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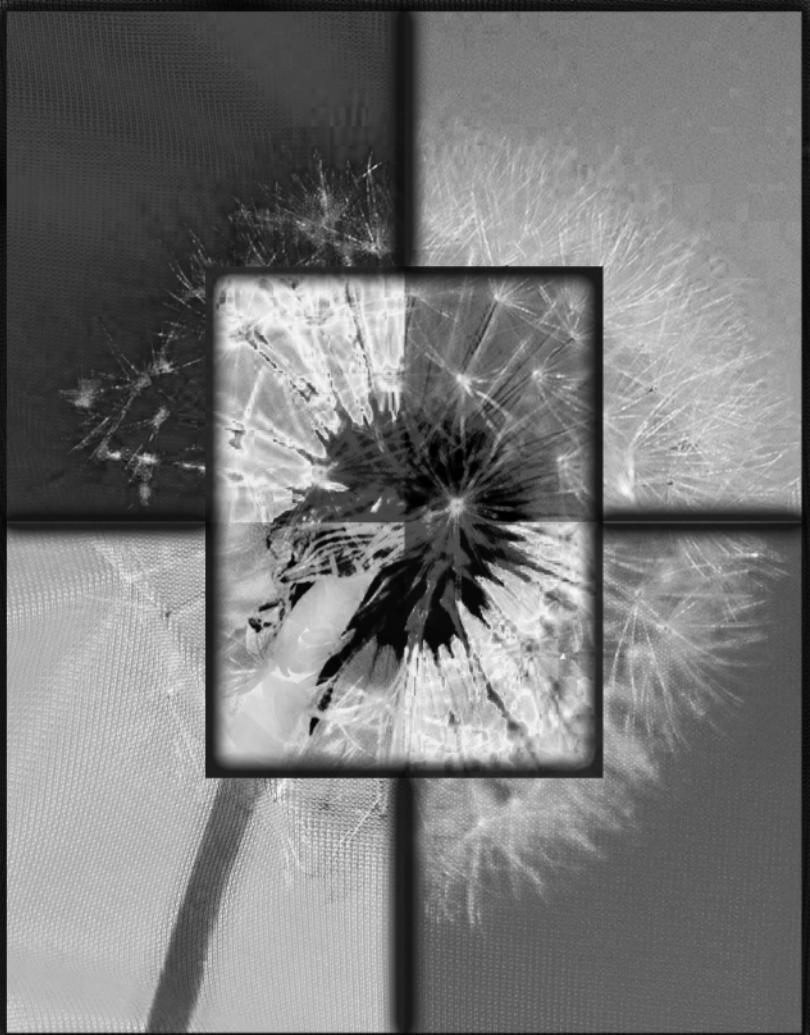

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GLISODIN®†, A VEGETAL SOD WITH GLIADIN, AS PREVENTATIVE AGENT VS. ATHEROSCLEROSIS, AS CONFIRMED WITH CAROTID ULTRASOUND-B IMAGING

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SUMMARY: Prevention of cardiovascular disease should target high-risk subjects based on genetic/familial factors, blood chemistry, blood pressure, body mass index (BMI), and a history of/or current cigarette smoking. We selected active adults (n=76) aged 30-60 and investigated these risk factors, in order to recommend preventive measures.
Another interesting variable is the preclinical status or atheroma of the arterial (carotid) wall or lumen. We also investigated the presence of oxidative stress in, and the anti-oxidant status of these subjects.
We studied the anti-oxidative efficacy of superoxide dismutase (SOD) and variations of malondialdehyde (MDA). Supplementation with GliSODin®, a vegetal SOD associated with gliadin, was effective in controlling the thickness of the carotid artery intima and media layers as measured by ultrasonography-B.
We could demonstrate the preventive efficacy of GliSODin at a preclinical stage in subjects with risk factors of cardiovascular disease.

Abbreviations: SOD: superoxide-dismutase; IMT: intima media thickness; ECG: electrocardiogram; qd: once daily; CRP: C-reactive protein; MDA: malondialdehyde; GPx: glutathione peroxidase.

