Proof of Concept in Humans

Investigating **O**ils **W**ith respect to **A**rterial flexibility (Photoplethysmography)

IOWA Study Results

Remarkable Experimental Results of Arterial Compliance Improvement with PEO Formulation (EZTREK™ precursor)

Professor Brian S. Peskin* with David Sim, M.D.*

* Brian Peskin received an appointment as an Adjunct Professor at Texas Southern University in the Department of Pharmacy and Health Sciences (1998-1999).

Dr. Sim is a practicing Interventional Cardiologist.

Long-term Results

IOWA: Investigating Oils With Respect to Arterial Flexibility

Significant differences in biological age compared with physical age

Brian Peskin, BSEE: Founder Life-Systems Engineering Science
with David Sim, M.D., Interventional Cardiologist

Long-term Results in Subjects using PEO Formulation (**EZTREK**™ precursor).

Significant differences (p=0.0015) with an experimental error of the mean +- 5 years. Subjects' cardiovascular biological age (average of) 8.8 years lower than their actual physical age.

Long-term (48-month maximum) PEO (**EZTREK**™ precursor) use

The effects of long-term PEO supplementation were evaluated in thirty-four (34) subjects with a daily dosage of 2,900 mg PEO formulation (**EZTREK**™ precursor). The sub-groups were as follows: twlve (12) male subjects and twenty-two (22) female subjects aged 35-75, with a *median age of 62-years-old*, utilizing the formulation a minimum of three (3) months to a maximum of 48 months. The median duration usage was twenty-four (24) months with half of the subjects using the PEO formulation less than 2 years and the remaining half utilizing the formulation over 2 years but less than 4 years. Vascular assessment was made via Photoplethysmography measuring arterial flexibility.

Overall Improvement = 73% Effectiveness–Highly Significant

Twenty-five (25) of the 34 subjects in the trial improved. **This corresponds to a seventy-three (73)%) effectiveness rating**. The average improvement in arterial flexibility was 9 years improvement meaning the average subject utilizing the PEO formulation (**EZTREK**™ precursor) had a cardiovascular system with the arterial flexibility of a subject representative of nearly a decade younger.

The best subject measured 39 years less (improvement) than his physical age waveforms would suggest. Of the 34 subjects, there was only one (1) subject who worsened.

NNT Effectiveness = 1.4—A "Remarkable" Result

The number needed to treat (**NNT**) is calculated as follows: 34 subjects / 25 improved subjects = **1.4**.

NNT quantifies how many patients have to be treated to obtain one successful outcome. An NNT of less than 50 is considered effective in the pharmaceutical industry.

Comparison with Statins

As a comparative example, statins, as reported by the pharmaceutical industry, have NNTs > 80 in preventing a cardiovascular event. This means a minimum of 80 patients would need to be treated to see a single (1) positive outcome when using statins.

In contrast, the PEOs improve a much more direct physiologic measure, i.e., arterial flexibility, in a profound way resulting in a **remarkable 1.4 NNT**.

Statistics (Highly Significant)—99.8% Accuracy

Long Term Results—No Baseline

IOWA: Investigating Oils With respect to Arterial Blockage
Significant differences in biological age compared to physical age

Brian Peskin, BSEE: Founder: Life-Systems Engineering Science with David Sim, M.D., Interventional Cardiologist

(Based on 34 patients using the PEOs (EZTREK™ precursor) over 3 months)

Age: 35-75	**Median age: 62**	22 females, 12 males

Paired t-test. Median: 24 months PEO use / Maximum: 48 months PEO (EZTREK™ precursor) **use**

Significant differences (**p 0.0015**) with standard error of the mean +- 5 years.
Subjects' **biological age being (average of) 8.8 years lower than their actual physical age.**

Note: This experiment has a 99.85% accuracy—30 times more accurate than the 5% standard error used in most clinical trials. Therefore, this result is *not* due to possible error and is *highly* significant with patient CV health 8.8 years better than physical age predicts.

Analysis by Alex Kiss, Ph.D. (statistics) — January 21, 2010
Analysis Variable : agediff

N	Minimum	Maximum	**Mean**	Std Dev	Pr > |t|
34	-39.00	22.00	**-8.82**	14.84	0.0015

Short-term Results

IOWA: Investigating Oils With respect to Arterial Flexibility

Significant differences in biological age compared with physical age

Brian Peskin, BSEE: Founder Life-Systems Engineering Science
with David Sim, M.D., Interventional Cardiologist

Short-term Improvement in Subjects using PEO Formulation (EZTREK™ precursor).

