm Hg)	127.54 (18.54)	127.67 (17.47)	0.55	127.61 (12.94)	125.56 (14.93)	0.21	132.56 (19.46)	128.24 (10.02)	0.26
Diastolic BP (<80 mm Hg)	**76.44 (10.11)**	**74.46 (9.80)**	**0.03**	**81.04 (8.23)**	**74.78 (8.92)**	**<0.001**	**79.15 (10.25)**	**74.00 (10.02)**	**0.02**

Table S1: The measurements of markers and blood pressure are represented as mean (standard deviation) for the Placebo group, TA-65 250 units group and TA-65 1000 units group at baseline and after 12 months (The other time points are not represented here). The bold numbers indicate the significant changes at 12 months compared to baseline.