

Jogging Study
(Mild 15 minute Jog)

Trial	Subject	Outcomes	Protocol	Results	Conclusion
#H4	4 Subjects	Verify that the increase observed in HSP 27 in jogging is similar to that observed in scuba diving.	a) 4 subjects carried out a mild jog for a period of 15 minutes. 2 subjects were preconditioned with Tex-OE at 3 mg/Kg BW, 2 acted as controls. Blood is withdrawn before the start of the exercise, 15, 30 and 90 minutes from the start of exercise. b) HSP level is determined by ELISA	a) The degree of change in HSP 27 on average is 2.2 times as high in the preconditioned subjects compared to the control.	Subjects preconditioned with Tex-OE showed an increase in HSP levels within the first 15 minutes from the start of the exercise. This indicates that Tex-OE can provide cellular protection very early from the onset of exercise thus minimizing the prolonged effect of cellular damage.

**Professional Cyclists Controlled Study
(Long Distance Road Race)**

Trial	Subject	Outcomes	Protocol	Results	Conclusion
# H5- a	10 Subjects. Professional 1 Cyclists.	Verify that the increase observed in HSP 72 in cycling during a field event is similar to that observed in scuba diving.	<p>a) 10 cyclists compete in a long distance road race. 5 subjects are preconditioned with Tex-OE (3mg/kg). 5 subjects are not preconditioned with Tex-OE. Blood is withdrawn 120 minutes into the race.</p> <p>b) HSP levels determined by Elisa.</p>	With Tex-OE preconditioning, there was, on average, a two-fold increase in the level of HSP 72 in cyclists within 120 minutes from the start of the exercise. There was no change in the HSP 72 level in the control subjects.	Subjects preconditioned with Tex-OE showed a significantly different rate of increase in HSP 72 levels within a relatively short period of gentle exercise. This type of race lasts for several hours for a number of days. An early increase in HSP level provides better protection and likely minimizes physical damage that induces fatigue.

**Professional Cyclists Controlled Study
(Repetitive Track Race)**

Trial	Subject	Outcomes	Protocol	Results	Conclusion
# H5-b	10 Subjects. Professional Cyclists	Verify that the increase observed in HSP 72 in cycling during a track event is similar to that observed in scuba diving.	<p>c) 10 cyclists compete in a track race. The cyclists carry out a 2-minute race every 10 minutes for one hour. 5 subjects are preconditioned with Tex-OE (3mg/kg). 5 subjects are not preconditioned with Tex-OE. Blood is withdrawn 45 minutes and 105 minutes after the start of the race.</p> <p>d) HSP levels determined by Elisa.</p>	With Tex-OE preconditioning, there was, on average, a two-fold increase in the level of blood HSP 72 in cyclists within 45 minutes from the start of the exercise. After 105 minutes, there was an increase in blood HSP 72 levels of 250% in the preconditioned subjects and a 50% increase in non-preconditioned subjects.	Subjects preconditioned with Tex-OE showed a significantly higher rate of increase in HSP 72 levels within a very short period of time following severe exercise, thus reducing the risk of cellular and tissue damage.

**Thermal Chamber Crossover Clinical Study
10 day washout period)**

Trial	Subject	Outcomes	Protocol	Results	Conclusion
#H1	2 Subjects 1 male 1 female	a) Effect of Tex-OE on serum HSP 27 without thermal shock b) Effect of thermal shock on serum HSP 27.	Thermal shock 85°C (185°F) X 15 min. 25°C (77°F) X 10 min. 105°C (225°F) X 15 min. Blood collected at 30' before shock and Q30min X 3 hrs. Same experiment at 24 and 48 hrs. Ten days later, entire experiment repeated but subjects took Tex-OE orally @6mg/Kg. 3 hrs. prior to initial thermal shock only. HSP 27 levels determined by ELISA.	a) No change in HSP27 levels in absence of thermal stress with or without Tex-OE. b) Increase in HSP 27 of 1.5 times baseline 2 ^o after shock under normal conditions. c) When treated with Tex-OE HSP 27 increased to 1.5 times baseline within 30 min. d) With successive stresses, subjects not preconditioned with Tex-OE were unable to mount a full response.	a) HSP 27 levels are not modified in the absence of thermal shock even with the ingestion of Tex-OE. b) HSP 27 protection is established at 2 hours after initiation of stress. c) Tex-OE accelerates syntheses of HSPs within 10 – 20 minutes of initiation of stress. d) Subjects who had ingested Tex-OE and were exposed to recurrent stress maintained high levels of HSPs for 3 days after the initial ingestion.