INTRODUCTION

Oxidative stress is known to be a risk factor of atherothrombosis (1-2). A number of studies have attempted to demonstrate the efficacy of anti-oxidants in primary prevention of this condition (3). The recent introduction of ultrasonography-B for the detection of the early stages of arterial damage is a major advance for detecting the preclinical stages of atherothrombotic disease (4-5). It is now possible to identify and study populations at risk, have a precise appreciation of their anti-oxidant status, measure the intima/media thickness of their carotid arteries (6), and evaluate the effects of the anti-oxidant supplementation with GliSODin, the first orally effective SOD (7-11). This new automated technology is a major improvement in primary prevention and it helps to appreciate the eventual important role played by anti-oxidants in this prevention; it allows for a follow up on carotid arteries, with an automated method that measures the intima-media thickness.

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MATERIAL AND METHODS

Subjects and Design of Study

The population selected for the study was made of seventy-six patients without clinical signs/symptoms of cardiovascular disease, but who were considered to be at risk because of:
- Family history of stroke;
- Height/weight ratio >20-30% above normal range, considering BMI.

Inclusion criteria included:
- Systolic arterial blood pressure >160mm Hg
- Diastolic arterial blood pressure: >90mm Hg;
- Total serum cholesterol: >2.5g/l;
- Serum triglycerides: >1.28g/l;
- LDL Cholesterol: >1.4g/l;
- Intima media thickness: >0.7mm.

All these values are considered to be associated with a significant clinically-relevant risk of atherothrombosis (1).

All medications that could influence or modify any clinical criterion, serum maker value, or affect the assays were excluded for the whole duration (~3 years) of the trial. Each subject had to answer a comprehensive list of questions related to his/her eating habits and was enrolled in a personalized diet program in accordance to the SU.VI.MAX study standards, the French Coordinating Committee for Research on Atherosclerosis and Cholesterol, and the Lyon Heart Study (12).

All subjects had to report weekly on diet details and had to avoid significant variations, both qualitative and quantitative, in their diets for the duration of the study (ANPM questionnaire); they had to submit to a medical evaluation every 3 months for the duration of the study.

These stringent requirements resulted in a drop-out of 42 subjects.

All enrolled subjects were fully informed on the details on the study, the risks involved, and signed informed consent forms.

The total duration of the study was three years:

➢ After sessions focusing on nutritional education, 76 subjects were initially selected;

➢ Six months later, a comprehensive medical evaluation was performed to appreciate the consequences on identified risk factors;

➢ Six months later (before inclusion), the anti-oxidant status was evaluated, and ultrasonography-B of the carotid arteries was performed to measure IMT (intima media thickness);

➢ As a result, 34 subjects were finally included and randomized:

- group A continued to adhere to the diet recommendations only,
- group B was also prescribed GliSODin (500UI-NBT SOD/day) as 2 capsules qd.

➢ Both groups (A&B) were followed for two more years in order to evaluate the effects of GliSODin.

METHODS

■ **Evaluation of the anti-oxidant status** of all subjects was conducted by Dr. Nicolas Zamaria; he assayed all samples within seconds of drawing to prevent any oxidation. He had previously investigated a regional (Ile-de-France) male & female population of 2,400 adults (age: 17-89) living under the same conditions: nutritional status, pollution, stress due to transportation and noise, etc. This previous study resulted in standards (Table 1: normal range) for enzymes and vitamins based on a subgroup of 1,278 healthy subjects.

In the current study, we used the protocol of the SU.VI.MAX study (13):

- Enzyme assays: CuZn-SOD (Riansod, Randox Labs., Crumlin, UK), GPX (Randox Labs, Crumlin, UK), GSH (HP chromatography);
- Vitamins: retinol, alpha-tocopherol (liquid chromatography; fluorometry), carotenoids (UV), ascorbic acid (clinical enzymology);
- Oxidation markers: malonyl-dialdehyde (MDA kit, spectrofluorometry) (13).

Enzyme; Vitamin	Lab Method	Unit	Range
Malonyl-dialdehyde (MDA)	Fluorometry	µmol/L	1.3-2.7
Superoxide-Dismutase (SOD)	Clinical enzymology	UI/gHb	1092-1817
Glutathione-Peroxidase (GPX)	Clinical enzymology	UL/gHb	27-74
Reduced Glutathione (GSH)	High pressure chromatography	µmol/L	850-950
Total Carotenoids (α &β, carotens, lycopene, lutein)	UV	µmol/L	1.5-3.7
Retinol (vitamin A)	Fluorometry	µmol/L	1.6-3.6
α tocopherol (vitamin E)	Fluorometry	µmol/L	25-45
Ascorbic acid (vitamin C)	Clinical enzymology	µmol/L	40-95

Table 1: Anti-Oxidant Status: Range from a Group of 1,278 Normal Healthy Adults.

