Premium ingredient

ViNitrox™

Time to power up!

- Ignite muscle performance
- Break through the fatigue barrier
- Amp up Nitric Oxide level
- Apple & grape polyphenols

nexira HEALTH

Healthy performance enhancer

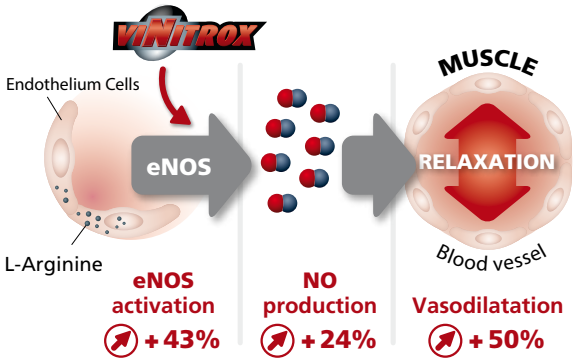
ViNitrox™ is a unique and proprietary synergistic combination of apple and grape polyphenols. It has been specifically developed to answer the demands of athletes in search of natural dietary supplements.

Amp up Nitric Oxide level: Mechanism of action

Vasodilation is linked to the production of **Nitric Oxide** (NO) by an enzyme called eNOs (endothelial Nitric Oxide synthase).

In vitro[1] and *ex vivo*[2] studies show that ViNitrox™ increases:

- **eNOs activation by 43 %**
- **NO production by 24 %**
- **Vasodilation by 50 %**



New clinical study

ViNitrox™ offers a number of exceptional sports nutrition properties including enhanced and lasting performance. Nexira's latest clinical study[3] on 50 athletes, 25-45 years old, demonstrated that under intensive effort 500 mg/day of ViNitrox™ improves physical capabilities.

- **INCREASES PHYSICAL TRAINING TIME BY 10%**
- **DELAYS THE FATIGUE BARRIER BY 13%**

IGNITE MUSCLE PERFORMANCE

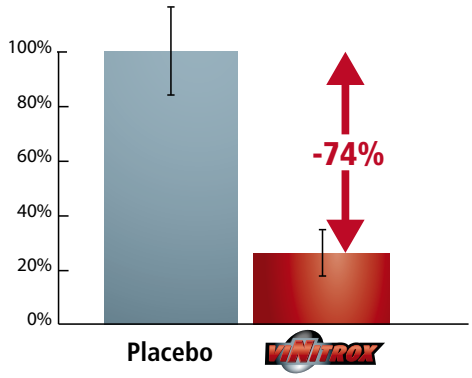
A powerful antioxidant

NO is an unstable molecule. It easily generates harmful free radicals (namely peroxynitrites) which can damage the muscle tissue. With higher vasodilation this is a "side-effect" that must be controlled.

Thanks to its unique polyphenols content ViNitrox™ is also a powerful antioxidant. An *in vivo* study[4] shows protective properties:

- **74% decrease of oxidative stress**
- **Minimum guaranteed ORAC value 6000 µmol TEq/g**

Level of oxidative stress marker in plasma (nitrotyrosin)
In vivo study on hamsters - dosage of 55 mg/kg body weight



- **High content in fruit polyphenols**
- **Safe and free from doping substances**
- **Suggested dose: 500mg/day**
- **ViNitrox™ can be used in tablets, capsules, gels, etc.**

[1] Effect of ViNitrox™ on the activation of eNOs through the phosphorylation of "Serine 1177" from endothelial cells (HUVECs) as measured by flow cytometry. 2010

[2] Products containing grape and apple extracts stimulate the production of nitric oxide (NO) by vascular endothelium. *Ex vivo* study on rat aorta. 2004

[3] Double blind, crossover, placebo controlled study

[4] The effects of ViNitrox™, a formulation containing grape and apple extracts and its effect on the production of peroxinitrites in hamsters subjected to aerobic physical activity. 2004

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NUTRACEUTICAL BUSINESS & TECHNOLOGY
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A Natural Ingredient for Sports Nutrition

27 April 2013 by Kevin Robinson in Articles - No Comments

Nexira Health presents the results of a recent clinical study of ViNitrox

Nexira recently released the results of a recent clinical study with Vinitrox, a healthy performance enhancer. ViNitrox is a unique and proprietary synergistic combination of apple and grape polyphenols. This all-natural ingredient for sports nutrition has been specifically developed to satisfy the demands of athletes in search of natural dietary supplements and healthy performance enhancers. ViNitrox offers a number of sports nutrition properties including enhanced and lasting performance thanks to its powerful antioxidant and vasodilating effects.

