Arterosil® Supports a Healthy Glycocalyx and Endothelial Function

"Perhaps the arterial glycocalyx will become the most important [drug target] for future early prevention of people at risk of cardiovascular disease."

A.J. Drake-Holland, and M.I.M. Noble: Update on the Important New Drug Target in Cardiovascular Medicine – the Vascular Glycocalyx.

Arterosil® is the only dietary supplement that helps maintain optimal endothelial function through regeneration of the vascular endothelial glycocalyx. Loss of glycocalyx-mediated endothelial function has been implicated in the development of a wide variety of diseases such as atherosclerosis, diabetes, sepsis, and trauma.[1]

THE ENDOTHELIAL GLYCOCALYX

The endothelial glycocalyx is a thin gel-like layer that coats the entire luminal side of vascular endothelium. It is a meshwork mainly of glycoproteins, proteoglycans and glycosaminoglycans at a thickness of approximately 1 μm magnitude.[2-4]

Syndecans and glypicans are the core proteins of heparan sulfate (aglycosaminoglycan) proteoglycans bound to endothelial cells identified in the glycocalyx.

Glycoproteins such as selectins and integrins are also anchored on endothelial cells while some other soluble proteins and proteoglycans simply dock in glycocalyx.[5]

FUNCTIONS OF THE ENDOTHELIAL GLYCOCALYX

Extensive research has revealed the importance of glycocalyx-mediated endothelial function in vascular and microvascular health.

For example, the endothelial glycocalyx:

- Regulates vascular permeability and fluid balance due to the large size and negative charge of glycosaminoglycans.[6,7]
- Provides a physical barrier against inadvertent adhesion of platelets and leukocytes to the vascular wall.[8]
- Regulates coagulation as many of mediators of coagulation pathway are buried inside the glycocalyx under normal physiological condition.[5]

Most intriguingly, the glycocalyx is found to be a mechano-sensor and -transducer of the shear-force inside blood vessels.[3] The signal is believe to be transduced to endothelial nitric oxide synthase (eNOS) via heparan sulfate in the glycocalyx to either up- or down-regulate the synthesis of nitric oxide (NO) in response to the blood flow.[9,10]

Figure 1 below illustrates the chemical structure of the endothelial glycocalyx and its signal transduction to eNOS and subsequently sGC (soluble guanynyl cyclase) to induce smooth muscle relaxation via shear stress.

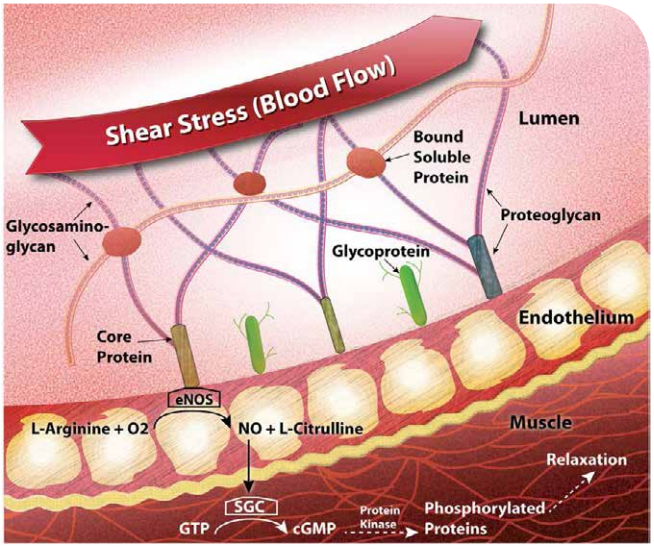

Figure 1. Structure of the endothelial glycocalyx and its activation of vascular muscle relaxation via NO in response to increased shear force

DAMAGE TO THE ENDOTHELIAL GLYCOCALYX

The endothelial glycocalyx is a delicate structure and can be damaged under many pathological conditions. It has been demonstrated that oxidized LDL reduces the endothelial glycocalyx and results in cholesterol penetration and leukocyte recruitment to endothelium in lab animals.[11,12]

HYPERGLYCEMIA AND THE ENDOTHELIAL GLYCOCALYX

Hyperglycemia is another condition that has been firmly identified to cause disruption of the endothelial glycocalyx.[13,14] In fact individuals with hyperglycemia and diabetes are known to have less endothelial glycocalyx.[15] Such a change may explain the endothelial dysfunction and increased microvascular permeability that lead to major complications in the diabetic population.[16,17]

OTHER PATHOLOGIES ASSOCIATED WITH IMPAIRED ENDOTHELIAL GLYCOCALYX

Impaired endothelial glycocalyx is also demonstrated in the patients with:

- Coronary heart disease[18]
- Renal diseases[19]
- Lacunar stroke (a small vessel disease)[20]
- Severe trauma[21]

PHARMACEUTICAL INTERVENTION

Given the vital role the endothelial glycocalyx plays in the pathology of many vascular and micro-vascular related diseases,it naturally became a target for pharmaceutical intervention.[1,22,23] The glycocalyx drug development is still in its infancy and no substantial progress has been made up to date.

