Food Health Claims - Cardiovascular Health

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1 INTRODUCTION

Atlantia Food Clinical Trials Ltd has considerable experience in conducting clinical trials in the area of cardiovascular health, and in this whitepaper reviews some of the newer techniques that have been used in studies for which EFSA has given a positive health claim opinion.

Cardiovascular diseases (CVD) currently account for 17.3 million deaths per year, a number that is expected to grow to >23.6 million by 2030 (Laslett, 2012). Therefore heart health is a key driver of many of today's buying decisions in the supermarket aisle, but consumers are wary of believing all the health and nutrition messages that have been circulating and are becoming more discriminating with their food purchases.

Heart-healthy foods can be either foods with an absence or reduction of ingredients that have a negative correlation to heart health (e.g. saturated fat, cholesterol or sodium), or functional foods that contain ingredients or components that have been clinically shown to positively impact the heart (e.g. plant stanol esters or fibre). This latter category presents an opportunity for innovative food companies that are willing to invest in ingredients that have been clinically proven to assist with heart health.

What defines a food or beverage as being "good for the heart"?

Regulatory agencies across the globe have approved cardiovascular health claims for a number of food products. In the USA, the FDA has approved health claims (those that meet significant scientific agreement (SSA) and those described as qualified) linking consumption of specific foods or food ingredients to reducing one's risk of developing coronary heart disease (CHD). CHD is the most-common and serious form of cardiovascular disease, and includes diseases of the heart and circulatory system, such as heart attack and stroke. As an example, FDA SSA claim (21 CFR 101.75) reads "Diets low in saturated fat, cholesterol and total fat may reduce the risk of heart disease. Heart disease is dependent upon many factors, including diet, a family history of the disease, elevated blood low-density lipoprotein (LDL) cholesterol levels and physical inactivity". There are a number of approved wording variations to this claim, for foods with specific nutrient content requirements as set out in Title 21 of the Code of Federal Regulations (CFR), Part 101, Section 62 for a "low-saturated-fat," "low-cholesterol" and "low-fat" food.

In Europe, the European Food Safety Authority (EFSA) has issued guidance on the scientific (i.e. accepted clinical and surrogate marker (or 'endpoint')) requirements for health claims related to cardiovascular health (EFSA J, 2011, 9 (12): 2474), for claimed effects that are considered to be physiologically beneficial for cardiovascular health. The (long term) clinical endpoints morbidity and mortality are widely accepted, however not all surrogate markers are, as these markers need to meet acceptance

criteria. A prerequisite for acceptance is that health claims should be based on specific and scientifically accepted and validated outcomes and there should be proven efficacy of the intervention using the given biomarker. Efficacy for cardiovascular health claims include favorable modification of blood lipid profile (as indicated by a reduction in fasting blood LDL-cholesterol or triglyceride levels, or an increase in fasting HDL-cholesterol levels), reduction of blood pressure, an improved vascular reactivity (as measured by brachial flow mediated dilatation endothelial function tests), or a decrease in platelet aggregatory potential or 'stickiness'. These latter two areas, endothelial function and platelet function, are less well known, which is unfortunate, as these methods can provide food manufacturers with effective and validated tools to meet the increasingly stringent acceptance criteria for heart health claims.

Hence this paper will overview these two areas and their methodologies, as used in the setting of clinical trials for functional food ingredients.

2 CLINICAL TRIALS METHODOLOGIES

Endothelial Function

In the last few years EFSA has approved a number of food health claims for products which improve endothelial function, such as the following positive opinion on walnuts submitted under article 13.1 by the EU Commission "Walnuts contribute to the improvement of endothelium-dependent vasodilation" (EFSA Journal 2011;9(4):2074) and the positive opinion on a cocoa flavanols dossier submitted by Barry Callebaut Belgium nv under article 13.5 "Cocoa flavanols help maintain endothelium-dependent vasodilation, which contributes to normal blood flow" (EFSA Journal 2012;10(7):2809).