Nexira conducted in 2012 a randomized, crossover, double-blind, placebo controlled study. The aim was to evaluate and prove with a high level of evidence, that the intake of ViNitrox will improve athletic performance. The muscle performance in this test is defined by the intensity and the time of exercise. This was a clinical study based on constant high-intensity aerobic exercise over a specified time. To reduce variability, a crossover study design was implemented so that each subject was their own control reference. This clinical study was conducted on 50 athletes aged between 25 to 45 years old, and was composed of three experimental sessions with at least seven day intervals between each session. During the first experimental session, subjects performed a maximal test on an ergocycle to determine their maximal aerobic power.

During the two following testing sessions, subjects realized an endurance test at 70% of the maximal power determined during the first session. Subjects were requested to pedal until exhaustion, that is, until they were unable to maintain the power. Two hours before each test, subjects took a standardized breakfast. Moreover, the preceding evening and one hour before the endurance test, each volunteer absorbed either two capsules with 250 mg ViNitrox or two placebo capsules according to randomization. Subjects that took Vinitrox during phase I took the placebo during phase II and inversely.

The primary endpoint was the time-limit defined by the maximum time that intense effort was maintained on the ergocycle at a power equal to 70% of maximal aerobic power. During this test, maximal and mean heart rate, maximal blood pressure, maximal and mean VO2 and maximal and

mean ventilation were also measured. Every four minutes during the all-out test, the Borg scale was used to determine subjects' perceived exertion.

This clinical study demonstrates that under intensive effort, 500 mg/day of ViNitrox significantly improves physical capacity and ignites muscle performance. The results of the study show a significant increase in the maximum duration of intense effort by subjects who took ViNitrox (+10%; $p < 0.05$) compared to those who took the placebo. The maximal perceived exertion and pain was delayed by 13% ($p < 0.05$) with ViNitrox.

These significant results are all consistent and reflect the increased resistance to effort due to an increase in the aerobic potential of athletes taking ViNitrox. No significant differences were recorded for the maximal and mean heart rate, maximal blood pressure, maximal and mean VO2, maximal and mean ventilation and oxygen saturation between the two groups. These results suggest that the vasodilating effects of ViNitrox might be implicated. We can assume that the vasodilating effect of ViNitrox leads to an increase in muscle perfusion, which results in an increase in oxygen available to muscle cells which in turn allows greater and longer aerobic utilization of glycogen.

The results of this clinical study showed the significant beneficial effects of ViNitrox for athletes looking for performance. On one hand, ViNitrox improves physical capacity by increasing the capacity to maintain an intense effort by 10%. On the other hand, ViNitrox helps to push physical limits by significantly delaying the perception of fatigue by 13%. These results are particularly interesting for athletes who have to maintain high intensity throughout training sessions and competitions. ViNitrox is useful for athletes who must maintain high intensive efforts throughout their competition or even throughout their training. This product concerns endurance sports, team sports, combat sports, etc. Thanks to Vinitrox, athletes are more resistant to intense effort and can stay ahead of their competitors (www.nexira.com).

Nexira polyphenols Sports nutrition ViNitrox

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Centre d'Expertise de la Performance G. Cometti

Newsletter N°7 – January 2013

EDITORIAL

It is essential to objectify physical training. This step involves testing sessions or even daily evaluations to determine a state of fitness. It is important to take certain precautions for classic evaluation sessions so that the recorded data is reliable and reproducible. The evaluation of any physical quality, even the most simple, may be subject to traps that would bias the interest. This new newsletter will therefore seek to provide a non-exhaustive list of key recommendations and will give some examples.

This edition will also present a few of the CEP's findings and some documentation. In particular, these results concern the effect of dietary supplements on performance and more specifically the effect of polyphenols on endurance.