ARTEROSIL : A NEW GLYCOCALYX DIETARY SUPPLEMENT

Now enters Arterosil®, a glycocalyx dietary supplement many years in the making. It started when a medical doctor in the United States searched for a natural alternative of heparin for his heart patients some 20 years ago. That journey led him to a rare green seaweed, monostroma nitidum, in east Asia.

THE ACTIVE INGREDIENT: RHAMNAN SULFATE

Monostroma nitidum is rich in rhamnan sulfate, a negatively charged polysaccharide, that has been shown to possess a wide range of biological activities including anticoagulant and antithrombotic activities with potential health benefits.[24-28] Rhamnan sulfate has a similar chemical structure to heparan sulfate found abundantly in the human endothelial glycocalyx and may exert its bioactivity by regenerating the glycocalyx.

We first tested this hypothesis in lab animals and showed rhamnan sulfate reversed the leukocytes adherence to endothelium induced by enzymatic removal of the endothelial glycocalyx (unpublished data).

Others also reported that rhamnan sulfate enhances the endothelial glycocalyx and decreases the LDL permeability of human coronary artery endothelial cells.[29]

A Japanese study demonstrated that daily supplementation of a crude rhamnan sulfate extract for 6 weeks significantly lowered total and LDL cholesterol in borderline or mild hypercholesterolemia human subjects.[30]

Another study also from Japan later showed that oral administration of crude extract of rhamnan sulfate to carbohydrate-loaded rats significantly reduced their blood glucose level compared to the control animals.[31]

CLINICAL STUDY OF ARTEROSIL®

To evaluate Arterosil® for supporting healthy endothelial function, we sponsored a randomized double blinded clinical study at a CRO in California. Twenty healthy human subjects were placed on ArterosilHP®* for 4 weeks. At the beginning and the end of the study, the subjects were challenged with a high fat high sugar meal in the morning and then followed up for 8 hours at the clinics.

The endothelial glycocalyx thickness was estimated by the measurement of sublingual capillary blood flow as described by Nieuwdorp et al.[32] Endothelial function is evaluated by reactive hyperemia index (RHI). At the beginning of the study, the subjects experienced a compromised glycocalyx at 1.5 hours after consumption of the high fat high sugar meal. With 4 weeks of Arterosil® supplementation, the same subjects showed a significantly improved glycocalyx at 1.5 hours after the high fat high sugar meal. In both visits, the glycocalyx recovered back close to normal 8 hours after the meal. Similar trend was also observed with RHI in the subjects (see Figure 2 below). Before Arterosil® supplementation (blue baseline), subjects experienced a dramatic drop of RHI 1.5 hours following the high fat high sugar meal.

After 4 weeks on Arterosil® supplementation (red Arterosil®), the drop of RHI for the same subjects 1.5 hours after the high fat high sugar meal was significantly reduced. Again RHI showed significant recovery after 8 hours in both visits. These results clearly demonstrate that Arterosil® supplementation ameliorates the damage of endothelial glycocalyx and the loss of endothelial function caused by a high fat high sugar meal in the healthy human subjects.

