



**UNIVERSITY OF HAVANA, CUBA**

*PHARMACY AND FOOD INSTITUTE*

Pharmacology Department/Center for Research and Biological Evaluation

**“EFFICACY OF OZONE OXIDATIVE POSTCONDITIONING  
IN PATIENTS WITH HERNIA DISC”**

**EUROPEAN COOPERATION OF MEDICAL OZONE SOCIETIES**

*OZONE IN MEDICINE*

*SPONSORING 50 JAHRE Dr. HÄNSLER OZONOSAN*

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# **INTRODUCTION**

**LOW BACK PAIN (LBP) MAY BE EITHER ACUTE OR CHRONIC. THE CHRONIC FORM IS THE MOST EXPENSIVE BENIGN ILLNESS IN THE INDUSTRIALIZED WORLD AND IS THE MAIN CAUSE OF DISABILITY IN PEOPLE UNDER 45 YEARS OF AGE. IT IS DEFINED AS A BIO-PSYCHO-SOCIAL SYNDROME WHERE THE BIOLOGICAL ASPECT HAS A VERY REDUCED DEFINITION COMPARED TO THOSE PSYCHOLOGICAL AND SOCIALS.**

**THE LONG TERM EFFECTS OF THE HERNIA DISC (HD) COMPROMISE THE CAPACITY OF THE SPINE TO HANDLE SUBSEQUENT DEMANDS AND CAN LEAD TO PROGRESSIVE INCAPACITY.**

**DESPITE THE GROWTH IN KNOWLEDGE OF THE PATHOLOGIC PROCESSES AND MICROMECHANICAL DISORDERS THAT GIVE RISE TO LBP THE LINKS BETWEEN BIOMECHANICS, DISC DEGENERATION AND BACK PAIN ARE DISPUTED AND STILL POORLY DEFINED.**

**OZONETHERAPY HAS BEEN WIDELY USED WITH CLINICAL EFFICACY IN HD AND SOME EXPLANATIONS HAVE BEEN EVOKED. SINCE HAS BEEN POSTULATED THE RELATIONSHIP BETWEEN HD AND DAMAGE TO SOME STRUCTURAL PROTEINS (COLLAGENS, OSTEONECTIN AND OTHERS) AND THE FACT THAT OXIDATIVE STRESS MAY PROMOTE INJURY TO PROTEINS SUCH AS INCREASING OF CARBONYL GROUPS, PROTEIN GLYCATION, ADVANCED OXIDATION PRODUCTS AND OTHERS**

**THE AIM OF THIS WORK HAS BEEN:**

- 1. TO CHARACTERIZE THE SYSTEMIC REDOX STATUS OF PATIENTS WITH HD, AND**
- 2. TO STUDY IF OZONE OXIDATIVE POSTCONDITIONING (OzoneOxPost) MODIFIED THE PATHOLOGICAL OXIDATIVE STRESS, PROTECTED AGAINST OXIDATIVE PROTEIN DAMAGE AN IF THERE WAS ANY RELATION BETWEEN THESE OXIDATIVE CHANGES WITH LBP.**

# **MATERIAL AND METHODS**

## ***Study Design***

**THIS RANDOMIZED CLINICAL TRIAL WAS APPROVED BY INSTITUTIONAL REVIEWERS BOARD (SCIENTIFIC AND ETHIC COMMITTEES).**

**ADULTS PATIENTS OF BOTH SEXES AND DIFFERENT ETHNIC ORIGINS WITH DIAGNOSIS OF HD BY AXIAL COMPUTARIZED TOMOGRAPHY, NUCLEAR MAGNETIC RESONANCE AND CLINICAL EVALUATION WERE ELIGIBLE TO PARTICIPATE IN THIS STUDY. EXCLUSION CRITERIA WERE PREGNANCY, MENTAL DISORDERS, ANY DISEASE ASSOCIATED TO OXIDATIVE STRESS OR IF THE SUBJECTS HAD/WERE RECEIVED/ING DRUGS WITH ANTIOXIDANT PROPERTIES. FOR THE CALCULATION OF THE SIZE OF THE SAMPLE THE MEDSTAT SYSTEM (VERSION 2.1, 1989) WAS USED. 33 PATIENTS WERE STUDIED.**

## ***Treatment description***

**PATIENTS RECEIVED 20 SESSIONS (ONE CYCLE) OF OZONE (GENERATED BY OZOMED EQUIPMENT, CUBA) BY PARAVERTEBRAL ROUTE TWICE A WEEK. 3-8 mg OF OZONE IN STEPPED WAY AND INCREASING ORDER WAS USED.**

## **THE MAIN VARIABLES CONSIDERED WERE:**

- 1. *PRIMARY VARIABLE.* PAIN PERCEPTION CLASSIFIED FROM “1 (MINIMUM INTENSITY AND “10” (MAXIMUM INTENSITY). IT WAS CONSIDERED AS “0” ANY PAIN.**
- 2. *SECONDARY VARIABLES.* PLASMATIC LEVELS OF PHOSPHOLIPASE A (PLA), AS INDICATOR OF EICOSANOIDS PROINFLAMMATORIES; TOTAL HYDROPEROXIDES, AS ROS ABLE TO PROMOTE INJURY TO MACROMOLECULES AND PRECURSOR OF FREE RADICALS; MALONDIALDEHYDE, PEROXIDATION POTENTIAL AS MARKERS OF LIPID PEROXIDATION AND PROTEIN INJURY; FRUCTOLYSINE, ADVANCED OXIDATION PROTEIN PRODUCTS AS A MEASURE OF PROTEIN DAMAGE. FINALLY, REDUCED GLUTATHIONE, SUPEROXIDE DISMUTASE AND CATALASE AS MEMBERS OF ANTIOXIDANT ENDOGENOUS SYSTEM.**

## Evaluation of the response

**AFTER THE PATIENT'S DIAGNOSIS AND BEFORE AND AFTER OF OzoneOxPost THE PAIN PERCEPTION WAS RECORDED THROUGH BACKILL SCALE AS WELL AS WERE DETERMINED THE REDOX PARAMETERS BEFORE MENTIONED.**

*Therapeutical Success (positive change).* **WHEN PATIENTS EXPRESSED A DECREASE OF THE PAIN = 30% IN 70% OR MORE PATIENTS AND THE REDOX PARAMETERS MOVED TOWARD OR RETURNED TO REFERENCE INTERVAL AFTER OzoneOxPost WITH STATISTIC DIFFERENCE ( $p < 0.05$ ) IN 70% OR MORE OF SUBJECTS INCLUDED IN THE CLINICAL TRIAL.**