The capacity of the endothelium of arteries to respond to an increase in blood flow by dilating is designated as flow-mediated dilation (FMD), or endothelium dependent flow mediated dilation (ED-FMD). To measure endothelial function, the brachial artery is often used as it is tends not to be prone to atherosclerotic change and can be well visualized by means of ultrasound imaging. Developed in 1992 by Celermayer at al, the brachial flow-mediated dilation test is now the most commonly used noninvasive assessment of vascular endothelial function in humans. In 2002 Corretti et al published international guidelines for the ultrasonic assessment of ED FMD of the brachial artery, in an attempt to standardize the technique, and these recommendations are widely used today.

Endothelium-dependent vasodilation is the widening of blood vessels resulting from a relaxation of the smooth muscle cells within the vessel walls. The endothelium is the thin layer of cells that lines the inner surface of blood vessels for the entire circulatory system, from capillaries to the heart, and which senses and responds to a plethora of internal and external stimuli through complex cell membrane receptors and signal transduction mechanisms. This leads to the synthesis and release of various vasoactive, thromboregulatory and growth factor substances. The endothelium plays many roles, including the regulation of smooth muscle tone, vasoconstriction/vasodilation, control of thrombosis, inhibition of leukocyte and platelet cell adhesion, and promotion of intra-arterial permeability. In addition, there are numerous vasoactive substances released from the endothelium, including endothelial cell growth factors, interleukins, plasminogen inhibitors, and nitric oxide (NO) which is a principal mediator of FMD (Ryan, 2010; Radegran, 1999).

In a brachial FMD test, arterial vasodilation occurs after an acute increase in blood flow (hyperaemia), typically induced by temporarily reducing circulation in the artery by inflating a blood pressure cuff above systolic pressure for a period of 5 minutes. Immediately after cuff release the hyperemia increases shear stress forces on mechanoreceptors of the endothelium, resulting in a cascade of chemical signals, and culminating in relaxation of the smooth muscle and subsequent vasodilation (arterial lumen diameter increase). The arterial lumen diameter is measured by ultrasound imaging. The vasodilation upon flow increase is defined as the difference between the resting lumen diameter prior to cuff occlusion, and the lumen diameter at maximum vasodilation after cuff release. The maximum increase is compared to the pre-cuff occlusion resting diameter and calculated as a percentage lumen change (%FMD).

As the brachial artery has a lumen size between 3 and 6 mm and a typical flow mediated dilation ranges from 5 and 10%, ultrasonographic assessment of brachial artery reactivity is challenging. To perform FMDs our equipment is technically optimized and a trial-specific ultrasound instrument application protocol is used. As the positioning of the ultrasound probe is critical, training and certification of sonographers is key to good image quality and the patient's arm is stabilized in an arm rest which also holds the ultrasound probe device stable. Image acquisition is ECG-triggered (R-wave), and image size is maximized to provide greatest accuracy, using near field vascular ultrasound transducers.