■ **Serology:** Total cholesterol, triglycerides, HDL- and LDL-cholesterol.

All assays were performed at the same location (CIEM Lab, F-75006 Paris, France; member of ARCOL/NSFA) in patients fasting >12 hours; time: 0830-0930. Patients gave full information on their diet.

■ **Schedule:** The study was initially conducted over a period of 360 days (D0-D360). 76 subjects were started on a controlled diet (12) on D0, were evaluated (lab tests) on D180, and on D360. Each subject was under the care of his/her attending physician. All clinical and biological data were registered into individual files with subject's ID, description of symptoms or conditions, past personal and family history, current and previous treatments, risk factors, and details on the personalized controlled diet (12).

■ **Statistical analysis:**

We used the following:

➢ Initial comparison of groups A & B, qualitative variables with χ^2 and analysis of variance (Kolmogorov-Smirnov) allowed for comparison as a function of time, and provided reliable information on the improvement or deterioration of the subject's status attributable to the treatment;

➢ Changes in groups A & B were analyzed by Student's test for paired data was used to evaluate the efficacy of the treatment with GliSODin.

■ **Carotid artery evaluation by ultrasonography-B;** All subjects were examined by the same physician (FL), specially trained in ultrasonography-B, at their workplace, with a real-time B-mode ultrasound imager. Imaging of the intima media thickness (IMT) and of the diameter of the lumen of the distal segment of the common artery, on both sides, was conducted according to a standard procedure (15). IMT images from the far wall of the artery consisted of two parallel echogenic lumen-intima and media-adventitia interfaces (double line pattern) visible on at least 1cm longitudinally. Such an image did not include intrusive plaque, since its presence would have disturbed the double-line pattern. Hence our results of IMT apply to carotid wall thickness before intrusion of lesions into the vessel (pre-intrusive IMT).
Each subject enrolled in the study had a longitudinal segment of common carotid artery >1cm free of intrusive plaque, allowing for enough visible IMT double-line pattern.
The lumen diameter was imaged between the near and far wall intima-media interfaces on the same segment explored for IMT.
Once the optimal quality of these images (far wall IMT and lumen diameter) had been visualized, they were frozen at end-diastole by ECG-triggering. They were then transferred to a computer equipped with a digitization software, and stored on disk for centralized off-line reading and interpretation.

■ **Summary:** All these data, before the diet phase, are presented in columns **D-360** of following tables. All subjects had a high BMI, elevated systolic blood pressure, metabolic biological abnormalities (elevated serum levels of lipids, and glucose; low HDL), and high IMT. Data at **D0** (randomization) are presented in Table 2; there are no differences between the **C** (control) group and the **GL** (GliSODin) group.

	C: n=18 13 men, 5 women	GL: n=16 10 men, 6 women
Age (y)	43	43
Body Mass Index	27.6 +/- 0.6	27.9 +/- 0.9
Blood Pressure in mm/Hg		
Systolic	151 (+/- 3)	149 (+/- 4)
Diastolic	82 (+/- 3)	80 (+/- 4)
Total Cholesterol in g/L	2.26 +/- 0.20	2.30 +/- 0.19
LDL Cholesterol in g/L	1.51 +/- 0.16	1.49 +/- 0.18
HDL Cholesterol in g/L	0.53 +/- 0.10	0.50 +/- 0.09
Triglycerides in g/L	1.38 +/- 0.11	1.34 +/- 0.10
Glycemia in g/L	1.10 +/- 0.09	1.09 +/- 0.08
Carotid IMT in mm	0.669 (0.012)	0.672 (0.010)

Table 2: Data of control and GliSODin groups at D0.
Values & data of subjects at randomization (D 0).