**ArterosilHP is the high potency version of Arterosil, containing twice the active ingredient*

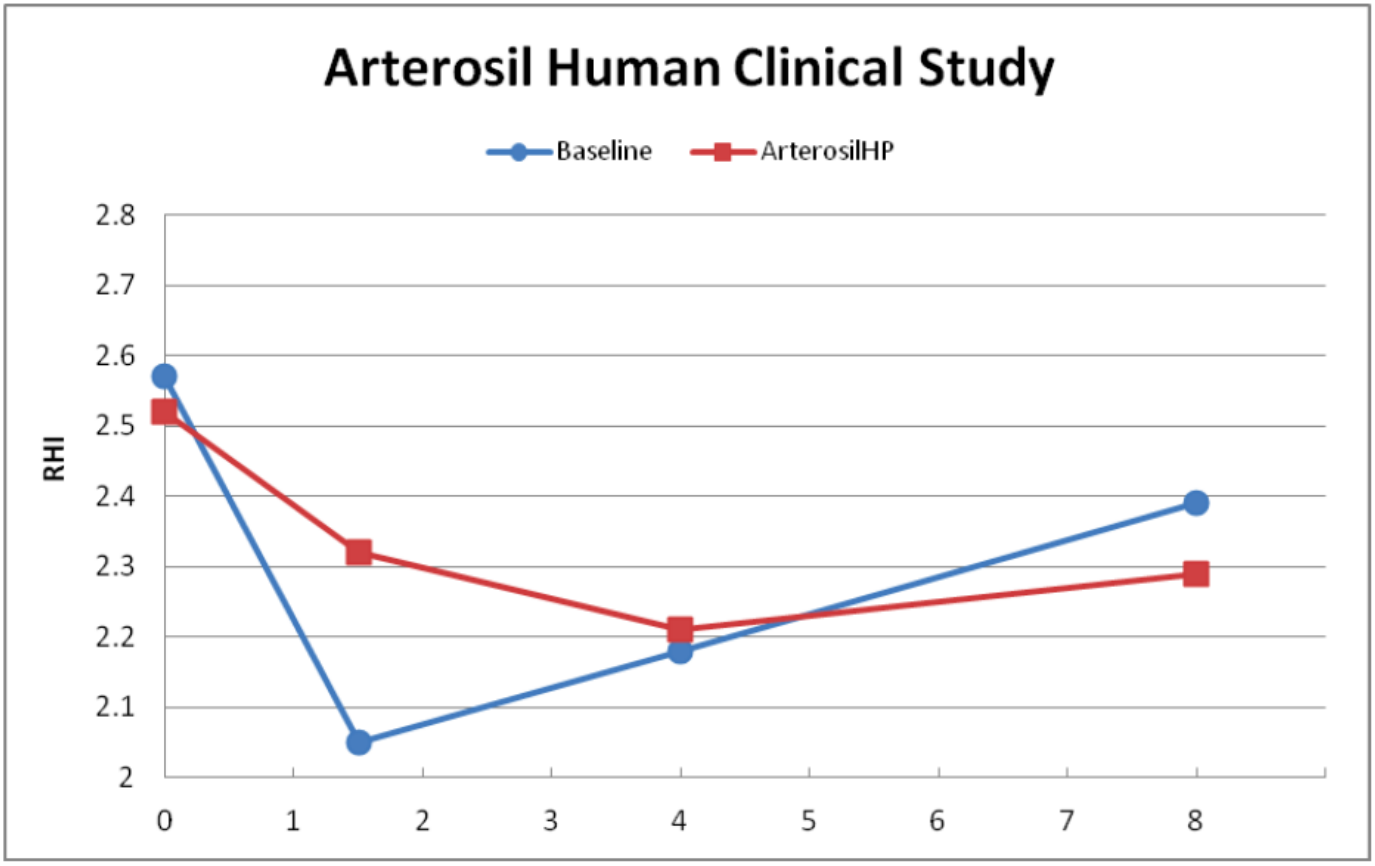

Figure 2. RHI data for subjects within 8 hours after the challenge of a high fat high sugar meal during their clinical visits at the beginning and the end of Arterosil® human study.

SAFETY ASSESSMENT

To assess the safety of Arterosil®, fasting blood samples were taken during both visits and tested for complete metabolic panel (CMP), thyroid stimulating hormone!"TSH), complete blood count (CBC), and partial thromboplastin time (PTT).

No significant changes were observed for any of these tests and all results fell in normal ranges. For example, the normal range for PTT is 17-33 seconds. The mean PTT at the baseline is 25.83 seconds and the mean PTT after Arterosil supplementation is 25.16 seconds with a standard deviation of 3.15 seconds for both. Safety and tolerability were also assessed by reviewing vital signs during the visits and the adverse event reports. There was no serious adverse event reported for the study and Arterosil was well tolerated by all subjects.

SUMMARY

Arterosil is a dietary supplement containing rhamnan sulfate derived from green seaweed monostroma nitidum. It supplies phytonutrients that can be used by the human body as building blocks for its vascular and/or microvascular glycocalyx.

Arterosil has been thoroughly studied and researched. It is the first and only product that has been proven to be safe and effective to help maintain a healthy endothelial glycocalyx and its mediated
endothelial function.

REFERENCES

1. Bernhard F. Becker, Daniel Chappell, Dirk Bruegger, Thorsten Annecke & Matthias Jacob. Therapeutic Strategies Targeting the Endothelial Glycocalyx: Acute Deficits, but Great Potential. Cardiovas cular Research (2010) 87:300–310.