PHYSIOLOGICAL TESTS FOR ATHLETES

During athletes' conditioning, evaluation is a crucial step, in particular to objectify and individualize physical training. Although the principle of evaluation appears simple, there are many pitfalls that need to be avoided, on the one hand to obtain the most accurate values possible and on the other hand, to make correct interpretations. The purpose of this article is not to make a list of tests that can be implemented but above all to give the necessary elements to make correct assessments. We will only discuss the evaluation of physical qualities. The evaluation of technical and tactical parameters, of a psychological profile, of medical aspects etc. will allow you to have the fullest knowledge of the athlete and thus provide relevant content.

WHY?

There are several purposes of evaluations. We can mention three which we consider fundamental. 1. knowing the athletes, their strengths and weaknesses and adapting training. 2. detecting any particular risk of injury by calculating any disequilibrium. 3. establishing a complete profile with objective information to guide physical training.

WHEN?

All the time! The evaluation process must be continuous but in different ways, depending on the parameter considered and the complexity of the evaluation. For example, in the case of strength sessions, after a simple/quick assessment, the load can be adapted. Similarly, daily assessments will determine the fitness of your athletes. This aspect will be the subject of an upcoming newsletter and therefore will not be discussed here.

For more complex parameters, dedicated evaluation sessions are required. The question then is when these sessions should be set. Of course, the answer depends on their number. One answer could be at the beginning, in the middle and towards the end of the season. Due to the time consuming nature of this process, repeated assessments, are all too often reduced to a minimum or even reduced to nothing. It seems important, however, to go through this process and to ensure the means. Indeed, a single assessment during one sports season is not enough to really know an athlete. Conversely, repeating them over time in order to compare them will be of certain use and will avoid misinterpretation. Indeed, the lack of comparative values can easily lead to errors, especially due to the use of reference "values" which do not take into account the context, history, equipment ...

WHAT?

All physical qualities can be evaluated. Depending on the temporal, human, material, and financial means, but also, of course, depending on the sport, age, level and sport policy, a series of tests of varying complexity can be carried out integrating a wide variety of physical qualities. Here are a few: strength, power, speed, vertical elasticity, aerobic fitness, flexibility, balance, coordination ... Each can be measured in simple or complex ways using laboratory equipments. For example, strength can easily be evaluated on standard weight machines, but can also be quantified on an isokinetic ergometer (specific equipment, but expensive). Aerobic fitness can also be determined from a simple test of maximal aerobic speed (fast, group ...) or by using equipment that measures and analyzes gas exchanges (maxVO2). The accuracy is, of course, more precise with complex equipments. However, depending on the level, this equipment provides superfluous information. Note that, regardless of the type of test or parameter evaluated, constraints mean that the athlete is often confronted with situations far from their daily practice. Tests close to the field can then be proposed. However, there is a danger of being on the border between physical quality assessment and the evaluation of multifactor aspects that may involve technical skills.

HOW?

Which test? The question is to know what each test can help to evaluate and to apply the test that most closely matches the sport concerned. To illustrate this, we can rely on aerobic tests with which the maximal aerobic velocity (MAV) can be evaluated. Indeed, in recent years many authors (see Schnitzler et al., 2010) have sought to develop different tests (initial velocity, velocity increments, duration of levels, continuous or intermittent form ...). Without trying to compare and judge these tests, we can simply say that each one will assess a form of MAV and will be used in training that is the closest possible to the test done. Thus, MAV evaluated through intermittent "long" and progressive VAMEVAL type exercise should be weighted toward higher speed if the training is “short” and intermittent.

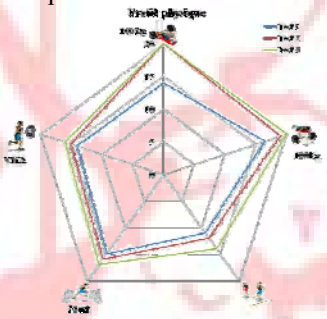
Which measurement procedure? A rigorous protocol, performed under identical conditions, will produce usable and comparable values. To illustrate this point we will use a simple example of vertical jump tests. Even though jumping as high as possible seems simple, rigorously quantifying this is quite different. In fact, most electronic equipment determines the height of a jump by the flight time. A landing with bent legs and flat feet may overestimate the height by several centimeters. Similarly, a Squat Jump type test (jumping from a semi-flexed position) is often poorly performed due to the difficulty of not making downward movements just before the leg extension. Many variables related to execution variables linked to the test must be mastered in order to make the protocol reliable. Although obvious, the environmental aspect also needs be taken into account. For example, aerobic tests can be performed indoors but also outdoors. The quality of the ground and the weather can easily influence the results.