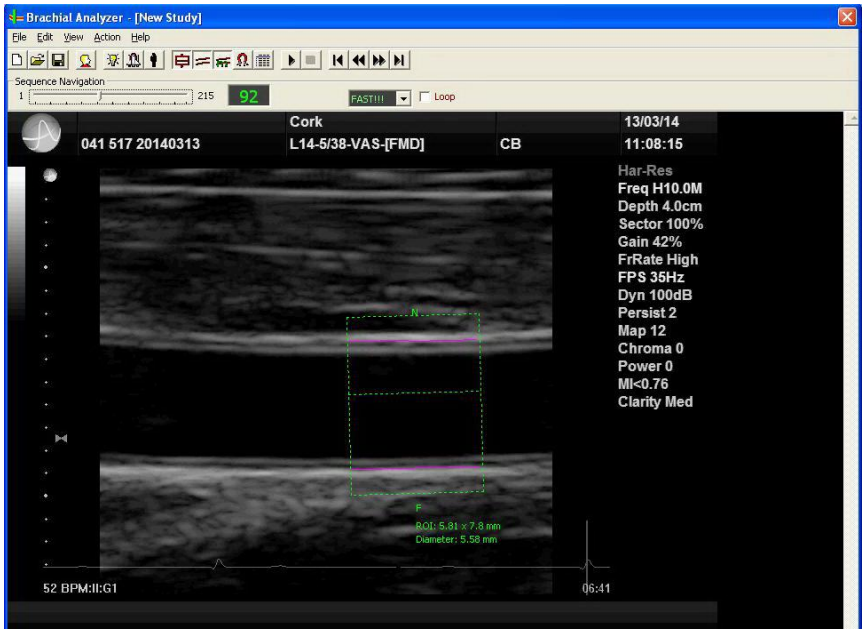

Figure 1: A typical image of the brachial artery, after download into image analysis software ('Brachial Analyzer', Prof. Milan Sonka, MIA-LLC, Iowa USA). The green box indicates the region of interest; the pink lines show the contour detection. The lumen diameter is defined as the average distance between the pink lines.

To ensure reproducible FMD measurements at different time points, we use the following recommendations for our studies. FMD measurements are performed in the morning after an overnight fast of at least 10 hours (water is permitted) as high-fat and high-carbohydrate meals have been shown to attenuate FMD (Padilla, 2006; Vogel, 1997). The fast also reduces the effects of pharmacologically active food ingredients that may affect dilation, such as caffeine (Papamichael, 2005). All subsequent scans for each subject are carried out at the same time in the morning, to minimize circadian variations. The changes in hormones across the menstrual cycle also have an effect on vasodilation (Mendelsohn, 1999) and hence measurements should be carried out at the same point in the menstrual cycle – this

will dictate the duration of the intervention period, which should be a multiple of 28 days. Subjects should refrain from smoking and smoke exposure for 30 minutes prior to measurements, as even second hand smoke has been shown to attenuate FMD measurements (Celermajer, 1993; Kato, 2006). It is recommended that subjects not exercise for 6h before the FMD measurement, as a single bout of exercise can improve FMD (Clarkson, 1999). As the goal of FMD is to compare baseline (or resting) arterial diameter with the peak vasodilation, a true baseline must be achieved. Hence subjects should have a 20 minute pre-scan rest lying in a quiet, darkened room, with the temperature controlled between 20°C and 26°C. Subjects should rest comfortably in the horizontal position (a small pillow is allowed). The subject's arm is comfortably immobilized in the extended position, allowing for ultrasound scanning of the brachial artery 5–10 cm above the antecubital fossa. We use the right arm because for cardiovascular studies usually ambulatory blood pressure is also an endpoint, and if so the blood pressure cuff is attached to the left arm for the 24-48hrs before FMD measurements. A baseline sonographic image, from which to measure arterial lumen diameter, is taken. Then the cuff is inflated to suprasystolic pressure (40 to 50 mmHg above systolic pressure) for 5 minutes, in order to induce temporary vascular occlusion. The cuff should be appropriate for the limb size and should be placed distal to the ultrasound probe. It is recommended that the measurements (i.e. sonographic image capture) are initiated at cuff release and are performed for 3 minutes, to ensure the peak FMD is captured. After the scan, the image is sent by a secure internet connection from the ultrasound instrument to a core image analysis lab where experienced image analysts provide immediate feedback to the sonographers on scan quality.

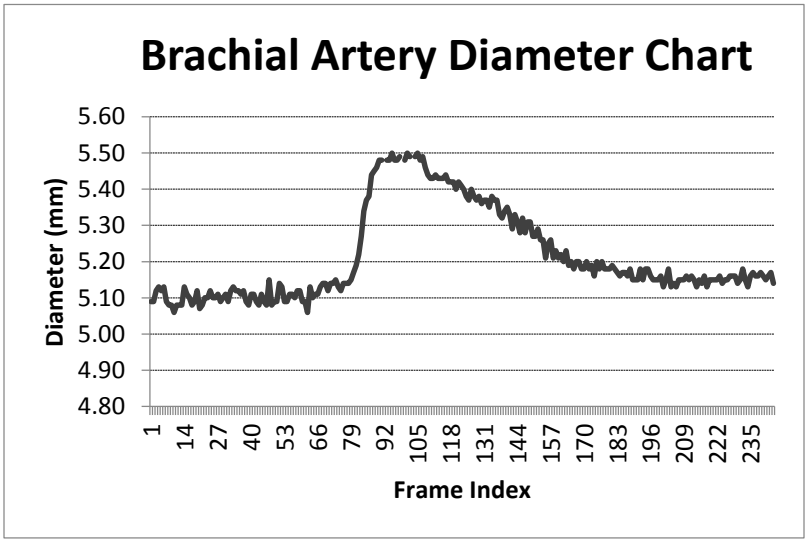

Figure 2: An FMD readout. In the chart, the lumen diameter is depicted on the Y-axis; the number of frames (one R-wave triggered frame/heartbeat) is depicted along the X-axis. In the 5 minute cuff occlusion period, no frames are acquired.

