"NORDENAU PHENOMENON" - APPLICATION OF NATURAL REDUCED WATER TO THERAPY.
FOLLOW UP STUDY UPON 411 DIABETES PATIENTS.

Z. GADEK[1], T. HAMASAKI[2] and S. SHIRAHATA[2]
[1] *Center for Holistic Medicine and Naturopathy.57392 Nordenau, Germany*
[2] *Department of Genetic Resources Technology, Faculty of Agriculture, Kyushu University, Fukuoka 812-8581, Japan*

Abstract. This prospective observation study examines changes in the relevant tests parameters of 411 diabetes patients drinking natural reduced water from the "Nordenau Spring", as well as a correlation of these changes with the fluctuation of the reactive oxygen species in their blood. The average age of the test persons is 71.5 years and the daily consumption of reduced water is as much as two liters. The average duration of stay in Nordenau is 6 days.
The diagnostic parameters such as blood sugar, HbA1c, cholesterol, LDL, HDL, and serum creatinine concentration are tested twice – at the beginning and at the end of the participants stay in Nordenau.
Additionally a random sample of reactive oxygen species in the blood of 136 patients is taken in order to find out its possible causal connections to the diabetes relevant test parameters.
HbA1c has been considered as the substantial test parameter in order to break down the whole group into responder and non-responder categories. One hundred and eighty six tested persons or 45% of the total have been assigned to the responder group, meaning that the patients' HbA1c **and** blood sugar improved significantly.
Furthermore we evaluated among the responder group a portion of patients who in the same time significantly improved their cholesterol, LDL, HDL and serum creatinine concentration average value.
This stage of our follow up study regarding type II diabetes patients estimates number needed to treat on four patients in order to achieve the significant improvement of all diabetes relevant parameters. This is a very good quotient; moreover it could be achieved entirely without side effects.
The significant improvement of diabetes relevant parameters like blood fats and creatinine can be also beneficial to other diseases like high blood pressure, circulatory disturbance, renal insufficiency or atherosclerotic dementia.
In addition to our previous tests, we administered to a random sample group of 136 patients a blood free oxygen radicals test (FORT). The test resulted in a decrease of the ROS of 70.6%.of the group or 96 patients.
Taking account of the fact that the natural reduced water as well as the electrolyzed reduced water obviously improves in a very short time and entirely without side effects very important metabolic parameters, it can be said that the reduced water shall be considered a useful supplement to the usual orthodox medication of ROS-associated diseases.

1. Introduction

Reactive oxygen species (ROS) are known to cause various diseases including diabetes mellitus. In the type II diabetes they cause reduction of glucose uptake by inhibiting the

insulin-signaling pathway in muscle cells and adipocytes. The anti-oxidative waters (reduced waters) such as electrolyzed reduced water and several natural reduced waters e.g. Nordenau water in Germany, Hita Tenryosui water in Japan and Tracote water in Mexico scavenge ROS in insulin responsive L6 myotubes and mouse 3T3/L1 adipocytes and stimulate uptake of 1-deoxy-D-glucose into both L6 and 3T3/L1 cells in the presence or absence of insulin. This insulin-like activity is mediated by the activation of PI-3 kinase, resulting in stimulation of translocation of glucose transporter GLUT4 from microsome to plasma membrane (Oda et al.1999).
Measurement of insulin secretion from beta cells of pancreas (HIT-T15 cell line derived from Syrian hamster) shown in the medium containing Nordenau reduced water 2.9 times higher concentration of insulin compared to control, respectively 2.2 for Hita Tenryosui water. Animal experiments using type II diabetes model mice (C57BL/Ks.J.Db+/Db+) exhibited the significant improved results in the sugar tolerance tests under administration of natural waters such as Nordenau water and Hita Tenryosui water (Shirahata et al.2001).
This prospective observation has been done to find out if an antioxidant operating mechanism of reduced water could really be in a position to suppress the concentration of reactive oxygen species in the blood of diabetes patients and by it to improve their diabetes relevant blood test parameters.
Furthermore this study searched for if the results of clinical trials correlate with those of the basic researches and animal experiments.