Which equipment? The main aspect to consider is the reliability of the equipment used (quality of measures, reproducibility ...). Again using the example of the vertical jump, there is a lot of equipment using different technologies. Some is reliable since it has been validated by independent scientific studies while some has never really shown its reliability. Whatever the equipment you choose, you must ensure that you always use the same in order to be able to make comparisons. Indeed, substantial differences can be obtained mainly because of the type of calculation.

Which variable? Each piece of equipment uses more or less complex algorithms to analyze the values obtained during a particular test. Multiplying calculations induces errors which will gradually accumulate and make the test less reliable. By keeping the gross values, you can avoid such errors. In addition, for the same equipment, different calculation methods for the same test and for similar settings may show inconsistencies and lead to different conclusions (e.g. increased jump height together with a decrease in the power mechanics of the lower limb). Finally, which variable is actually given by the equipment used? For example, to evaluate muscle strength on weight machines, some equipment gives average power values, whereas the peak power seems more relevant.

WHICH INTERPRETATION?

Interpreting the evaluations is the most complex stage of this process. Correctly presenting (see representation of radar) and interpreting assessments in order to focus or refocus training is often more complex. Interpreting a test is not, in itself, too complicated, but looking at all the tests together is quite different. Therefore, it’s necessary to know well what a physical test represents and what it’s used to evaluate. We can give a simple example with the Counter Movement Jump (CMJ). This test is often considered to involve the elastic properties of muscle whilst height gain would be attributed to a prolonged muscle active state (see, Bobbert et al., 1996). In the case of repeated assessments, interpretation is easier since values can be compared.



Radar representation of different testing sessions (velocity, strength, vertical jump and MAV tests).

Indicators can be calculated from various tests or evaluations. For example, using isokinetic ergometers, a ratio between the strength of the quadriceps and hamstrings is determined (on this type of device, one does not speak of strength, but torque). There are conventional and functional ratios. Standard values are well known (cf. Crosier et al., 2008) to diagnose a significant imbalance between these muscles. However, depending on the sport practiced, this ratio should be higher or lower. So rather than generalizing, you should adapt to your public. This aspect also raises the problem of “threshold" values, through which it’s possible to detect an imbalance between the right and left. Due to constraints related to the sport practiced, we cannot state whether an imbalance of 10% or 20% is a significant value...

TAKE HOME MESSAGES

- **Evaluation consists of going through a regular and complex process which makes it possible to objectify physical training.**
- **Adapt test(s) to target objectives. Know the meaning of each proposed test.**
- **Many errors related to the test protocol, but also to the equipment can make any evaluation process useless.**
- **Replace the values in context (sports, history, technical skills, trauma...) to make consistent interpretations.**

REFERENCES

Bobbert MF et coll (1996) Med Sci Sports Exerc. 28:1402-12
Croisier JL et coll (2008) Am J Sports Med 36:1469-75
Schnitzler C et coll (2010) J Strength Cond Res 24:2026-31

AT THE CENTRE D'EXPERTISE DE LA PERFORMANCE

Effect of a single dose of polyphenols (Vinitrox®) on endurance and recovery in healthy subjects

N Babault, G Deley, FA Allaert

Introduction.
The aim of this work was to study the effect of acute intake of polyphenols (grape and apple extracts; Vinitrox®) on physical performance during an endurance test with time to exhaustion performed on a cycle ergometer.

Methods.
48 physically active subjects (31 ± 6 years) participated in two experimental sessions, each corresponding to a test with time to exhaustion on a cycle ergometer at 70% of the maximal aerobic power. The night before and 2 hours before each endurance test, the subjects randomly took either a placebo or polyphenols (Vinitrox®: grape and apple extracts).
Time to exhaustion as well as some physiological parameters such as gas exchanges (K4B2), blood pressure, heart rate... were quantified. The rate of perceived exertion (RPE) was determined using the Borg scale.

Results.
Compared with the placebo, the study shows that the time to exhaustion was significantly improved with an acute intake of Vinitrox ® (+9.7 ± 6.0%). The maximum RPE was felt 2.7 minutes later (+12.8 ± 6.8%). Finally, the time of half recovery of VO2 was extended by 8.5 ± 11.4 seconds.