RESULTS

We observed minor improvements in clinical (BMI, systolic and diastolic BP) and biological criteria (total and LDL-cholesterol) at D.0 in all subjects; this was due to modifications of their diet (12) and lifestyle when compared to D-360 (Table 3). However we did not find changes in their anti-oxidant status –that remained poor- (Table 4), or their IMT with numbers too high when considering the age of these subjects. Conversely, during the treatment period, all clinical and biological numbers remained stable (Table 5), and modifications of the anti-oxidant status became significant at D270 but only the GliSODin group (Table 4).
Changes in the carotid IMT started to be visible at D545, but became statistically significant at or after D730.
We compared subjects with diet (12) changes only and slight increase in IMT with subjects of the GliSODin-treated group; in GliSODin-treated subjects, IMT decreased and reached significance at D365; and at D545 and D730 it reached $p<0.001$ (Figure 1).

DISCUSSION

This study was conducted in young adults and investigated the potentials of a personalized diet associated to a new vegetal compound in preventive medicine; we evaluated the resulting effects by using non invasive ultrasonography-B, and biological assays conducted immediately to avoid subsequent oxidations.

NR OF SUBJECTS: 76	@ D -360	@ D -180	@ D0
Age (years)	42 +/- 0.5		43 +/- 0.5
Body Mass Index Kg	28.3 (0.3)	28.2 (0.4)	27.8 (0.3)
Blood pressure mmHg			
Systolic	156 (2)	153 (3)	150 (2)
Diastolic	90 (1)	89 (2)	80 (2)
Total Cholesterol g/l	2.38 +/- 0.32	2.35 +/- 0.30	2.20 +/- 0.26
LDL-Cholesterol g/l	1.53 +/- 0.26	1.50 +/- 0.21	1.46 +/- 0.20
HDL-Cholesterol g/l	0.52 +/- 0.10	0.50 +/- 0.09	0.51 +/- 0.12
Triglycerides g/l	1.58 +/- 0.30	1.38 +/- 0.32	1.35 +/- 0.26
Glycemia	1.10 +/- 0.12	1.12 +/- 0.10	0.86 +/- 0.11
Family History of Diabetes	+ in 34 subjects		
Family History of Myocardial Infarction	+ in 40 subjects		
Family History of Cerebrovascular Accident	+ in 32 subjects		
Carotid IMT mm	0.668 (0.012)	0.666 (0.010)	0.670 (0.010)

Table 3: Data before Treatment/Control.
Original data on subjects at recruitment, i.e. when ALL subjects enrolled in the personalized diet phase.

GliSODin® is a water-soluble superoxide-dismutase extracted from a special strain of *Cucumis melo* LC (cantaloupe melon) chemically combined to wheat gliadin for efficacy after oral administration (7-11).
Recent studies have confirmed its activity vs. oxidative stress; e.g. the study by Muth CM et al vs. hyperbaric oxygen-related cell damage was very demonstrative.
We have been studying populations of the Paris, France area (Ile-de-France) since 1961 through our center, focusing on preventive medicine. 3,400 subjects undergo a regular annual evaluation in this center. The biological risk factors are evaluated by the NSFA-certified reference laboratory that conducts assays within seconds of sampling; the carotid arterial status is measured by ultrasonography-B using special software registered in France and in the US (SYNARC). We have built up references and standards for oxidative stress based on >2.400 subjects.
By using new and affordable technology, we can monitor activity on anti-oxidant stress and the reduc-

	MDA		SOD		GPx	
D0	3.020		1.118		43.360	
	C	GL	C	GL	C	GL
D60	3.114	2.816	1,084	1,326	46.704	49.22
D120	3.468	2.340	1,076	1,428	39.860	58.160
D270	3.326	2.04	1,069	1,580	40.520	62.420
D480	3.230	2.01	1,080	1,576	41.410	63.720
D730	3.180	2.09	1,066	1,630	42.664	66.82

Table 4: Anti-oxidant status.
Anti-oxidant status in subjects at D0, 60, 120, 270, 480 and 730 of the treatment phase (day 0-730). Number of Subjects: 34. Group C (Control): 13 males, 6 females; group GL (GliSODin 500mg qd): 10 males, 6 females. Age: ~42-43.