*Therapeutic Failure.* **WHEN MORE THAN 30% OF PATIENTS SUBMITTED TO OzoneOxPost DID NOT IMPROVE THE PAIN INTENSITY (CHANGES < 30% OF PAIN PERCEPTION) AFTER OZONE TREATMENTS.**

## *Statistical analysis*

❖ **OUTLIERS PRELIMINARY TEST**

❖ **ONE-WAY ANALYSIS OF VARIANCE (ANOVA)**

❖ **HOMOGENEITY VARIANCE TEST (BARTLETT-BOX)**

❖ **MULTIPLE COMPARISON TEST (DUNCAN TEST)**

❖ **LEVEL OF SIGNIFICANCE WAS ACCEPTED AT  $p < 0.05$ .**

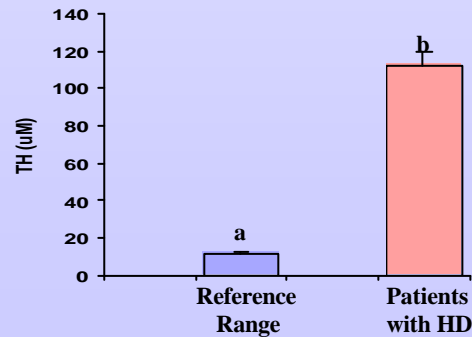
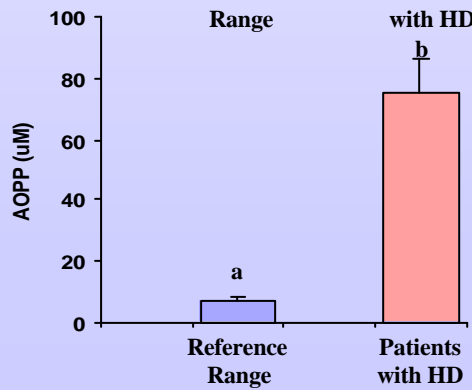
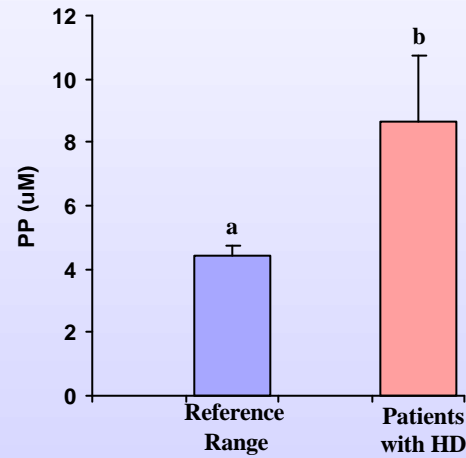
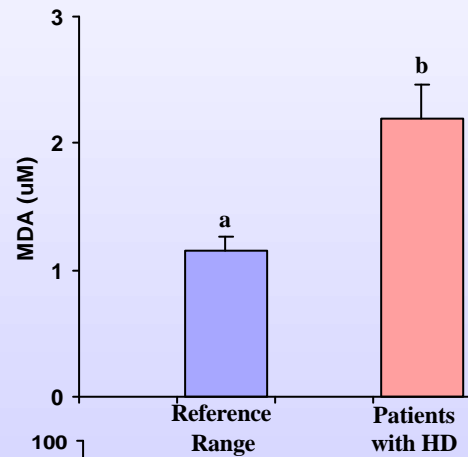
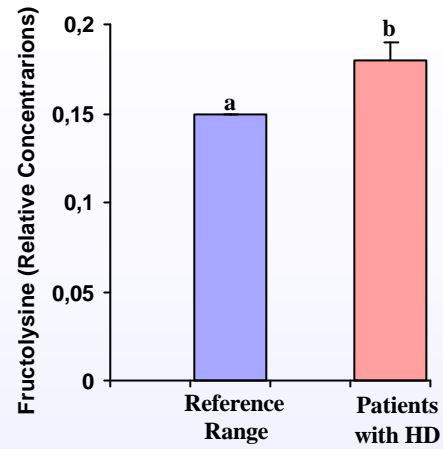
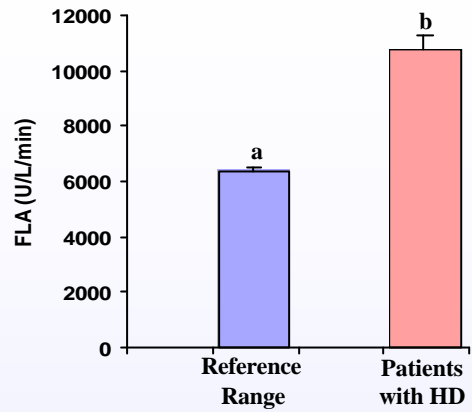
❖ **REGRESSIONS WERE ANALYZED USING PEARSON'S AND SPERMAN'S CORRELATIONS.**