Discussion.
Acute intake of Vinitrox® improves endurance in an all-out test performed on a cycle ergometer and delays the onset of fatigue (evidenced by the RPE). Vinitrox® is therefore useful for athletes who must maintain high intensive efforts throughout their competition or even throughout their training. The product concerns endurance sports, team sports, combat sports, etc. For more information, please refer to the longer version of this study at the end of this newsletter.

NB. This product is certified not to contain any doping substance.

IN THE SCIENTIFIC LITERATURE

Acute effects of static stretching on peak torque and the hamstrings-to-quadriceps conventional and functional ratios

Costa PB et coll. Scand J Med Sci Sports 2013; 23:38-45

Aim.
One of the goals of stretching whilst warming-up is to prevent the occurrence of injuries. However, little evidence exists on this aspect. The aim of this study is to examine the effects of static quadriceps (Q) and hamstring (HS) stretching on the hamstring-quadriceps ratio (this ratio is commonly used as a sign of potential HS injury).

Methods.
Subjects: 22 physically active women.
Stretching: 3 different experimental conditions. Static stretching (3 assisted and one unassisted) of only Q, only HS, or Q+HS simultaneously. Each exercise lasted 4 × 30" with 15" recovery intervals. The total duration of stretching was 18 to 36 minutes per session respectively where only one muscle group was stretched, or both were stretched simultaneously.
Main outcome. Isokinetic tests on Q and HS: concentric and eccentric (60 and 180 °.s^{-1}). Calculation of functional ratio (eccentric of HS divided by concentric of Q) and conventional ratio (concentric of HS divided by concentric of Q)

Results.
The results show that the conventional ratio decreases for the condition with only HS stretched. The functional ratio significantly decreases for the condition only Q stretched and for Q + HS stretched (Figure 1).

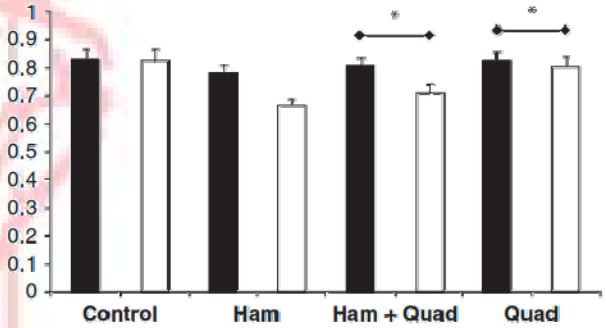


Fig. 1: Evolution of the functional ratio HS-Q before and after static stretching of HS (Ham), Q (Quad) and HS + Q (Ham + Quad). * Significant difference between values obtained before and after static stretching (regardless of the angular velocity).

Conclusion.
The results of this study clearly show that doing static stretching before exercise results in a significant decrease in HS-Q ratios. This greater imbalance between agonist and antagonist thigh muscles increases the risk of injury, in particular during activities requiring sprints.

On the field: Testing examples

Vertical jump tests on volleyball players

Tests.
Squat Jump (SJ), Counter Movement Jump (CMJ) with and without arms, Drop Jump from a fixed height of 30 cm (DJ), specific volleyball tests (block, smash).

Equipment.
1. Optojump Next to measure the flight time, contact time and hence the jump height and power. Combined use with 2 cameras to quantify knee joint angle (sagittal and frontal planes).
2. Vertec to measure the height reached during specific volleyball tests.

Recommendations.
For standard tests, thoroughly check:
1. Body position and above all knee angles,
2. Position during landing (knee angle, feet position ...)
3. Quality of execution (in particular speed, absence of counter movement during SJ…)
4. …

Values.

Test	Max Value
SJ	46.8 cm
CMJ (arm)	61.8 cm
Block	3.25 m
Smash	3.45 m

Example of values obtained with top volleyball players (A League). For specific tests, values correspond to heights attained.

.

Index and interpretations.
It's possible to guide training by using the difference between the different jumps to highlight a "deficit" of explosive strength, strength, coordination or use of muscle elasticity. A direct return on the physical organization (orientation of the trunk, knees ...) can be obtained by using the cameras; this can help to improve the quality of execution and also determine attitudes that can be signs of potential injury (diagnosis of a risk to the cruciate ligaments).