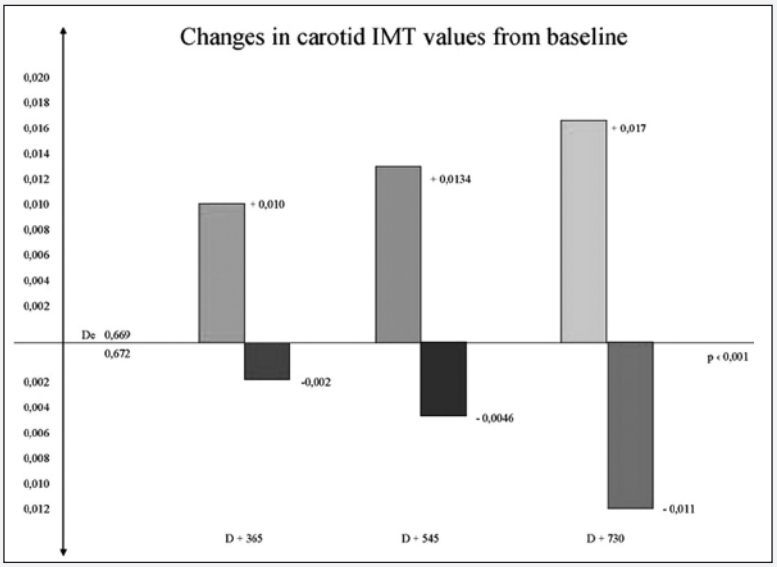

Figure 1: Changes in carotid IMT values from baseline.
Changes of IMT at baseline, D365, D545, and D730.
Upper columns: IMT in control group; lower columns: IMT in GliSODin group. While the control group experienced significant thickening of IMT, the GliSODin group experienced a significant reduction of IMT.

	D 180		D 360		D 730	
	C	GL	C	GL	C	GL
B.M.I.	27.4 +/- 0.4	27.6 +/- 0.3	27 +/- 0.4	26.8 +/- 0.2	26.7 +/- 0.3	26.5 +/- 0.5
Blood Pressure mm/Hg						
Systolic	152 +/- 8	150 +/- 6	146 +/- 5	148 +/- 6	146 +/- 3	149 +/- 6
Diastolic	84 +/- 3	83 +/- 4	82 +/- 3	84 +/- 2	80 +/- 5	83 +/- 4
Total Cholesterol g/L	2.34 +/- 0.31	2.29 +/- 0.29	2.40 +/- 0.27	2.38 +/- 0.22	2.33 +/- 0.18	2.36 +/- 0.16
LDL Cholesterol g/L	1.52 +/- 0.10	1.49 +/- 0.08	1.40 +/- 0.11	1.38 +/- 0.10	1.36 +/- 0.18	1.34 +/- 0.16
HDL Cholesterol g/L	0.54 +/- 0.11	0.52 +/- 0.09	0.49 +/- 0.10	0.51 +/- 0.08	0.50 +/- 0.009	0.48 +/- 0.12
Triglycerides g/L	1.40 +/- 0.20	1.38 +/- 0.22	1.42 +/- 0.19	1.39 +/- 0.17	1.37 +/- 0.21	1.36 +/- 0.15
Glycemia g/L	1.11 +/- 0.10	1.10 +/- 0.9	1.10 +/- 0.8	1.09 +/- 0.8	1.09 +/- 0.8	1.06 +/- 0.10
CRP mg/L	1.2 +/- 2	1.06 +/- 1.6	1.02 +/- 1.8	1.24 +/- 1.70	1.00 +/- 0.9	1.2 +/- 0.7

Table 5: Evolution of clinical and biological data during treatment phase (D0-D730).
Evolution of physical/clinical and biological data in randomized subjects of Control and GliSODin-treated groups at days 180, 360 and 730. No significant difference was observed.