# RESULTS AND DISCUSSION

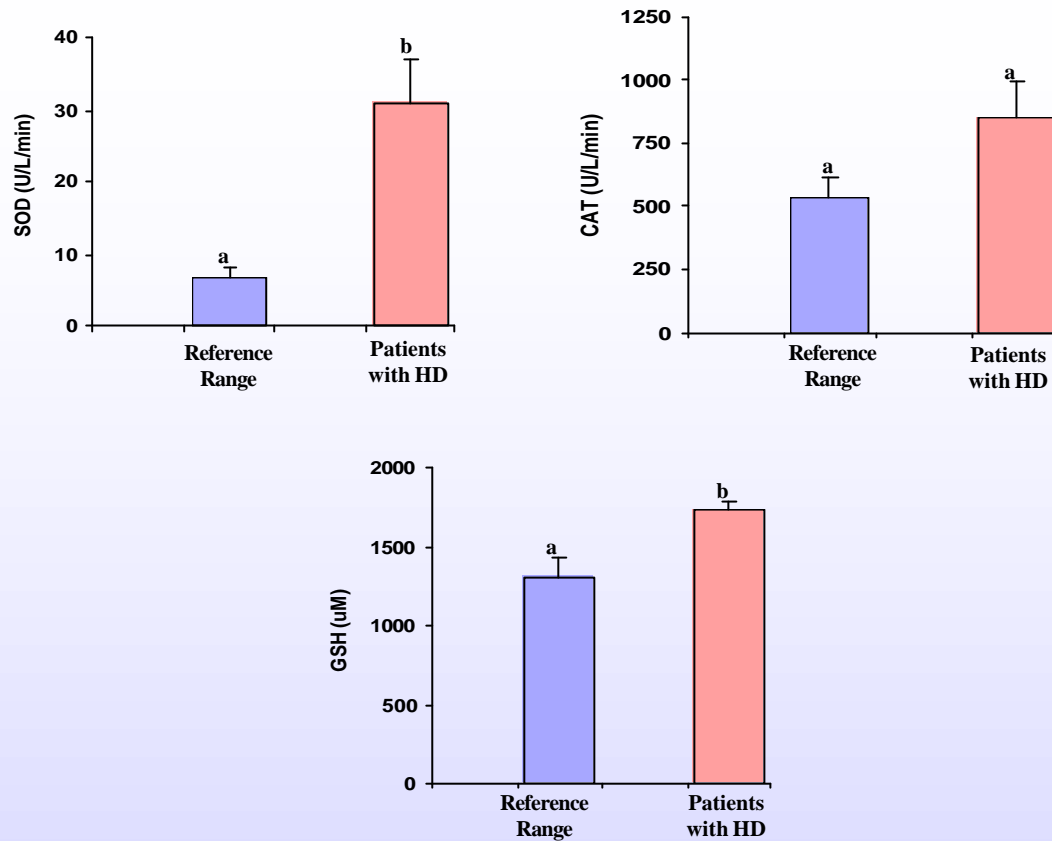
**Table 1. Clinical picture of the patients with hernia disc**

Demographic data/ patient's histories			%
- Men	18/33		54.5
- Women	15/33		45.5
- Age		43 ± 11	
- Previous therapies	non-steroidal antiinflammatories	8/33	24
- Previous operations		Non	
- Evolution time of the disease:			
< 3 years	20/33		60.6
> 3 years	13/33		39.4
- HD severity			
. Prolapse	17/33		51.5
Protrusion	16/33		48.5
-Lumbar hernia disc (L <sub>5</sub> -S <sub>1</sub> )	21/33		64

Mean age was 43 years old. There were not observed differences between sexes and with regard to the severity of the hernia disc (prolapse and protrusion). Patient's group with evolution time of the disease < 3 years were larger than > 3 years.



**Figure 1. Plasma Injury Markers in patients with HD before OzoneOxPost.**



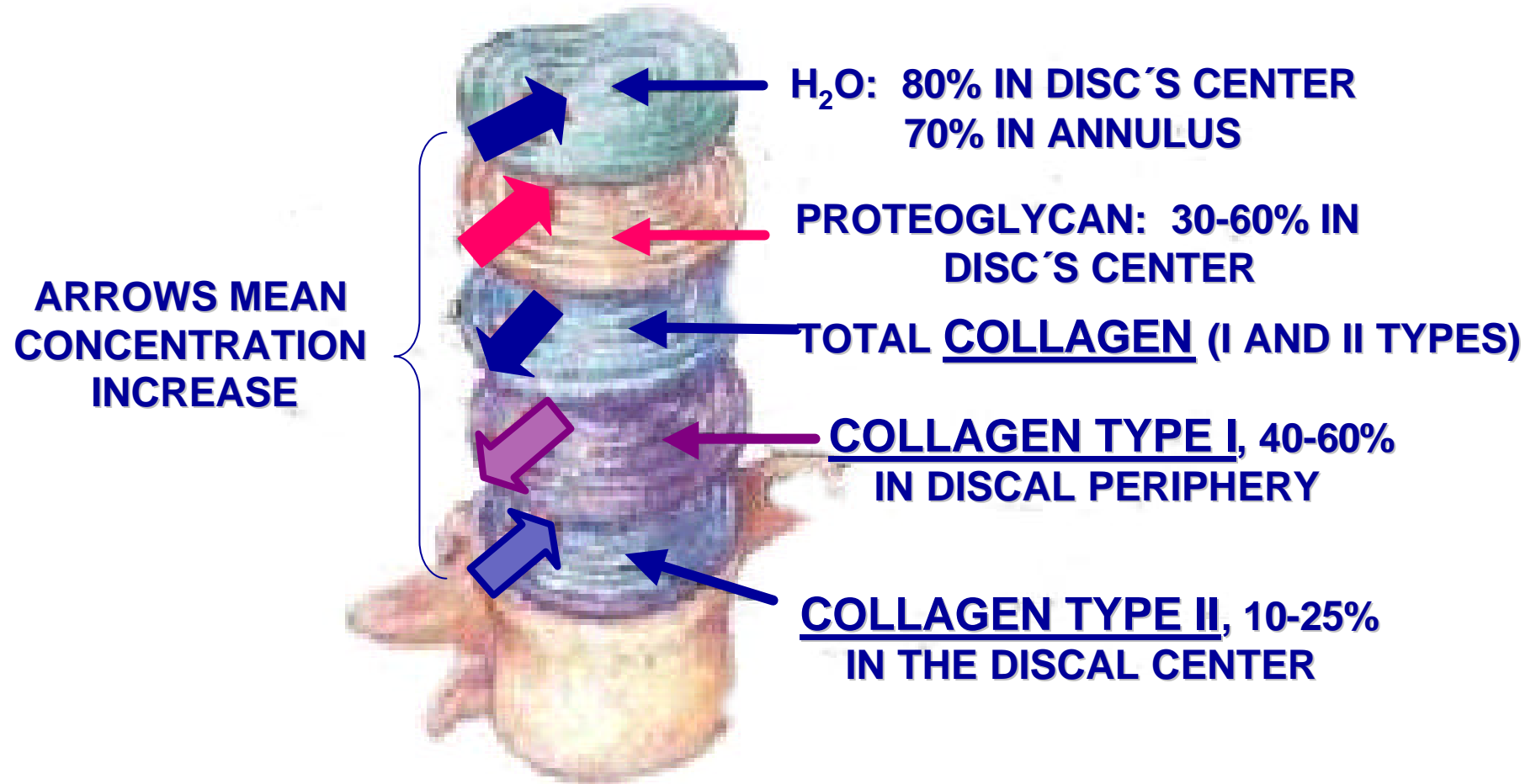
**Figure 2. Plasma Antioxidant Markers in patients with HD before OzoneOxPost.**

8 from 9 redox parameters were increased with regard to reference range. Catalase was the only marker which didn't show difference. Major changes were observed for protein damage (AOPP), TH generation and SOD activity which produce  $H_2O_2$ .