In addition, in order to refine the findings and provide more objective guidance for training, complementary measures are needed to quantify the strength or muscular power on the different movements and joints (press, ½ squat, calves). For this, you should use an isokinetic ergometer or power wire sensors.

Aerobic capacity evaluation

Tests.
A triangular test (with a progressive increase in intensity) can be done on the ground or on different ergometers (depending on the sport specialty).

Equipment.
1. Cones or ergometer (bike, treadmill, rower …)
2. K4b2 to continuously measure and record the gas exchange, via a mask.
3. Cardiofrequencemeter to measure changes in the heart rate during the test and during recovery.
4. Blood pressure, pulse oximeter... to obtain more precise information concerning the body's adaptation to exercise.

Recommendations.
1. Check values at the start of the test (rest values)
2. Choose the test based on the specificity of each discipline (muscle mass involved)
3. Individualize the protocol: a continuous test must last between 8 to 15 minutes
4. Check out the test conditions (REPRODUCIBILITY: climatic conditions …)
5. …

Values.

Test	VO2max (mL/min/kg)	maxHR (batt/min)
45/15 treadmill	53.9	199
Rowing	52.7	186
Boxing	52.7	193

Example of values obtained by a top-level boxer, during 3 different maximal tests.

Index and interpretations.
These values listed above show observable differences depending on the test procedure used. Note that for non-specialists, the rowing test is often limited by the technique, which prevents the athlete from reaching their maximum capacity and underlines the importance of the choice of the protocol. VO2max is the best known and most frequently taken into account in this type of evaluation: it tells us about the athlete's "power capacity". However, this is not the only parameter to be considered. Indeed, ventilatory threshold, performance, respiratory efficiency… can be determined during this test, thereby giving other valuable information to the coach.

ADVERT BREAK…

INFORMATION

November 2012: a complete testing session for professional volleyball players CVB52 (Chaumont – Ligue A). Some tests: speed, vertical jump, power and isokinetic tests.

December 2012: a complete testing session for high-level Olympic valid and Paralympics table tennis players.

October and November 2012: CEP members talk during conferences (sport and nutrition, physical activities for old people…).

The CEP is a partner of the association 'Dijon Performance Sport et Santé' for sport knowledge promotion…

EVENTS

18 – 19 January 2013: Strength development seminar during the 'Diplôme Universitaire de Préparation Physique *Gilles Cometti'*.

15 – 16 march 2013: Pliometrics seminar during the 'Diplôme Universitaire de Préparation Physique *Gilles Cometti'*.

12 and 13 april 2013: 3rd conference Gilles Cometti – *Athletes conditioning: from lab to field.* Some conferences from: R. Enoka (USA), J. Duchateau (Belgium)… More information online: www.cepcometti.com

THE CEP NEWSLETTER

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Publication : Nicolas Babault, Gaëlle Deley, Christos Païzis

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web

The effects of a single intake of Vinitrox® on exercise endurance and recovery in healthy subjects: a controlled, randomized, cross-over, double-blind study versus placebo

Nicolas Babault, Gaëlle Deley

Centre d'Expertise de la Performance Gilles Cometti, Dijon, France

Introduction

Exercising involves adaptations of all the systems of the body (pulmonary, cardiac, muscular and vascular) in order to ensure optimal oxygen furniture and metabolites removal. This requires an increase in peripheral blood flow and a vasodilation. Among the various mediator of this vasodilation, nitric oxide (NO) is one of the most important (Bescos et al. 2012). Indeed, it is now well known that NO plays an important role in many functions in the body regulating vasodilation, blood flow, mitochondrial respiration and platelet function (Shen et al. 1995). Moreover, NO beneficial effects have been demonstrated on muscle strength and endurance (Folland et al. 2000) but also during recovery following an effort (Bloomer 2010). For all these reasons, NO has extensively been studied and it has been shown that synthesis and bioavailability of NO were influenced by arginine, Nitric Oxide Synthase (NOS) and superoxide anion (Drexler 1999). Supplements such as polyphenols are, therefore, thought to be an interesting ergogenic aid (Petroczi and Naughton 2010). Polyphenols, abundant in human diet, have protective effects on the cardiovascular system (Erdman et al. 2008) but also activate endothelial Nitric Oxide Synthase (eNOS) which will increase synthesis and bioavaibility of NO (Engler et al. 2004). For example, previous works (Muller et al. 2009, Nexira 2008a) showed that Vinitrox®, composed of grape and apple extracts, activate NOSe and increases NO production by 24% (Nexira 2008b).