2. Material and method

All the participants of the study were patients who had already been diagnosed by specialists and were receiving adequate medication as well as suitable diet guidelines. The inclusive criteria were defined only so far that all the tested persons were suffering by diabetes type II, no matter to age, sex, form of medication and diet.
The average age of the test persons is 71.5 years and the daily consumption of reduced water is as much as two liters. The average duration of stay in Nordenau is 6 days.
The test persons were particularly reminded to continue the medication prescribed by their specialists, to stick to their individual diet plans and not to alter any of their behavioral patterns.
During their stay in Nordenau the patients drank two liters natural reduced water from “Nordenau spring” daily.
We were looking after the patients diagnostically within the scope of what is known as “course control”.
The investigators strictly kept to the requirements of neutrality and didn’t alter either regular medication of the tested persons or their diet instruction. The diagnostic parameters such as blood sugar, HbA1c, cholesterol, HDL, LDL and serum creatinine concentration were tested twice. Once at the beginning and once at the end of the participants stay in Nordenau.
Parallel to that the random sample of reactive oxygen species concentration in the blood of 136 patients had been additionally taken to find out the possible causal connections between the alteration of diabetes relevant parameters and the changes of their blood ROS.

The free oxygen radicals monitoring, which were applied, is based on so-called Haber-Weiss-reaction. This method is due to the fact that the peroxides, which come into being by lipid peroxidation as an interim product, create in the presence of free

transition metals (not bound on proteins) high aggressive hydroxyl radicals. These products can be measured in the presence of N, N-diethyl-para-phenylendiamin on the photometric way.
The oxygen species level (ROS) in the blood had been stressed in FORT Units.
Here 1 FORT Unit = 0.26 mg H_20_2 /l blood.
The values between 250 and 310 FORT Units are considered to be normal.

The statistical interpretation of this clinical data contains:

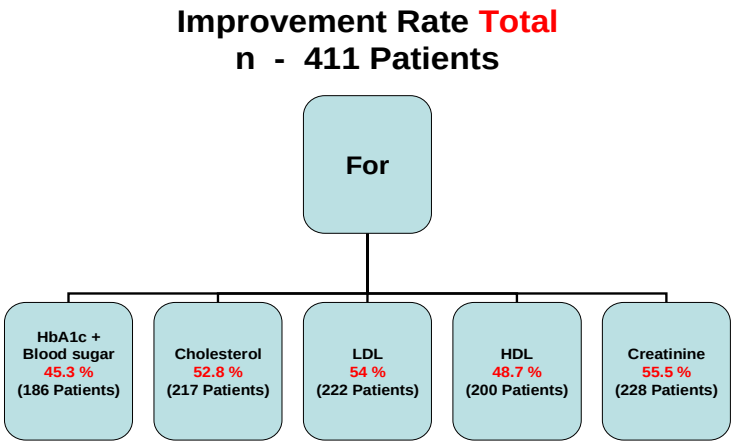
-The descriptive statistics of the whole group and of both subgroups.
-A pairing-directed T-Test.
-A proportional evaluation of the entire group and of both subgroups.
-Variance and co-variance analyses.

The allowance for error probability was set at 2.5 %.

3. Results and discussion

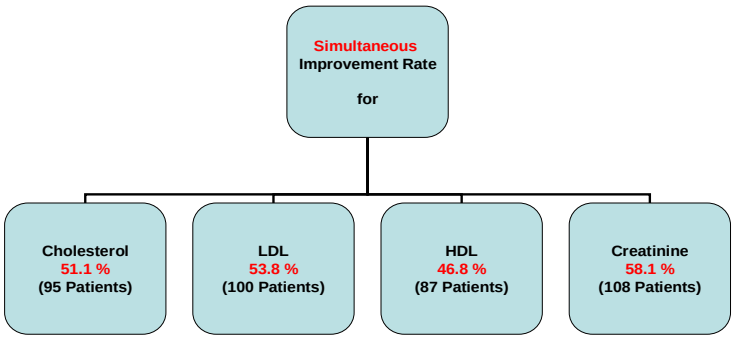
HbA1c has been considered as the substantial test parameter in order to break down the whole group into responder and non-responder categories. One hundred and eighty six tested persons or 45% of the total have been assigned to the responder group, meaning that the patients' HbA1c **and** blood sugar improved significantly.

The data evaluation focuses exclusive on this group, although the significant improvement of cholesterol, LDL, HDL and serum creatinine concentration values has been registered among over 50% of total collective.



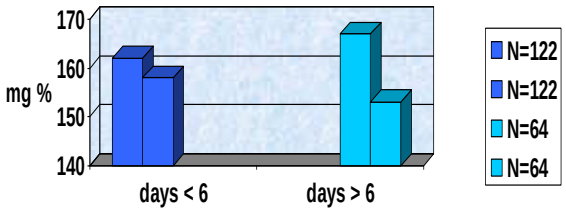
This restrictive criterion diminished in numbers the remaining patient's to 186, but the results of the database analysis of this smaller group win through this restriction much more evidence. Furthermore we evaluated among the responder group a portion of patients who in the same time significantly improved their cholesterol, LDL, HDL and serum creatinine concentration average value.