**These results have demonstrated that patients with HD have a severe oxidative stress. On the other hand, antioxidant defenses as GSH, and CAT were not able to avoid the overproduction of ROS in HD. It was observed a dramatic protein damage (AOPP and Fructolysine content) as well as lipids (MDA and PP). MDA promotes lysine adducts formation in proteins (collagens and others). These transformed proteins generate tissue injury.**

**These changes affect the structure and functions of those modified proteins and increase pathological cellular responses. In addition, there was a remarkable increase of TH (a measure of H<sub>2</sub>O<sub>2</sub>) which may oxidize essential –SH groups of proteins and also it is involved in the collagens cross-linking leading to lost of their stability and hardness. Collagens are the main components of extra-celular matrix and represent 40-60%and 10-15% in peripheria and center of the disc, respectively.**

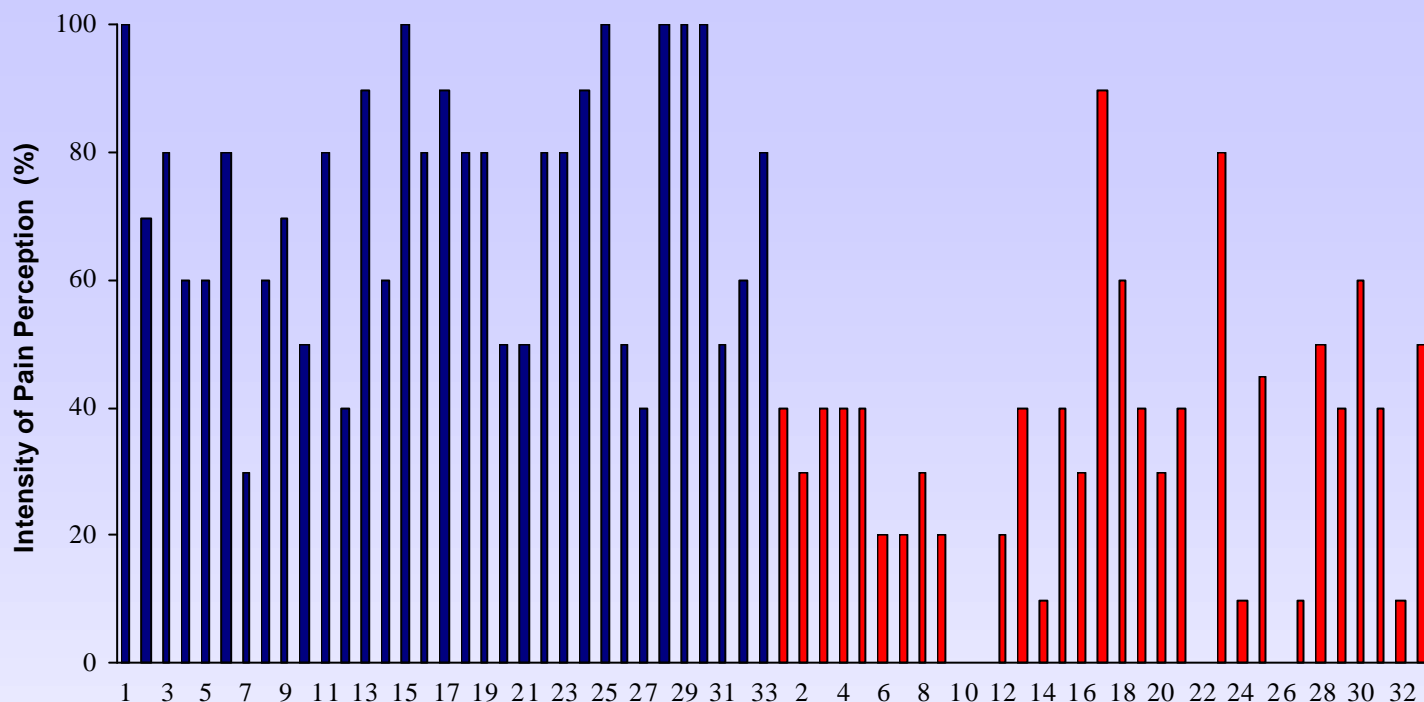
# CHEMICAL COMPOSITION OF THE INTERVERTEBRAL DISC



Disc's cushion function is dependent of elasticity of fibrous annulus and adequate hydration of the nucleus. Both are dependent of proteins integrity mainly the collagens.

**IT HAS BEEN DEMONSTRATED AN OXIDATIVE STRESS INVOLVED IN INFLAMMATORY PROCESSES AND PROTEIN DAMAGE IN HD WHICH PROVIDES SCIENTIFIC SUPPORT FOR OZONETHERAPY IN THIS NEUROLOGICAL DISORDER CONSIDERING THE WELL-KNOWN OXIDATIVE PRE/POST CONDITIONING MECHANISM ABLE TO REESTABLISH THE REDOX BALANCE.**

**Figure 3 shows the pain intensity expressed by the patients BEFORE and AFTER OzoneOxPost. 73% of patients reduced the pain in 41% of intensity AFTER Ozone treatment as may be observed in table 2. These results accomplished the efficacy criteria established in the clinical trial.**