**Hyperbaric Chamber Dive Crossover Study
(10 Day Washout Period)**

Trial	Subject	Outcomes	Protocol	Results	Conclusion
#H2	8 Subjects 1 Female 7 Males	<p>a) Confirm that levels of HSP 72 in serum increase after a mild stress from a dive.</p> <p>b) Determine time required for HSP 72 production with and without Tex-OE</p> <p>c) Determine period of protection with and without Tex-OE.</p>	<p>a) 8 divers exposed to hyperbaric chamber dive to depth of 35 m for 30 min controlled by dive computer to simulate normal dive curve with mild stress. Blood drawn from subjects before dive, at bottom, before ascent, at resurface and one hour post resurface. 10 day washout.</p> <p>b) 8 divers pretreated with Tex-OE the night previous to dive with Tex-OE 3 mg/kg. Rest of trial same as described part a.</p> <p>c) HSP 72 levels determined by ELISA.</p>	<p>a) Non-preconditioned subjects' HSP levels increased in 120 minutes from onset of stress (dive).</p> <p>b) Subjects preconditioned with Tex-OE had increased HSPs (3 times the baseline) within 6 - 18 minutes of onset of stress (dive).</p>	<p>Subjects preconditioned with Tex-OE showed an increase in HSP levels before they reached the bottom.</p> <p>This is important because although Tex-OE will not prevent a DCI (decompression incident), it provided cellular protection before the start of ascent, thereby providing earlier protection and hopefully minimizing the physical effects of a DCI.</p>

Cell Culture Using Human Skin Fibroblasts

Trial	Subject	Outcomes	Protocol	Results	Conclusion
#CC1	HSF – human skin fibroblasts	Synthesis of HSP 27, 70, and 90 by HSF in culture @ 37 ^o vs 43 ^o C (stress) with and without Tex-OE.	<p>a) Culture medium w/ HSF @ 37^o overnight without Tex-OE</p> <p>Culture medium w/HSF @ 37^o overnight with Tex-OE 1%</p> <p>b) Culture medium w/HSF @ 43^o for 15 minutes. Culture medium w/ HSF @ 43^o with Tex-OE 1% for 15 minutes.</p> <p>c) All levels measured by fluorescence.</p>	<p>a) No activity either control or Tex-OE</p> <p>b) b) Synthesis of HSPs 27, 70, & 90 activated within 10 to 20 min in presence of Tex-OE and heat</p> <p>c) HSPs 27, 70 & 90 increase 120 minutes after beginning of stress w/o Tex-OE.</p>	Tex-OE does not modify HSP 27, 70 or 90 levels in absence of stress. Tex-OE activated HSP 27, 70, and 90 within 10 – 20 min. of onset of stress.