tion of oxidative markers. This evidence of arterial wall protection could be considered as controlling the extension of atherosclerosis and cardiovascular risks.
Since the first publication of Salonen (1991) on ultrasonographically-assessed carotid artery morphology, progress in non-invasive medical imaging during this last decade has confirmed the value of and validated the use of this technology as clinical marker of atherosclerosis; the ultrasonographic measurement of the intima-media thickness (IMT) of the carotid artery wall is now accepted internationally (14, 16). Thickening of the intima-media is frequently observed in hypertensive patients (15, 17-18); it is also found in patients with cardiovascular risk factors; hyperlipidemia (19, 21) and IMT are correlated not only with increased risk of cerebrovascular disease, but also with increased risk of myocardial infarction.
Changes in vessel thickness appear very early in atherosclerosis (inflammatory initial phase). During the past few years, high resolution ultrasound imaging techniques have enabled investigators to quantify non-invasively the presence and the progression of early atherosclerosis in large peripheral arteries and in large populations. This ultrasound technology was developed by Simon and Gariépy (22-23) at the Hospital Broussais, Paris, France with the French company SYNARC; it allows for the following of changes of the IMT of the carotid wall as a function of age, gender, and atherosclerotic risk factors (14, 24, 27). Aging is the main factor influencing IMT; the normal IMT is found in subjects <18 years of age, free of cardiovascular risk factors. Adult men have higher IMT and reduced diameter values, and have a more rapid progression of atherosclerosis than women as a general rule. There are very limited data available on the influence of ethnicity on IMT; differences between ethnic groups may reflect different lifestyles, risk profiles, or sensitivity to risk factors.
This technology is a reliable diagnostic tool for predicting cardiovascular complications, and helps control the long-term effects of treatments (hypotensive or hypolipidemic) (28, 31). Reduction of IMT has been observed with angiotensin conversion enzyme (ACE) inhibitors (31) in hypertensive patients, and with aggressive lipid-lowering treatments (29-30). These studies have also shown that lipid profile and blood pressure may act synergistically in the progression of IMT.
The main result of the present study is the demonstration of the reduction of the progression of carotid IMT with a program of diet (12), and anti-oxidant supplementation. The Lyon Heart Diet (12) was administered for one year before inclusion, and for two more years until completion of this three-year study.
This was a primary prevention trial in subjects aged 40-46, with the same socio-professional background, residing in the same geographic area (Ile-de-France). Subjects were classified according to their cardiovascular risk factors, using the algorithms of the Framingham study modified by the European Society of Cardiology, and including a low anti-oxidant status (32), as well as the determination of IMT of the common carotid artery; the IMT was measured in these adults of both genders. We could select, within this population, subjects at risk with IMT>P90th percentile in the same age group. We have been using this technology since 1992, including an automated computerized edge detection program (Iotec) that makes SYNARC the leading company providing imaging for clinical trials. In the Cholesterol Lowering Atherosclerosis Study (CLAS), common artery IMT (33) provided earlier and better information on the effects of LDL-lowering treatment than angiographically-detected coronary stenosis, or carotid plaques; this was recently confirmed in a study on primates. A Finnish epidemiological study (34) reported that LDL-cholesterol was predictive of the rate of progress of carotid IMT only in subjects with high serum levels of pro-oxidant copper; the intracellular accumulation of modified LDL is promoted (or delayed) by serum pro-oxidants (or anti-oxidants). The Los Angeles Atherosclerosis Study investigated the role of serum and dietary anti-oxidants on the progression of IMT in subjects enrolled in 1994; the results were published in 2004 (35), and confirm the potential benefits of some anti-oxidants, although GliSODin was not included.
GliSODin is a well-known anti-inflammatory: in C57BL/6mice, GliSODin alone –but not the melon SOD or the gliadin alone- protected cells from the pro-inflammatory action of INF-gamma, with a significant increase in the production of IL-10 and a significant reduction in the production of TNF-alpha after activation of macrophages by immune complexes (11). It also prevented tumor progression promoted by inflammation through scavenging inflammatory cell-derived superoxide anion (36).
Since it is now generally accepted to consider atherosclerosis and resulting cardiovascular disease to be started and developed through an inflammatory process, we can understand why –and somewhat how- GliSODin, associated with a proper diet (12), is active against atherosclerosis; there were no side effects reported with or without GliSODin. Our study also demonstrates that non-invasive modern technologies can diagnose, follow, and predict the outcome of atherosclerosis in subjects with risk factors.
In summary, we have demonstrated that long-term primary prevention in cardiovascular disease is possible in (apparently) healthy adults with risk factors by combining: regular comprehensive clinical and biological evaluation including the anti-oxidant status and –more importantly- measurements of IMT by ultrasonography-B, a proper diet (12) that tends to reduce initially elevated risk factors, and GliSODin that

improves significantly the anti-oxidant status and diminishes remarkably carotid artery IMT.