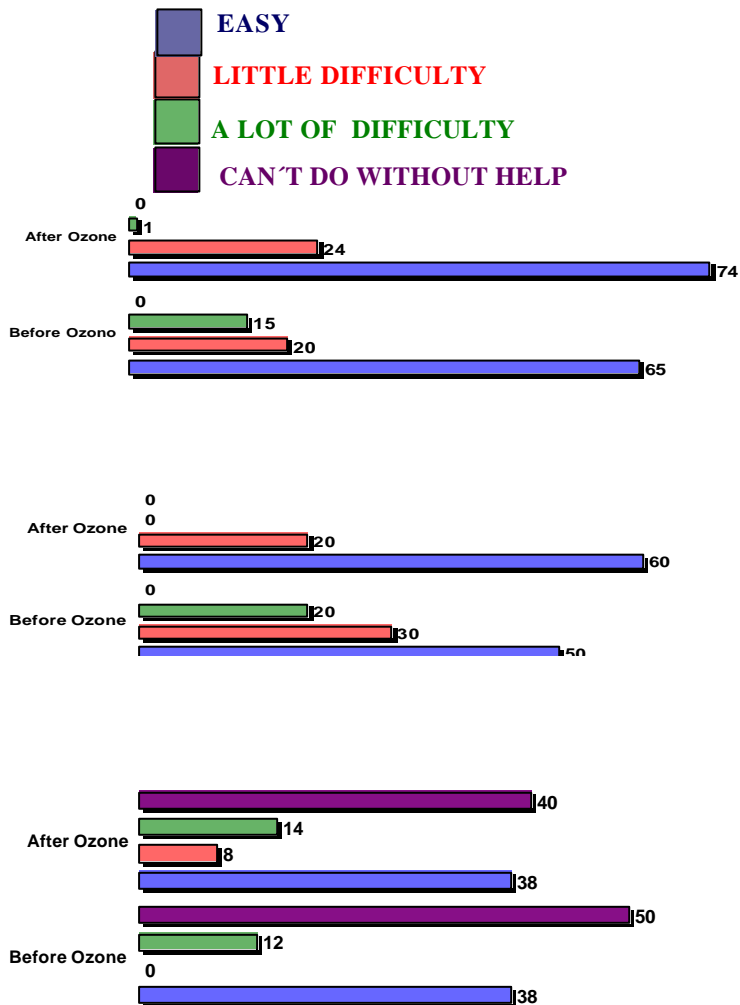
**Figure 3. Intensity of pain perception (%) in each patient with HD included in the study BEFORE (blue) and AFTER (red) of OzoneOxPost treatment.**

**Table 2. Mean score of pain in patients with HD BEFORE and AFTER OzoneOxPost (%)**

CONCEPT	BEFORE OzoneOxPost	AFTER OzoneOxPost	Pain Decreasing (%)
Pain	74.3 ± 3.5 <sup>(a)</sup>	33.4 ± 3.9 <sup>(b)</sup>	41

Each value is the mean ± SEM. Mean values having different subscript letters indicates significant difference (p<0.05).

**In order to evaluate not only the pain relief but also the quality of the pain improvement after OzoneOxPost BACKILL Scale was applied**



**IN FRONT OF FOLLOWING QUESTIONS**

How do you feel sitting for 30 min ?

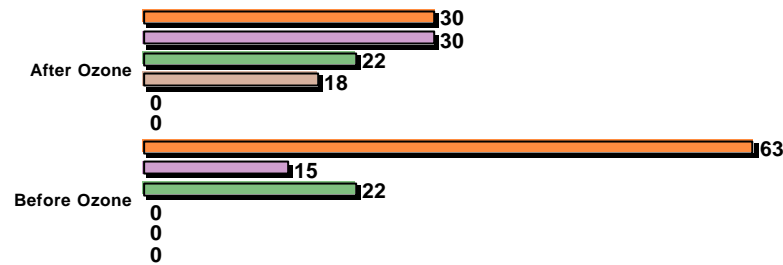
How do you feel standing for 30 min ?

How do you feel getting up from a low seat ?

**After OzoneOxPost the movility of the patients improved in the 3 evaluated items. It must emphasize In the question “How do you feel standing for 30 min”. Before Ozone treatment there was 20% of patients who expressed “A lot of difficulty”. After OzoneOxPost there was not any patients in this kind of answer With regard to “How do you feel getting up from a low seat”after ozone treatment there was a 10% of decrease In the answer “I Can’t do without help”.**

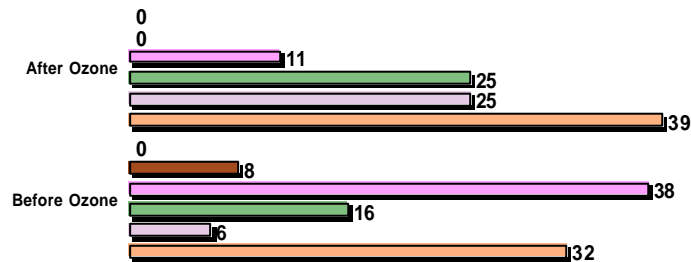
# ABOUT LIFTING, WALKING, TRAVELING AND PERSONAL CARE FUNCTIONS WERE OBTAINED THE FOLLOWING RESULTS

## LIFTING FUNCTIONS



- I cannot lift or carry anything at all
- I can't lift only very light weights
- I can only lift light to medium weights only if they are conveniently positioned
- I can't lift heavy weights only if they are conveniently positioned
- I can't lift heavy weights but I get extra discomfort
- I can't lift heavy weights without having extra discomfort

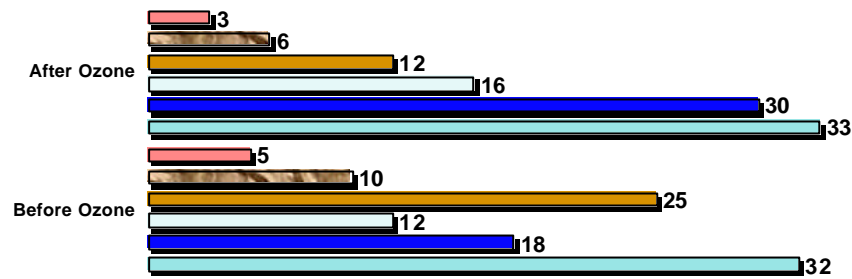
## WALKING FUNCTIONS



- I am able to walk any distance
- Discomfort prevent me from walking more than 1 mile
- Discomfort prevent me from walking more than 1/2 mile
- Discomfort prevent me from walking more than 1/4 mile
- I walk only a limited distance or use a cane, crutches or a walkers
- I am in bed most of the time or I use a wheelchair

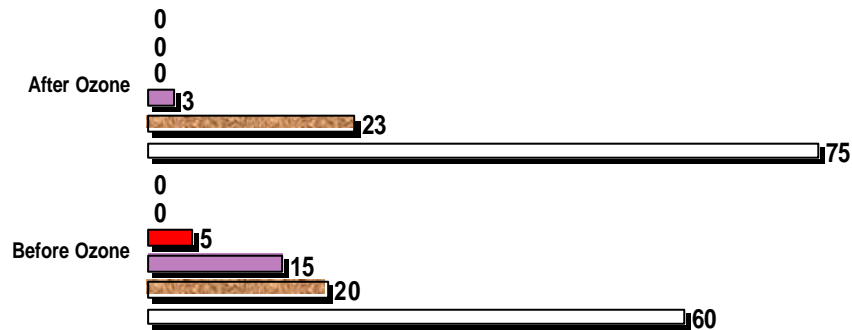
After OzoneOxPost there was a decrease more than 30% of patients who had answered “I cannot LIFT or carry anything at all”. In WALKING the necessity of crutches, walkers and other systems disappeared in the totality of the patients after OzoneOxPost.