Cell Culture Using Human Keratinocytes

Trial	Subject	Outcomes	Protocol	Results	Conclusion
#CC2	HK – human keratinocytes	Amount of time necessary for increases in serum levels HSP 27 with Tex-OE.	<p>a) 5 divers performing open sea dive (water temperature 14°C/ 57°F) to depth of 35 m for 30 minutes 2 preconditioned with Tex-OE 3 mg/kg and 3 not. Dive controlled by dive computer to assure normal dive curve with mild stress. Blood drawn from subjects just prior to dive, as soon as resurfaced, (one hour after the start of the dive) and one hour after resurfacing. 3 week washout</p> <p>b) Same 5 divers as part a. Crossover of those pretreated at same dose vs. those not during last dive. Rest of trial same as described in part a.</p> <p>c) All HSP levels determined by ELISA.</p>	<p>a) In the absence of stress, preconditioned and non-preconditioned subjects had no change in their HSP levels.</p> <p>b) Within one hour after start of dive treated subjects have maximal level of HSP 27.</p> <p>c) In the first hour a significant difference in the levels of HSP 27 observed between preconditioned and non-preconditioned subjects.</p> <p>d) At 2 hours after the start of the dive, HSP 27 levels were similar in all subjects without regard to preconditioning.</p>	Subjects preconditioned with Tex-OE showed an increase in HSP levels before the end of the dive. This is important because although Tex-OE will not prevent a DCI (decompression incident), it provided cellular protection before the dive was even completed, thereby providing earlier protection and hopefully minimizing the physical effects of a DCI.

**Open Sea Diving, Crossover Study
(3 week washout period)**

Trial	Subject	Outcomes	Protocol	Results	Conclusion
#H3-a	5 subjects	Amount of time necessary for increases in serum levels HSP 27 with Tex-OE.	<p>a) 5 divers performing open sea dive (water temperature 14°C/ 57°F) to depth of 35 m for 30 minutes 2 preconditioned with Tex-OE 3 mg/kg and 3 not. Dive controlled by dive computer to assure normal dive curve with mild stress. Blood drawn from subjects just prior to dive, as soon as resurfaced, (one hour after the start of the dive) and one hour after resurfacing. 3 week washout</p> <p>b) Same 5 divers as part a. Crossover of those pretreated at same dose vs. those not during last dive. Rest of trial same as described in part a.</p> <p>c) All HSP levels determined by ELISA.</p>	<p>a) In the absence of stress, preconditioned and non-preconditioned subjects had no change in their HSP levels.</p> <p>b) Within one hour after start of dive treated subjects have maximal level of HSP 27.</p> <p>c) In the first hour a significant difference in the levels of HSP 27 observed between preconditioned and non-preconditioned subjects.</p> <p>d) At 2 hours after the start of the dive, HSP 27 levels were similar in all subjects without regard to preconditioning.</p>	<p>Subjects preconditioned with Tex-OE showed an increase in HSP levels before the end of the dive. This is important because although Tex-OE will not prevent a DCI (decompression incident), it provided cellular protection before the dive was even completed, thereby providing earlier protection and hopefully minimizing the physical effects of a DCI.</p>

Open Sea Diving, Crossover Study
(3 week washout period)

Trial	Subject	Outcomes	Protocol	Results	Conclusion
#H3- b	12 Subjects	<p>Verify that the increase observed in HSP 27 corresponds to that observed for HSP 72.</p> <p>As the most critical period, as determined by the previous experiment, was at one hour from the start of the dive, outcomes were analyzed using data from blood drawn right before the dive and one hour after the start of the dive.</p>	<p>a) 12 divers performing open sea dive (water temperature 14°C/ 57°F) to depth of 35 m for 30 minutes 6 preconditioned with Tex-OE 3 mg/kg and 6 not. Dive controlled by dive computer to assure normal dive curve with mild stress. Blood drawn from subjects just prior to dive, and one hour after resurfacing. 3 week washout.</p> <p>b) Same 12 divers as part a. Crossover of those pretreated at same dose vs. those not during last dive. Rest of trial same as described in part a.</p> <p>c) All HSP levels determined by ELISA.</p>	<p>a) Without Tex-OE there was only a 10% average increase in the HSP 72 level at 1 hour after the start of the dive.</p> <p>b) With Tex-OE, in the first hour, all divers had a significant increase in the level of HSP 72 – 371%. (One subject ingested Tex-OE with a meal, counter to dosing regimen and only exhibited an increase in HSP 72 of 14%.)</p>	<p>Subjects preconditioned with Tex-OE showed an increase in HSP levels before the end of the dive. This is important because although Tex-OE will not prevent a DCI (decompression incident), it provided cellular protection before the dive was even completed, thereby providing earlier protection and hopefully minimizing the physical effects of a DCI.</p>