*In this example FMD is calculated as [(5.50 – 5.10)/5.10]*100% = 8%.*

There is now precedence for the use of FMD as an endpoint for cardiovascular studies in Europe. EFSA has published, both in their guidance document (EFSA J, 2011, 9 (12): 2474), and in a positive opinion (EFSA Journal, 2012;10(7):2809) that it considers that a sustained increase of *endothelium-dependent vasodilation* in fasting conditions, measured using well established techniques (e.g. FMD), in response to an intervention (e.g. regular consumption of a food/constituent) is a beneficial physiological effect and that maintenance of normal endothelium-dependent vasodilation is also a beneficial physiological effect. The positive opinion (EFSA Journal 2012;10(7):2809) was an article 13.5 application for cocoa flavanols submitted by Barry Callebut nv. The company sponsored a number of clinical studies to help

prove their cocoa flavanol claim. In their positive opinion, the EFSA panel "took into account that cocoa flavanols consumed for 12 weeks have been shown to increase fasting *ED-FMD* significantly in the target population in one human intervention study".

Platelet aggregation

The first article 13.5 health claim, for newly developed scientific evidence, to be approved by EFSA was for a dossier submitted by Provexis plc, linking water-soluble tomato concentrate consumption and platelet aggregation., The applicants claimed that "decreasing platelet aggregation in subjects with constitutive platelet activation would contribute to "normalise" or "restore" a "normal" platelet function, which may be relevant in the context of delaying atherosclerosis progression and cardiovascular complications. The EFSA panel accepted platelet function measurements as a good indicator of circulatory health, and judged that "maintaining normal platelet aggregation is beneficial to human health" (EFSA J, 2009, 1101, 1-15, EFSA Journal 2010; 8(7):1689).

Cardiovascular disease is a chronic disease influenced by many factors, with activated blood platelets being one of them. The main role of platelets is to contribute to hemostasis, or the controlling of bleeding where the endothelium is damaged, where platelets stick to the damaged endothelium (adhesion) and are then activated to turn on receptors and secrete chemical activators. Platelets then aggregate to form a platelet plug, which is followed by a coagulation cascade, and cessation of bleeding. But platelet activation and aggregation can also play an essential role in the development and progression of CVD by extending unstable or ruptured plaques within blood vessels, contributing to early inflammatory events (Palomo, 2008). Therapy with anti-platelet agents has been shown to significantly decrease the incidence of coronary events linked to CVD, and can result in a 15-30% reduction in the incidence of stroke after a transient ischaemic event (Antiplatelet Trialists' Collaboration, 1994; Antithrombotic Trialists Collaboration 2002; Smith, 2001; Weissman 2002).

Some practitioners suggest that the use of antiplatelet agents, combined with lipid-lowering measures and blood pressure monitoring, to all individuals over 55yrs, could help reduce the risk of number of heart attacks and strokes by up to 80% (Wald, 2003; Law, 2003). But there is general consensus from the limited number of primary studies that side effects from prophylactic drug regimens may outweigh the benefits (Pearson, 2002). Consumption of diets rich in plant-based products can protect against the development of cardiovascular disease. Polyphenols, which are plant metabolites found in a wide range of foodstuffs and beverages, may be partially responsible for these effects. Their protective properties include inhibitory effects on platelet function *in vitro* and *in vivo*.