## TRAVELING FUNCTIONS



- I can travel anywhere without extra discomfort
- I can travel anywhere without extra discomfort
- I manage trips over 2 h with some discomfort
- My discomfort limit me to trips of least than 1 h duration
- My discomfort limit me to short, necessary trips under 30 min
- My discomfort prevents me from traveling except to the doctor or hospital

## PERSONAL CARE FUNCTIONS



- I can look after myself normally without having extra discomfort
- I can look after myself normally but have extra discomfort
- It is uncomfortable to look after myself and I am slow and careful
- I need some help but I manage most of my personal care
- I need help every day in most aspects of self care
- I do not get dressed, I wash with difficulty and I stay in bed

**TRAVELING functions improved in general. With regard to PERSONAL CARE 15% of patients increased the capacity to do it without having extra discomfort and there was any patient in the category “I need some help.....” after OzoneOxPost.**

**Table 3. Patients with HD with “RESPONSE” or “NO RESPONSE” in the redox balance after OzoneOxPost**

REDOX MARKERS	RESPONSE TO (**) OzoneOxPost (73%)		NO RESPONSE TO OzoneOxPost (27%)	
	BEFORE	AFTER	BEFORE	AFTER
<b>Injury Markers</b>				
MDA (μM) [0.78-1.54]	<b>2.96 ± 0.5<sup>a</sup></b>	<b>1.23 ± 0.14<sup>b</sup></b>	1.19 ± 0.45 <sup>a</sup>	0.88 ± 0.55 <sup>a</sup>
PP (μM) [3.42-5.42]	<b>11.49 ± 4.5<sup>a</sup></b>	<b>3.79 ± 0.24<sup>b</sup></b>	12. ± 8.3 <sup>a</sup>	14.7 ± 10 <sup>a</sup>
AOPP (μM) [2.48-10.88]	88.31 ± 17.4 <sup>a</sup>	38.44 ± 7.4 <sup>b</sup>	39.8 ± 15 <sup>a</sup>	87.5 ± 22 <sup>a</sup>
TH (μM) [9.82-13.88]	104.5 ± 10.8 <sup>a</sup>	52.2 ± 7.5 <sup>b</sup>	81.9 ± 16.5 <sup>a</sup>	101 ± 16.8 <sup>a</sup>
FLA (U/L/min) [6128-6658]	11937 ± 1035 <sup>a</sup>	9085 ± 763 <sup>b</sup>	851 ± 454 <sup>a</sup>	10708 ± 742 <sup>a</sup>
Fructolysine(*) [0.14-0.15]	<b>0.19 ± 0.01</b>	<b>0.15 ± 0.01<sup>b</sup></b>	0.16 ± 0.01 <sup>a</sup>	0.21 ± 0.02 <sup>b</sup>
<b>Antioxidant Defenses</b>				
SOD(U/L/min) [4.36-11.56]	<b>43.8 ± 10.4<sup>a</sup></b>	<b>14 ± 2.5<sup>b</sup></b>	5.9 ± 2 <sup>a</sup>	30.4 ± 7.6 <sup>b</sup>
CAT (U/L/min) [336-730]	<b>848 ± 14<sup>a</sup></b>	<b>486 ± 106<sup>b</sup></b>	N. D.	N. D.
GSH (μM) [1181.6-1436]	1727 ± 54 <sup>a</sup>	1554 ± 29 <sup>b</sup>	153 ± 32 <sup>a</sup>	1615 ± 37 <sup>a</sup>

After OzoneOxPost there was a re-establish of redox indicators in the majority of markers such as for those of damage as well as for the ones of protection in 73%. It is important to emphasize that patients in the group of NO RESPONSE (27%) to Ozone treatment although there were not positive redox changes neither was observed an increase in the progression of oxidative stress since there were not significant differences. An exception was Fructolysine concentrations which seems to be critical in the protein damage associated to HD.

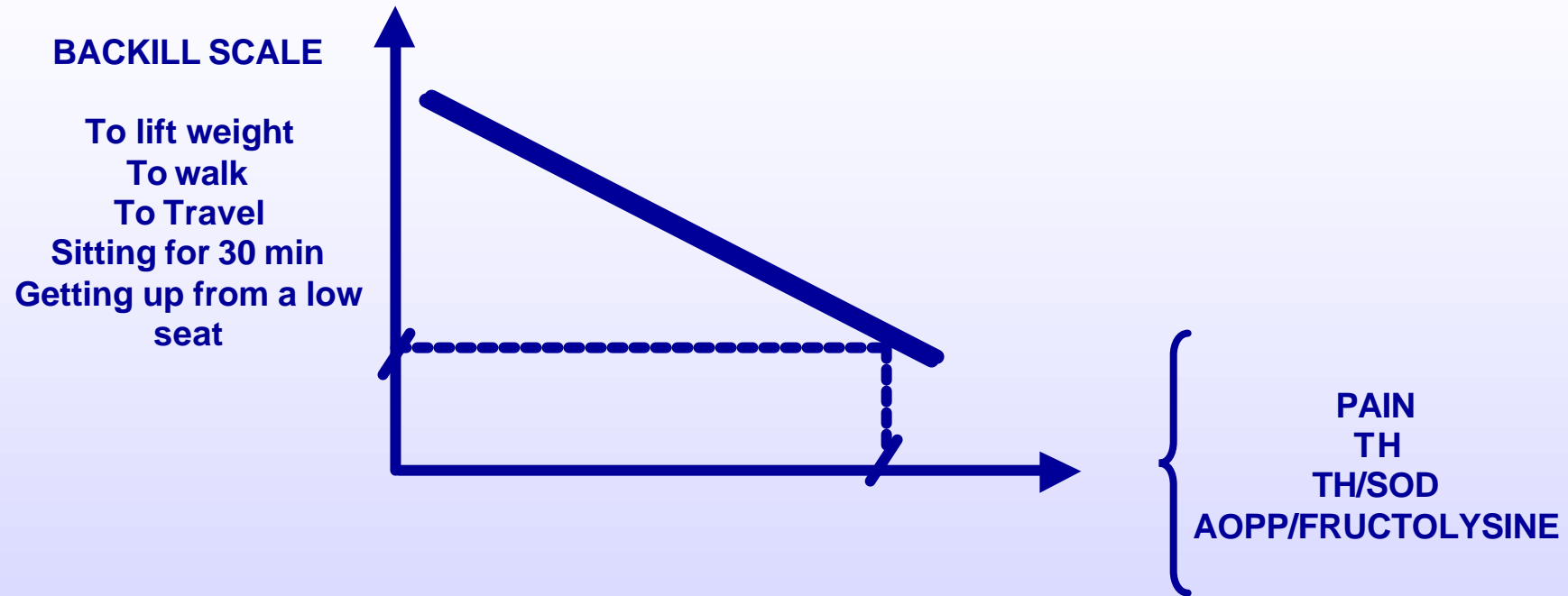
Pain and life quality measured according to BACKILL Scale are indicators of clinical efficacy. Therefore it was necessary to evaluate if there was any relation between the clinical responses and the redox status of the patients with HD.