2. C. S. Alphonsus & R. N. Rodseth. The Endothelial Glycocalyx: A Review of the Vascular Barrier. Anaesthesia (2014) 69(7):777-84.

3. Bingmei M. Fu & John M. Tarbell. Mechano-sensing and Transduction by Endothelial Surface Glycocalyx: Composition, Structure, and Function. Wiley Interdiscip Rev Syst Biol Med.(2013) 5(3): 381–390.

4.Hana Kolálová, Barbora Ambruzová, Lenka Švihálková Šindlerová, Anna Klinke & Lukáš Kubala. Modulation of Endothelial Glycocalyx Structure under Inflammatory Conditions. Mediators of Inflammation (2014) Article ID 694312, 1-17.

5. Sietze Reitsma, Dick W. Slaaf, Hans Vink, Marc A. M. J. van Zandvoort & Mirjam G. A. oude Egbrink. The Endothelial Glycocalyx: Composition, Functions, and Visualization. Eur J Physiol (2007) 454:345–359.

6. Bernard F. Becker, Daniel Chappell & Matthias Jacob. Endothelial Glycocalyx and Coronary Vascular Permeability: the Fringe Benefit. Basic Research in Cardiology (2010) 105:687–701.

7. F. E. Curry & R. H. Adamson. Endothelial Glycocalyx: Permeability Barrier and Mechanosensor. Annals of Biomedical Engineering (2012) 40:828–839.

8. A. W. Mulivor & H. H. Lipowsky. Role of Glycocalyx in Leukocyte-endothelial Cell Adhesion. American Journal of Physiology: Heart and Circulatory Physiology (2002) 283(4):H1282–H1291.

9. Eno E. Ebong, Sandra V. Lopez- Quintero, Victor Rizzo, David C. Spray & John M. Tarbell. Shear-induced Endothelial NOS Activation and Remodeling via Heparan Sulfate, Glypican-1, and Syndecan-1. Integr Biol (Camb). (2014) 6(3): 338–347.

10. Wanyi Yen, Bin Cai, Jinlin Yang, Lin Zhang, Min Zeng, John M. Tarbell & Bingmei M. Fu.!Endothelial Surface Glycocalyx Can Regulate Flow- Induced Nitric Oxide Production in Microvessels In Vivo. PLoS One (2015) 10(1):e0117133.

11. Bernard M. van den Berg, Jos A. E. Spaan & Hans Vink. Impaired Glycocalyx Barrier Properties Contribute to Enhanced Intimal LowdensityLipoprotein Accumulation at the Carotid Artery Bifurcation in Mice. Eur J Physiol (2009) 457:1199-1206.

12. Alina A. Constantinescu, Hans Vink, & Jos A. E. Spaan. Endothelial Cell Glycocalyx Modulates Immobilization of Leukocytes at the Endothelial Surface. Arteriosclerosis, Thrombosis, and Vascular Biology (2003) 23(90):1541–1547.

13. Max Nieuwdorp, Timon W. van Haeften, Mirella C.L.G. Gouverneur, Hans L. Mooij, Miriam H.P. van Lieshout, Marcel Levi, Joost C.M. Meijers, Frits Holleman, Joost B.L. Hoekstra, Hans Vink, John J.P. Kastelein & Erik S.G. Stroes. Loss of Endothelial Glycocalyx During Acute Hyperglycemia Coincides With Endothelial Dysfunction and Coagulation Activation In Vivo. Diabetes (2006) 55(2):480-486.

14. Bart J. M. Eskens, Thomas M. Leurgans, Hans Vink & Jurgen W. G. E. VanTeeffelen. Early Impairment of Skeletal Muscle Endothelial Glycocalyx Barrier Properties in Dietinduced Obesity in Mice. Physiol Rep. (2014) 2(1):e00194.

15. Claudia Nussbaum, Ana Cavalcanti Fernandes Heringa, Zuzana Mormanova, Alexandra F. Puchwein-Schwepcke, Susanne Bechtold-Dalla Pozza & Orsolya Genzel-Boroviczény. Early Microvascular Changes with Loss of the Glycocalyx in Children with Type 1 Diabetes. J Pediatr. (2014) 164(3):584-589.

16. Sandra V. Lopez-Quintero, Limary M. Cancel, Alexis Pierides, David Antonetti, David C. Spray & John M. Tarbell. High Glucose Attenuates Shear-Induced Changes in Endothelial Hydraulic Conductivity by Degrading the Glycocalyx. PLoS One. (2013) 8(11):e78954.