Improvement Rate among Responder-Group
according to the main criterion
(decrease of HbA1c and Blood sugar average value)
n - 186 Patients



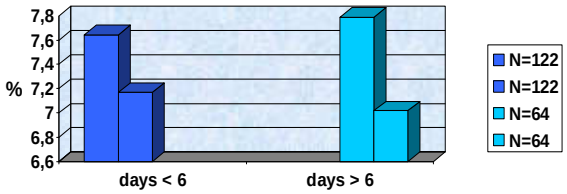
The statistical significant improvement of cholesterol value had been observed among 51% (95 patients) of responder group. As a group, 54.0% of the responders (comprised of 100 persons) demonstrated a significant downward trend of the LDL average value.The average value of HDL increased significantly among 87 patients (47.0%) of the responder group.
The serum creatinine concentration was reduced significantly by among108 patients (58%) of the responder group.
At the next step we tried to find out if the length of therapy period, that also means the quantity of consumed reduced water, can scale up the improvement.
The following graphs confirm the certain regularity which can be defined as follows:
Except the changes of the serum creatinine concentration, the improvement scales up parallel to the lengths of the therapy period.

Changes of blood sugar average value
depending of length of the therapy period



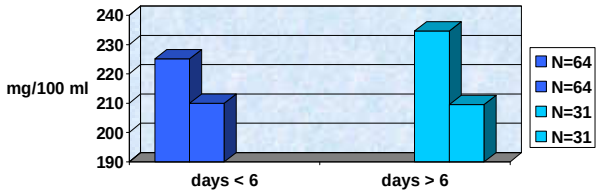
- Decrease in the short therapy period <6 days by 4 mg%
- Decrease in the longer therapy period >6 days by 14 mg%

Changes of HbA1c average value depending of length of the therapy period



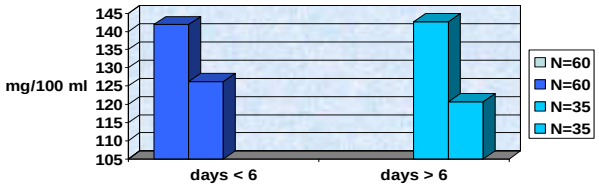
- Decrease in the short therapy period <6 days by 0.5 %
- Decrease in the longer therapy period >6 days by 0.8 %

Changes of cholesterol average value depending of length of the therapy period



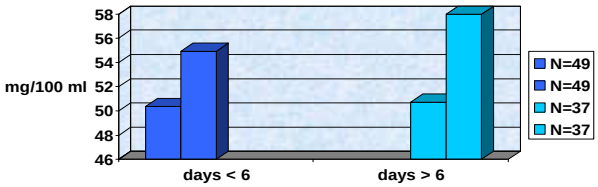
- Decrease in the short therapy period <6 days by 15 mg/100 ml
- Decrease in the longer therapy period >6 days by 25 mg/100 ml

Changes of LDL average value depending of length of the therapy period



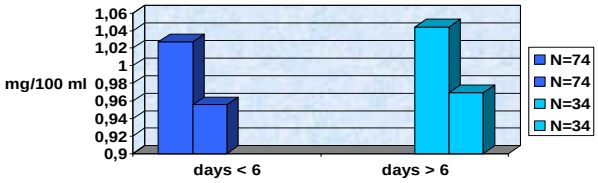
- Decrease in the short therapy period <6 days by 16 mg/100 ml
- Decrease in the longer therapy period >6 days by 22 mg/100 ml

Changes of HDL average value depending of length of the therapy period



- Increase in the short therapy period <6 days by 5 mg/100 ml
- Increase in the longer therapy period >6 days by 7 mg/100 ml

Changes of Creatinine average value depending of length of the therapy period



- Decrease in the short therapy period <6 days by 0.5 mg/100 ml
- Decrease in the longer therapy period >6 days by 0.4 mg/100 ml

In addition to our previous tests, we administered to a random sample group of 136 patients a blood free oxygen radicals test (FORT). The test resulted in a decrease of the ROS of 70.6%.of the group or 96 patients. However, the downward trend of ROS in the blood of the patients (a decrease by 2.3%) was below statistical significance.