**Table 4. Non parametric correlations between BACKILL items, Pain and the Redox variables**

BACKILL ITEMS	PAIN AND REDOX MARKERS	CORRELATION COEFFICIENT
To lift weights	PAIN TH	- 0.37 - 0.33
To Walk	PAIN MDA AOPP/Fructolysine TH/SOD	- 0.50 - 0.41 - 0.38 - 0.43
To Travel	PAIN TH TH/SOD	- 0.47 - 0.37 - 0.38
Sitting for 30 min	AOPP/Fructolysine	- 0.37
Geetting up from a low seat	PAIN MDA AOPP TH AOPP/Frutolysine TH/SOD	- 0.32 - 0.38 - 0.32 - 0.42 - 0.32 - 0.33

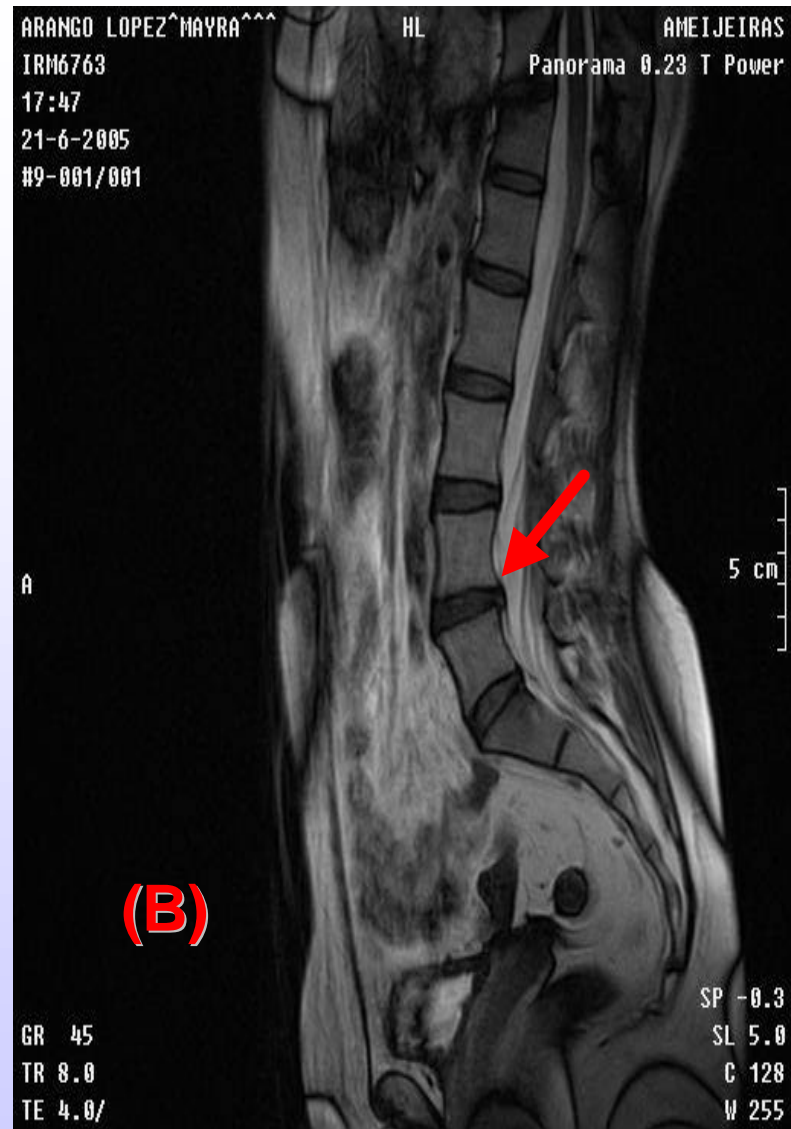
**$P < 0.05$  when Sperman's "R" > 0.31**

**Negative correlations means that whereas the Redox Variables and the Pain are higher the Disability of the patients will be higher too**



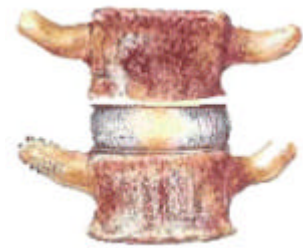
**Most interesting relations were obtained between:**





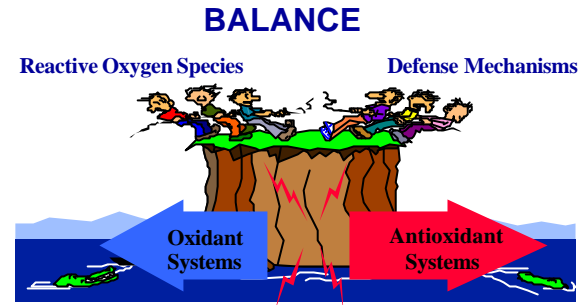
**Nuclear Magnetic Resonance of a patient (A) BEFORE and (B) AFTER Ozone Oxidative Postconditioning**