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†GliSODin and GliSODine are registered trade marks by Isocell Nutra, Paris, France. We used GliSODine in our study, but elected to use the international spelling GliSODin for this article. For more information check: http://www.glisodin.com/contact.php and www.glisodin.org.

References

1. Favier A. - Le stress oxydant : intérêt de sa mise en évidence en biologie médicale et problèmes posés par le choix d'un marqueur. *Ann Biol Clin* 1997;5:9-16.
2. Salonen J.-T. - Clinical trials testing cardiovascular benefits of antioxidant supplementation. *Free Rad Res* 2002;36:1299-1306.
3. O'Leary D.-H., Polak, J.-F. - Intima Media Thickness: a tool for atherosclerosis imaging and event prediction. *Am J Cardiol* 2002;90:18L-20L.
4. Greenland P., Abrams J., Aurigemma G.-P. et al. - Prevention Conference V: Beyond secondary prevention: identifying the high-risk patient for primary prevention; non-invasive tests of atherosclerotic burden. Writing group II. *Circulation* 2000;101:E16-E22.
5. Chambless L.-E., Folsom A.-B., Clegg L.-X. et al. - Carotid wall thickness is predictive of incident clinical stroke: The atherosclerosis risk in communities (ARIC) study. *Am J Epidemiol* 2000;151:478-487.
6. Salonen J.-T., Salonen R. - Ultrasound-B mode images in observational studies of atherosclerotic progression. *Circulation* 1993;87:1156-1165.
7. Dugas B. - Glisodin®, a nutraceutical product that promotes the oral delivery of superoxide dismutase. *Free Radic Biol Med* 2002;33: S64.
8. Muth C.-M., Glenz Y., Klaus M. et al. - Influence of an orally effective SOD on hyperbaric oxygen-related cell damage. *Free Radic Res* 2004;38: 927-132.
9. Vouldoukis I., Conti M., Kolb J.-P. et al. - Induction of Th1-dependent immunity by an orally effective melon superoxide dismutase extract. *Curr Trends Immunol* 2003;5:141-145.
10. Vouldoukis I., Conti M., Krauss P. et al. - Supplementation with gliadin-combined plant superoxide dismutase extract promotes antioxidant defences and protects against oxidative stress. *Phytother Res* 2004;18: 957-962.
11. Vouldoukis I., Lacan D., Kamate C. *et al.* - Antioxidant and anti-inflammatory properties of a Cucumis melo LC extract rich in superoxide dismutase activity. *J Ethnopharmacol* 2004;94 (1): 67-75.
12. De Lorgeril M., Renaud S., Mamelle N. *et al.* - Mediterranean alpha-linolenic acid rich diet in secondary prevention of coronary heart disease. *Lancet* 1994;343:1454-1459.
13. Hercberg S., Galan P., Preziosi P. *et al.* - The SU.VI.MAX Study: a randomized, placebo-controlled trial of the health effects of antioxidant vitamins and minerals. *Arch Intern Med* 2004;164:2335-2342.
14. Lefèvre G., Beljean-Leymarie M., Bonnefont-Rousselot D. *et al.* - Evaluation de la peroxydation lipidique par le dosage des substances réagissant avec l'acide thiobarbiturique. *Ann Biol Clin* 1998 ;56 :305-319.
15. Denarié N., Gariépy J., Chironi G. *et al.* - Distribution of ultrasonographically-assessed dimensions of common carotid arteries in adults of both sexes. *Atherosclerosis* 2000;148:297-302.
16. Gariépy J., Massonneau M., Levenson J. *et al.* - Evidence for in vivo carotid and femoral wall thickening in human hypertension. *Hypertension* 1993;22:111-118.
17. Pauletto P., Palatini P., Das Ros S. *et al.* - Factors underlying the increase in carotid intima-media thickness in borderline hypertensives. *Arterioscler Thromb Vasc Bi*ol 1999;19:1231-1237.
18. Zanchetti A., Crepaldi G., Bond M.-G. *et al.* - Systolic and pulse blood pressures (but not diastolic blood pressure and serum cholesterol) are associated with alterations in carotid intima media thickness in the moderately hypercholesterolaemic hypertensive patients of the Plaque Hypertension Lipid Lowering Italian Study (PHYLLIS study group). *J Hypertens* 2001;19:79-88.