17. Andrew H. J. Salmon & Simon C. Satchell. Endothelial Glycocalyx Dysfunction in Disease: Albuminuria and Increased Microvascular Permeability. J Pathol (2012) 226: 562–574.

18. Ties A. Mulders, Max Nieuwdorp, Erik S Stroes, Hans Vink & Sara-Joan Pinto- Sietsma. Non-invasive Assessment of Microvascular Dysfunction in Families with Premature Coronary Artery Disease. Int J Cardiol. (2013) 168(5):5026-5028.

19. Carmen A. Vlahu, Bregtje A. Lemkes,Dirk G. Struijk, Marion G. Koopman, Raymond T. Krediet & Hans Vink. Damage of the Endothelial Glycocalyx in Dialysis Patients. J Am Soc Nephro (2012) l23:1900–1908.

20. Remy J.H. Martens, Hans Vink, Robert J. van Oostenbrugge & Julie Staals. Sublingual Microvascular Glycocalyx Dimensions in Lacunar Stroke Patients. Cerebrovasc Dis (2013) 35:451–454.

21. Elaheh Rahbar, Jessica C Cardenas, Gyulnar Baimukanova, Benjamin Usadi, Roberta Bruhn, Shibani Pati, Sisse R Ostrowski, Pär I Johansson, John B Holcomb & Charles E Wade. Endothelial Glycocalyx Shedding and Vascular Permeability in Severely Injured Trauma Patients. J Transl Med. (2015) 13:117.

22. Angela J. Drake-Holland & Mark I. Noble. The Important New Drug Target in Cardiovascular Medicine – the Vascular Glycocalyx. Cardiovascular & Haematological Disorders-Drug Targets (2009) 9:118-123.

23. Angela J. Drake-Holland & Mark I. Noble. Update on the Important New Drug Target in Cardiovascular Medicine the Vascular Glycocalyx. Cardiovascular & Haematological Disorders-Drug Targets (2012) 12:76-81.

24. Masaakira Maeda, Tutomu Uehara, Naoki Harada, Masayuki Sekiguchi & Atsushi Hiraok. Heparinoids-active Sulphated Polysaccharides from monostroma nitidum and Their Distribution in the Chlorophyta. Phytochemistry (1991) 30(11):3611-3614.

25. Naoki Harada & Masaakira Maeda. Chemical Structure of Antithrombinactive Rhamnan Sulfate from monostrom nitidum. Biosci Biotechnol Biochem. (1998) 62(9):1647-1652.

26. Wen-Jun Mao, Fang Fang, Hong-Yan Li, Xia-Hui Qi, Hai-Hong Sun, Yan Chen & Shou-Dong Guo. Heparinoidactive Two Sulfated Polysaccharides Isolated from Marine Green Algae monstroma nitidum. Carbohydrate Polymers (2008) 74:834-839.

27. Jung-Bum Lee, Seiji Koizumi, Kyoko Hayashi & Toshimitsu Hayashi. Structure of Rhamnan Sulfate from the Green Seaweed monostroma nitidum and Its Anti-herpetic Effect. Carbohydrate Polymers (2010) 81:572-577.

28. Supatra Karnjanapratum & Sanguan You. Molecular Characterieristics of Sulfated Polysaccharides from monostroma nitidum and Their in vitro Anticancer and Immunomodulatory Activities. Int. J. Biol. Macromol. (2010), doi:10.1016.

29. Limary Melissa Cancel & John M Tarbell. Rhamnan Sulfate Enhances the Endothelial Glycocalyx and Decreases the LDL Permeability of Human Coronary Artery Endothelial Cells In Vitro. The FASEB Journal (2013) 27:896.3

30. Masakatsu Nishikawa, Masayuki Mitsui, Koji Umeda, Yoshikuni Kitaoka, Yoshie Takahashi & Shigeo Tanaka. Effect of Sulfated Polysaccharides Extract from Sea Algae (monostroma latissium and monostroma nitidum) on Serum Cholesterol in Subjects with Borderline or Mild Hypercholesterolemia. J. New Rem. & Clin. (2006) 55(11):95-102 (Japanese).

31. Antidiabetic Compounds from Marine Organisms and Their Properties (2012) Page 11. (In Marine Pharmacognosy: Trends and Applications. Edited by Se- Kwon Kim)

32. Max Nieuwdorp, Marijn C. Meuwese, Hans L. Mooij, Can Ince, Lysette N. Broekhuiizen, John J. P. Kastelein, Erik S. G. Stroes & Hans Vink. Measuring Endothelial Glycocalyx Dimensions in Humans: A Potential Novel Tool to Monitor Vascular Vulnerability. J Appl Physiol (2008) 104(3):845-852.