4. Conclusion:

- Given the results of the clinical follow up studies on 411 diabetes II patients, it can be said that reduced water consumption seems to have a beneficial effect upon the most important diabetes parameters.
- The evaluation of the clinical data estimates number needed to treat on four patients in order to achieve the significant improvement of all diabetes relevant parameters. This is a very good quotient; moreover it could be achieved entirely without side effects.
- This improvement scales up parallel to the quantity of consumed reducer water.
- The significant improvement of diabetes relevant parameters like blood fats and creatinine can be also beneficial to other diseases like high blood pressure, circulatory disturbance, renal insufficiency or atherosclerotic dementia.

- Taking account of the fact that the natural reduced water as well as the electrolyzed reduced water obviously improves in a very short time and entirely without side effects very important metabolic parameters, it can be said that the reduced water shall be considered a useful supplement to the usual orthodox medication of ROS-associated diseases

5. References

Borg LA, Eide SJ, Anderson A and Hellerstrom C (1979) Effect *in vitro* of alloxan on the glucose metabolism of mouse pancreatic ß-cells. Biochem J 182: 797-802.

Halliwell B and Gutteridge JMC (1990) Role of free radicals and catalytic metal ions in human disease: an overview. Methods in Enzymol 186: 1-85.

Hanaoka K (2001) Antioxidant effects of reduced water produced by electrolysis of sodium chloride. J Appl Electrochem 31: 1307-1313.

Kim H, Rho H, Park B, Park J, Kim J and Kim UH (1994) Role of Ca^{2+} in alloxan-induced pancreatic ß-cell damage. Biochim Biophys Acta 1227: 87-91.

LeBel CP, Ishiropoulos H and Bondy SC (1992) Evolution of the probe 2',7'- dichlorofluorescein as an indicator of reactive oxygen species formation and oxidative stress. Chem Res Toxicol 5: 227-231.

Li Y., Nishimura T., Teruya K., Maki T., Komatsu T., Hamasaki T., Kashiwagi T., Kabayama S., Shim S.-Y., Katakura Y., Osada K., Kawahara T., Otsubo K., Morisawa S., Ishii Y., Gadek Z., Shirahata S. (2003) Cytotechnology 40: 139-149

Malaisse WJ and Lea MA (1982) Alloxan toxicity to the pancreatic ß-cell. A new hypothesis. Biochem Pharmacol 31: 3527-3534.

Masumoto N, Tasaka K, Miyake A and Tanizawa O (1990) Superoxide anion increases intracellular free calcium in human myometrial cells. J Biol Chem 265: 22533-22536.

Oda M, Kusumoto K, Teruya K, Hara T, Maki T, Kabayama Y, Katakura Y, Otsubo K, Morisawa S, Hayashi H, Ishii Y and Shirahata S (1999) Electrolyzed and natural reduced water exhibit insulin-like activity on glucose uptake into muscle cells and adipocytes. Animal cell Technology: Products from Cells, Cells as Products (eds. Bernard A et al.), pp. 425-427, Kluwer Academic Publishers, the Netherlands.

Rho H, Lee J, Kim H, Park B and Park J (2000) Protective mechanism of glucose against alloxan-induced ß-cell damage: pivotal role of ATP. Exp Mol Med 32: 12-17.

Shirahata S (2000) Regulation of functions of animal cells by reduced water and its medical application. Nippon Nogei Kagaku Kaishi 74: 994-998.

Shirahata S (2002) Reduced water for prevention of diseases. Animal Cell Technology: Basic & Applied Aspects, Volume 12, pp. 25-30, Kluwer Academic Publishers, the Netherlands.

Shirahata S, Kabayama S, Nakano M, Miura T, Kusumoto K, Gotoh M, Hayashi H, Otsubo K, Morisawa S and Katakura Y (1997) Electrolyzed-reduced water scavenge active oxygen species and protects DNA from oxidative damage. Biochem Biphys Res Commun 234:269-274.

Shirahata S, Nishimura T, Kabayama S, Aki D, Teruya K, Otsubo K, Morisawa S, Ishii Y, Gadek Z and Katakura Y (2001) Anti-oxidative water improves diabetes. Animal Cell Technology: From Target to Market (eds. Linder-Olsson E et al.), pp. 574-577, Kluwer Academic Publishers, the Netherlands.

Takasu N, Asawa T, Komiya I, Nagasawa Y and Yamada T (1991) Alloxan-induced DNA strand breaks in pancreatic islets: Evidence for H_2O_2 as an intermediate. J Biol Chem 266: 2112-2114.

Tashiro H (1999) Clinical examination of alkaline ion water. Abstract book of Symposium "Electrolyzed functional water in therapy" in 25th Meeting of Japanese Medical Society. pp. 6-7.