Significant differences (p=0.0099) with an experimental error of the mean +- 5 years. Subjects' cardiovascular biological age (average of) 7.2 years lower than their actual physical age.

Short-term (3-month) PEO (EZTREK™ precursor) use

The effects of short-term PEO supplementation were evaluated in sixteen (16) subjects with a daily dosage of 2,900 mg PEO formulation. The sub-groups were as follows: seven (7) male subjects and nine (9) female subjects aged 46-84, with a *median age of 64 years old*, utilizing the formulation a median of 2.5 months (half of the subjects with less duration and half of the subjects with more duration) and mean average of 3 month's usage. Minimum PEO formulation usage was one (1) month and the maximum subject usage was 8 months PEO usage. Vascular assessment was made via Photoplethysmography measuring arterial flexibility.

Overall Short-term Improvement = 43% Effectiveness–Highly Significant

Seven (7) subjects of the sixteen (16) subjects in the trial improved. **This corresponds to a forty-three-percent (43%) effectiveness rating over a very short period of time**. The average improvement in arterial flexibility was 7.2 years improvement, meaning the average subject utilizing the PEO formulation had a cardiovascular system with the arterial flexibility of a younger subject.

NNT Effectiveness = 2.3–A "Remarkable" Result

The number needed to treat (**NNT**) is calculated as follows: 16 subjects / 7 improved subjects = **2.3**, an outstanding result for such a short period of time.

Comparison to Statins

As a comparative example, statins, as reported by the pharmaceutical industry, have NNTs > 80 in preventing a cardiovascular event.

This means a minimum of 80 patients would need to be treated to see a single (1) positive outcome. In contrast, the PEOs (EZTREK™ precursor) improve a much more direct physiologic measure, i.e., arterial flexibility, in a profound way resulting in a **remarkable 2.3 NNT**.

Statistics (Highly Significant)—99% Accuracy

Short-Term Results—With Baseline

IOWA: Investigating Oils With respect to Arterial Flexibility
Significant differences in biological age compared with physical age (short-term)

Brian Peskin, BSEE: Founder: Life-Systems Engineering Science with David Sim, M.D., Interventional Cardiologist

(Based on 16 patients using the PEO formulation (EZTREK™ precursor) 1 month - 8 months)

Age: 46-84	**Median age: 64**	9 females, 7 males

Paired t-test. Median: 2.5 months PEO formulation use / Mean: 3 months PEO formulation use

---

Significant differences (**p 0.0099**) with an experimental error of the mean +-5 years.
Subjects' **biological age being (average of) 7.2 years lower than their actual physical age.**

Note: This experiment has a 99.00% accuracy—5 times more accurate than the 5% standard error used in most clinical trials. Therefore, this result is *not* due to possible error and is significant with patient CV health 7.2 years better than physical age predicts.

Analysis by Alex Kiss, Ph.D. (statistics) — March 26, 2010
Analysis Variable : agediff

N	**Mean**	Std Dev	Pr > |t|
16	**-7.24**	10.19	0.0099

PEOs versus Fish Oil

IOWA: Investigating Oils With respect to Arterial Flexibility
Significant differences in biological age compared with physical age

Brian Peskin, BSEE: Founder Life-Systems Engineering Science
with David Sim, M.D., Interventional Cardiologist

Subjects discontinued fish oil supplementation, replacing it with PEO Formulation (EZTREK™ precursor).

Significant differences (p=0.0001) with an experimental error of the mean +-5 years. Subjects' cardiovascular biological age (average of) 11.1 years lower than their actual physical age.

PEOs versus fish oil

The effects of the PEOs (EZTREK™ precursor) were evaluated in subjects who ceased fish oil supplementation, replacing it with a daily dosage of 2,900 mg PEO formulation. The effects of the PEO formulation were measured in 15 subjects: seven (7) male subjects and eight (8) female subjects aged 46-74, with a *mean age of 60 years old*, utilizing the formulation an average duration of 3.5 months. Vascular assessment was made via Photoplethysmography measuring arterial flexibility.

Overall Improvement

Thirteen (13) of the fifteen (15) subjects improved with the PEOs for an **87% effectiveness** rating and an **NNT of 15 / 13 = 1.2. Improvement was 11.1 years** as measured by standard population samples.