For platelet aggregation studies, it is important to use well defined exclusion criteria for the selection of participants in order to reduce the confounding factors affecting platelet aggregation, such as low platelet number in whole blood (< 170 x 109/L), abnormal platelet response to agonist (around 10%), haematocrit below 40% for males or below 35% for females, or haemoglobin below 120 g/L for males or below 110 g/L for females. Washout periods of at least 4 weeks should be used after donation of 0.5l of blood or more for transfusion purposes, and at least 6 weeks if consuming fish oils or evening primrose oil. Ideally the volume of active or placebo should not be >250mls, in order to prevent any dilution effects in the blood from the carrier volume.

Platelet aggregation is carried out *ex vivo*, and can be carried out in whole blood or platelet enriched plasma, and is measured in response to an agonist, as shown in figure 3 (ADP, collagen, thrombin or arachidonate) (Ostertag, 2010). Blood is collected with siliconised needles, and extreme care must be taken to restrict the number of venipunctures over the course of a study day in order to minimize activation of the hemostatic system. If more than 3 blood draws are required cannulation should be carried out. It is useful to check samples for activation during venipuncture, and to discard any samples shown to be activated in this way. As the individual response to agonist is very heterogenous, screening visits in whichthe optimal concentration of APD agonist is determined for each subject may be useful, where an optimal concentration is then used for subsequent interventions. Effects on platelet aggregation observed after treatment or control interventions can be expressed as the percentage change in measurement parameters (such as area under the aggregation curve, maximum extent of aggregation or slope of the aggregation curve) after consumption of intervention product or placebo, as compared with baseline values. Another valuable assessment which can be carried out as part of such studies is the time to primary clot formation using the PFA-100 platelet function analyzer, a point-of-care measurement device which provides unified information on the effects of the intervention on the fluidity of the circulating blood. This measurement links platelet function, endothelial function and shear stress, and so can provide valuable information on possible mechanism of action.

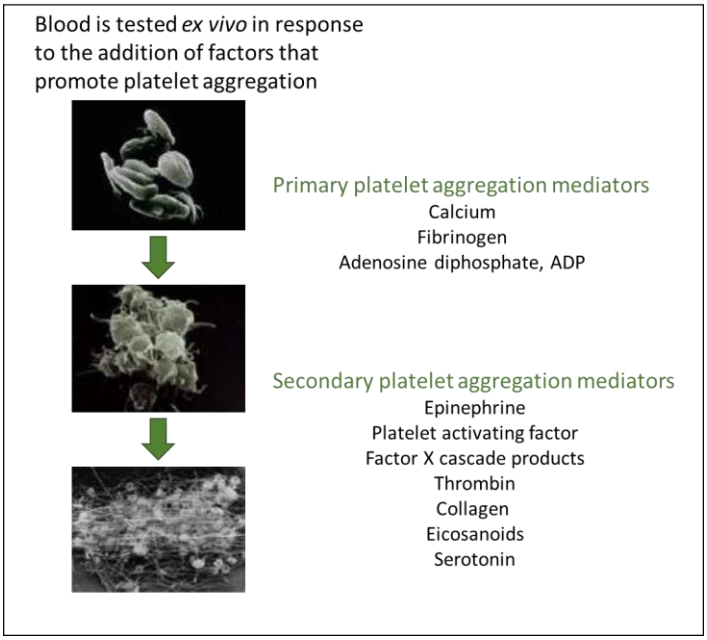

Figure 3: Simplistic overview of the mediators of platelet aggregation

A suppression of platelet function could be beneficial in preventing thrombotic and pro-inflammatory events associated with activated platelets.

Conclusion:

In proving the health benefits of a functional food ingredient, and in order to be successful with an EFSA health claim, it is essential to demonstrate a *beneficial physiological effect*. Two aspects of cardiovascular health which have been accepted by EFSA in the last few years are endothelial function

and platelet function. EFSA has indicated that maintenance of normal endothelium-dependent vasodilation is a beneficial physiological effect. The use of endothelium dependent flow mediated dilation (ED FMD) is a method that has been accepted by EFSA as a biomarker of cardiovascular health. The EFSA panel has also stated that maintaining normal platelet aggregation is beneficial to human health. These biomarkers provide effective and validated tools to clinically demonstrate a beneficial health effect and address the stringent acceptance criteria for heart health claims.

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