19. Ylitalo K., Syvanne M., Salonen R. *et al.* - Carotid artery intima-media thickness in Finnish families with familial combined hyperlipidemia. *Atherosclerosis* 2002;162:171-178.
20. Keulen E.-T., Knujshoop M., Schaper N.-C. *et al.* - Increased intima-media thickness in familial combined hyperlipidemia associated with apolipoprotein *B. Neth J Med* 2002;60:354-361.
21. Hurwith Eller N., Netterstrom B. - The intima media thickness and coronary risk factors. *Int Angiol* 2001;20:118-125.
22. Gariépy J., Simon A., Massonneau M. *et al.* - Wall thickening of carotid and femoral arteries in male subjects with isolated hypercholesterolemia. *Atherosclerosis* 1995;113:141-151.
23. Graf S., Gariépy J., Massonneau M. *et al.* - Experimental and clinical validation of arterial diameter waveform and intima-media thickness obtained from B-mode ultrasound image processing. Ultrasound Med Biol 1999;9:1353-1363.
24. Linhart A., Gariépy J., Massonneau M. *et al.* - Carotid intima-media thickness: the ultimate surrogate end-point of cardiovascular involvement in atherosclerosis. *Applied Radiology* 2000;25-39.
25. O'Leary D.-H., Polak J.-F., Kromal R.-A. *et al.* - Carotid artery intima and media thickness as a risk factor for myocardial infarction and stroke in older adults: Cardiovascular Health Study Collaborative Research Group. *N Engl J Med* 1999;340:14-22.
26. O'Leary D.-H., Polak J.-F., Kronmal R.-A. *et al.* - Thickening of the carotid wall. A marker for atherosclerosis in the elderly? Cardiovascular Health Study Collaborative Research Group. *Stroke* 1996;27:224-231.
27. Pignoli P., Tremoli F., Poli A. *et al.* - Intimal plus medial thickness of the arterial wall: a direct measurement with ultrasound imaging. *Circulation* 1986;74:1399-1406.
28. Mayet J., Stanton A.-V., Sinclair A.-M. *et al.* - The effects of antihypertensive therapy on carotid vascular structure in man. *Cardiovasc Res* 1995;30:147-152.
29. Smilde T.-J., Van Wissen S., Wollersheim H. *et al.* - Effect of aggressive versus conventional lipid lowering on atherosclerosis progression in familial hypercholesterolemia (ASAP: a prospective, randomized, double-blind trial. *Lancet* 2001;24(357):577-581.
30. Smilde T.-J., Van der Berkmortel F.-W., Wollersheim H. *et al.* - The effect of cholesterol lowering on carotid and femoral artery wall stiffness and thickness in patients with familial hypercholesterolaemia. *Eur J Clin Invest* 2000;30:473-480.
31. Hoogerbrugge N., de Groot E., de Heide L.-H. *et al.* - Doxazosin and hydrochlorothiazide equally affect arterial wall thickness in hypertensive males with hypercholesterolaemia (the DAPHN study). Doxazosin Atherosclerosis Progression Study in Hypertensives in the Netherlands. *Neth J Med* 2002;60:354-361.
32. Pincemail J., Defraigne J.-O., Limet R. - Oxidative stress in clinical situations: fact or fiction? *Europ J Anaesth* 1996;13:219-234.
33. Lonn E. - Carotid artery intima-media thickness – a new non-invasive gold standard for assessing the anatomic extent of atherosclerosis and cardiovascular risk? *Clin Invest Med* 1999;22:158-160.
34. Salonen J.-T., Nyysonen K., Salonen R. *et al.* - Antioxidant Supplementation in Atherosclerosis Prevention (ASAP) study: a randomized trial of the effect of vitamin E and C on a 3-year progression of atherosclerosis. *J Intern Med* 2000;248:377-386.
35. Dwyer J.-H., Paul-Labrador M.-J., Fan J. *et al.* - Progression of intima-media thickness and plasma anti-oxidants: the Los Angeles Atherosclerosis study. *Arterioscler Thromb Vasc Biol* 2004;24:313-319.
36. Okada F., Shionoya H., Kobayashi M. *et al.* - Prevention of inflammation-mediated acquisition of metastatic properties of benign mouse fibrosarcoma cells by administration of an orally available superoxide dismutase. *Br J Cancer* 2006;94:854-862.