On average, the PEO formulation (EZTREK™ precursor) quickly improved the cardiovascular system's arterial flexibility by over 11 years (younger) in the subjects. Thirteen (13) subjects improved; one (1) subject remained the same, one (1) subject worsened by 1 year. Results were highly statistically significant **(p=0.0001) — 99.99% accuracy**.

Subjects with "high cholesterol"

Of the seven (7) subjects previously diagnosed with high cholesterol levels replacing fish oil supplements with the PEO formulation (EZTREK™ precursor), six (6) subjects improved their cardiovascular biological ages. This translates to an **NNT of 7 / 6 = 1.2** for improvement in cardiovascular system compliance in subjects with high cholesterol manifestations of heart disease.

Subject with both diabetes and "high cholesterol"

One (1) subject who had both diabetes and high cholesterol diagnosis also improved.

Comparison to Statins

As a comparative example, statins, as reported by the pharmaceutical industry, have NNTs > 80

in preventing a cardiovascular event. This means a minimum of 80 patients would need to be treated to see a single (1) positive outcome.

In contrast, the PEOs (EZTREK™ precursor) improve a much more direct physiologic measure, i.e., arterial flexibility, in a profound way, resulting in a **remarkable 1.2 NNT**.

Statin user improvements

Two patients are taking statins, and both subjects improved their biological age by twenty (20) years for an **NNT = 1 in those patients taking statins**. NNTs of less than 50 are considered excellent. Even with the small number of subjects in this sub-group taken into account, the results of this trial are exceptional and not due to chance.

These results clearly show that the PEO formulation (EZTREK™ precursor) is superior to fish oil supplements in preventing and reversing cardiovascular disease. In fact, as this experiment shows, fish oil WORSENS arterial compliance because the improvement is greater with fish oil taken than nothing!

Statistics (Highly Significant)—99.99% Accuracy

Analysis by Alex Kiss, Ph.D. (statistics)—August 20, 2010

Mean of BIO_AGE_W_FO variable

Analysis Variable: BIO_AGE_W_FO

Mean	Std Dev
49.20	11.33

Mean of BIO_AGE_PEO variable

Analysis Variable: BIO_AGE_PEO

Mean	Std Dev
38.07	8.12

Paired t-test run: mean change (FO - PEO) was found to be 11.1 (sd=8.4).
This was statistically significant (p=0.0001)

Analysis Variable: diff

Mean	Std Dev	t value	Pr > |t|
11.13	8.37	5.15	0.0001

Plethysmography and Pulse Wave Velocity Research—as used in IOWA Screening Experiment

1. Application of heart rate variability in prognosis of patients with diabetes mellitus. Markuszewski L, Bissinger A. Pol Merkuriusz Lek. 2005 Oct;19(112):548-52.

2. A Measurement of Electrocardiography and Photoplethesmography in Obese Children. C. V. Russoniello, V. Pougtachev, E. Zhirnov and M. T. Mahar. Applied Psychophysiology and Biofeedback: Volume 35, Issue 3 (2010), Pages 257-259.

3. Guidelines for the management of arterial hypertension: the Task Force for the Management of Arterial Hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). Mancia G, De Backer G, Dominiczak A, et al. 2007. J Hypertens. 2007;25:1105-1187.

4. Noninvasive pulse wave analysis for the early detection of vascular disease. Cohn J, Finkelstein S, McVeigh G, et al. Hypertension. 1995;26:503-508.

5. Contour analysis of the photoplethysmographic pulse measured at the finger. Millasseau SC, Ritter JM, Takazawa K, Chowienczyk PJ. J Hypertens. 2006;24:1449-1456.

6. Pulse pressure and aortic pulse wave velocity are markers of cardiovascular risk in hypertensive populations. Asmar R, Rudnichi A, Blacher J, London GM, Safar ME. Am J Hypertens. 2001;14:91-97.

7. Better management of cardiovascular diseases by pulse wave velocity: combining clinical practice with clinical research using evidence-based medicine. Khoshdel AR, Carney SL, Nair BR, Gillies A. Clin Med Res. 2007;5:45-52.

8. Wave reflection in the systemic circulation and its implications in ventricular function. O'Rourke MF, Kelly RP. J Hypertens. 1993;11:327-337.

9. Pulse wave analysis and arterial stiffness. Wilkinson IB, Cockcroft JR, Webb DJ. J Cardiovasc Pharmacol. 1998;32:S33-S37.