**TAKEN TOGETHER THESE RESULTS SUGGEST  
THAT PROTEIN DAMAGE IS AN ESSENTIAL STEP  
IN HERNIA DISC, SO WE PROPOSE,**



FUNCTIONAL DISC

INFLAMMATORY DISEASES  
GENETIC DETERMINANTS  
OTHERS  
~~→~~  
 $O_3 / O_2$



**Imbalance: Oxidative Stress**  
Biomolecules Damage

**CHRONIC Oxidative Stress and ACCUMULATIVE DAMAGE**



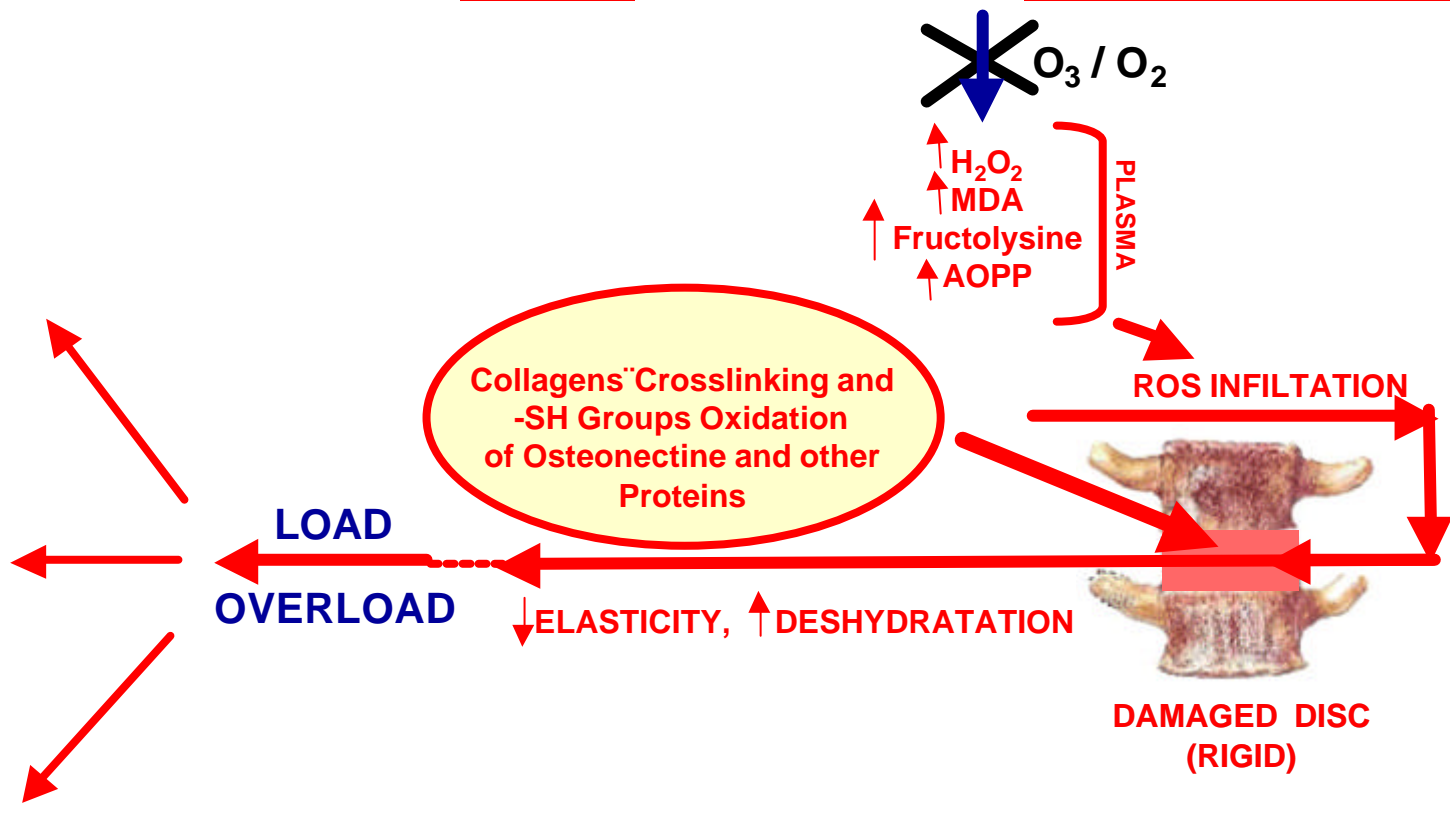
FISURE



PROLAPSE



PROTRUTION



**PROPOSAL OF DAMAGE MECHANISMS ON INTERVERTEBRAL DISC  
MEDIATED BY ROS AND THE PROTECTIVE EFFECTS OF OzoneOxPost**

## **THIS MECHANISM EXPLAINS SOME CLINICAL RESULTS,**

**IT IS FRECUENT THAT PATIENTS WITH HD MUST RECEIVE MORE THAN ONE CYCLE (20 TREATMENTS). IT IS IN CORRESPONDENCE:**

- A) WITH PATIENTS WHO DECREASE OXIDATIVE STRESS BUT THEY DIDN´T RETURN TO REFERENCE INTERVAL,**
- B) PATIENTS WHERE OXIDATIVE STRESS DID NOT PROGRESS BUT REDOX IMBALANCE WAS NOT REGULATED IN THE FIRST CYCLE (27%).**

**Therefore,**

- REDOX CHARACTERIZATION OF PATIENTS TREATED WITH OZONETHERAPY IS AN IMPORTANT DETERMINANT IN THE PROGNOSTIC OF HD,**
- IT MAY REPRESENTS A CRITERIA OF PATIENT´S RECOVER, AND**
- PROVIDE INFORMATION ABOUT THE NUMBER OF TREATMENT TO BE APPLIED.**

## **IN SUMMARY,**

**PATIENTS WITH HERNIA DISC ARE IN SEVERE OXIDATIVE STRESS. THESE RESULTS SUGGEST THAT THE DAMAGE TO FUNCTIONAL PROTEINS (COLLAGENS AND OTHERS) IS ONE OF THE PRIMARY STEPS ASSOCIATED TO LOST OF PROPERTIES OF THE INTER – VERTEBRAL DISC WHICH WHEN IT IS SUBMITTED TO LOAD OR OVERLOAD IS INJURED AND IS PRODUCED FISSURE THAT MAY PROGRESS TO PROLAPSE AND PROTRUSION. OzoneOxPost IS A REGULATOR OF CELLULAR REDOX BALANCE, PROTECT AGAINST OXIDATIVE INJURY TO ESSENTIAL PROTEINS AND PRESERVE THE INTEGRITY OF THE DISC.**



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***THANK YOU VERY MUCH***

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