10. Pulse wave velocity and the second derivative of the finger photoplethysmogram in treated hypertensive patients: their relationship and associating factors. Hashimoto J, Chonan K, Aoki Y, et al. J Hypertens. 2002;20:2415-2422.

11. The peripheral pulse wave: information overlooked. Murray WB, Foster PA. J Clin Monit. 1996;12:365-377.

12. Photoelectric plethysmography - some fundamental aspects of the reflection and transmission method. Nijboer JA, Dorlas JC, Mahieu HF. Clin Phys Physiol Meas. 1981;2:205-215.

13. Frequency analysis of the peripheral pulse wave detected in the finger with the photoplethysmograph. Sherebrin MH, Sherebrin RZ. IEEE Trans Biomed Eng. 1990;37:313-317.

14. Arterial stiffness: clinical relevance, measurement and treatment. Izzo JL Jr, Shykoff BE. Rev Cardiovasc Med. 2001; 2:29-34,37-40.

15. Determination of age-related increases in large artery stiffness by digital pulse contour analysis. Millasseau SC, Kelly RP, Ritter JM, Chowienczyk PJ. Clin Sci (Lond). 2002;103:371-377.

16. Waveform analysis of peripheral pulse wave detected in the fingertip with photoplethysmograph. Hlimonenko I, Meigas K, Vahisalu R. Measure Sci Rev. 2003;3:49-52.

17. Photo-electric plethysmography as a monitoring device in anaesthesia. Dorlas JC, Nijboer JA. Br J Anaesth. 1985;57:524-530.

18. An analysis of the relationship between central aortic and peripheral upper limb pressure waves in man. Karamanoglu M, O'Rourke MF, Avolio AP, Kelly RP. Eur Heart J. 1993;14:160-167.

19. A photoelectric plethysmograph for the measurement of cutaneous blood flow. Challoner AV, Ramsay CA. Phys Med Biol. 1974;19:317-328.

20. Annual progression of coronary calcification in trials of preventative therapies: a systematic review. McCullough PA, Chinnaiyan KM. Arch Intern Med. 2009;169:2064-2070.

21. Repeatability of peripheral pulse measurements on ears, fingers and toes using photoelectric plethysmography. Jago JR, Murray A. Clin Phys Physiol Measure. 1988;9:319-329.

22. Noninvasive determination of age related changes in the human arterial pulse. Kelly RP, Hayward C, Avolio A, O'Rourke M. Circulation. 1989;80:1652-1659.

23. Aortic pulse wave velocity as a marker of cardiovascular risk in hypertensive patients. Blacher J, Asmar R, Djane S, London GM, Safar ME. Hypertension. 1999;33:1111-1117.

24. Impact of aortic stiffness on survival in end-stage renal disease. Blacher J, Guerin AP, Pannier B, Marchais SJ, Safar ME, London GM. Circulation. 1999;99:2434-2439.

25. A double take on serial measurement of coronary artery calcification. O'Malley P. Arch Intern Med. 2009;169:2051-2052.

26. Aortic stiffness is an independent predictor of all-cause and cardiovascular mortality in hypertensive patients. Laurent S, Boutouyrie P, Asmar R, et al. Hypertension. 2001;37:1236-1241.

27. Studies of the arterial pulse wave and its modification in the presence of human arteriosclerosis. Lax H, Feinberg AW, Cohen BM. J Chronic Dis. 1956;3:618-631.

Photoplethysmography Overview

Photoplethysmography (PPG) is a simple and low-cost optical technique that can be used to detect blood volume changes in the microvascular bed of tissue. It is often used non-invasively to make measurements at the skin surface. The PPG waveform comprises a pulsatile ('AC') physiological waveform attributed to cardiac synchronous changes in the blood volume with each heart beat, and is superimposed on a slowly varying ('DC') baseline with various lower frequency components attributed to respiration, sympathetic nervous system activity and thermoregulation. It is generally accepted that a PPG can provide valuable information about the cardiovascular system. There has been a resurgence of interest in the technique in recent years, driven by the demand for low cost, simple and portable technology for the primary care and community based clinical settings, the wide availability of low cost and small semi-conductor components, and the advancement of computer-based pulse wave analysis techniques. The technology has been used in a wide range of commercially available medical devices for measuring oxygen saturation, blood pressure and cardiac output, assessing autonomic function, and also detecting peripheral vascular disease.