To date, most of the studies regarding the effects of polyphenols investigated several weeks supplementation and vascular or blood parameters (blood pressure, NO concentration, oxidative stress markers) but only few of them investigated the effects on immediate performance and recovery capacity (Morillas-Ruiz et al. 2005, Lafay et al. 2009). The present work therefore aimed to study the effects of an acute intake of polyphenol (grape and apple extracts; Vinitrox®) on physical performances. More specifically, subjects, in the present study, had to perform a high intensity all-out exercise until exhaustion revealing their capacity to maintain a constant strong effort hereafter named endurance (notably illustrated by the time to exhaustion).

Methods

48 men (31.0 ± 6.0 years), regularly involved in a physical activity (3.9 ± 1.0 hour per week) were included in this study. It was composed of three experimental sessions interspersed with at least seven days. All were informed about the experimental procedure and signed a written consent form. The protocol and nutritional components used during this study were validated by the local committee of human research and AFSSAPS. Subjects were requested to refrain from any alcohol consumption and exhaustive exercise at least 24 hours before each experimental session.

Experimental setup. During the first experimental session, subjects performed a maximal test on an ergocycle to determine their maximal aerobic power. The ergocycle setup (handlebars and saddle height and distance) was noticed and reused during the two other test sessions. During this test, pedaling rate was imposed at 80 revolutions per minute (rpm).

During the two other testing sessions (respectively noted phase I and II), subjects realized an endurance all-out test at 70% of the maximal power determined during the first session. Subjects were requested to pedal until exhaustion, i.e., until they were unable to maintain the power with the requested pedaling rate. During phase I, the pedaling rate was free but within a 80-95 rpm range. During phase II, phase I pedaling rate was rigorously reproduced. Two hours before each test, subjects took a standardized breakfast (80g whole-wheat bread, 20g butter, 20g marmalade and 25cL orange juice). Moreover, the preceding evening and one hour before the endurance test, volunteers had to absorb either two capsules with 250mg Vinitrox® each or two placebo capsules according to randomization: subjects that had Vinitrox® during the phase I took the placebo during the phase II and inversely. Both the subjects and the experimenters were blinded from the randomization.

Data analyses. The main parameter tested was the time to exhaustion measured during the endurance test. During this test, were also evaluated the maximal and mean heart rate, maximal blood pressure, maximal and mean VO_2 and maximal and mean ventilation.

Blood pressure, heart rate, oxygen saturation, VO_2 and ventilation were measured at exercise stop and during the recovery at 2 min, 3 min and 5 min. Half-recuperation time for VO_2 and heart rate (i.e., the time necessary to obtain half the value measured at exercise end) were also calculated. Every four minutes during the all-out test, Borg scale was used to determine subjects' perceived exertion. Muscle pain was finally evaluated 48 hours after each experimental session using a numerical 7-points scale.

Statistical analysis. All parameters were analyzed using the cross-over method with treatment effect, time effect and period effect. Mean values ± standard deviation are presented. $P < 0.05$ was taken as significative level for all condition.

Results

Time to exhaustion. In comparison with the placebo, the present study revealed a significant 2.5 min increase of the maximal duration of the endurance all-out test with Vinitrox® ($P < 0.05$) corresponding to a 9.7 ± 6.0 % increase.

Parameters registered during cycling. No significant differences were obtained for maximal and mean heart rate, maximal blood pressure, maximal and mean VO_2, maximal and mean ventilation and oxygen saturation when comparing Vinitrox® with placebo. In contradiction, the maximal perceived exertion was reached 2.7 min later (+12.8 % ± 6.8, $P < 0.05$) with Vinitrox® than with placebo

Recovery. At the end of the exercise, a 8.5 ± 11.4 seconds VO_2 half-recuperation time lengthening was observed with Vinitrox® (+16.2 % ± 7.5%, $P < 0.05$). No other differences were noticed between Vinitrox® and placebo for the parameters registered during the recovery period after the end of the test.

Muscle pain perception, evaluated 48 h after each experimental session, was not different between conditions.

Discussion/interpretation

The main results of the present study are the increased time to exhaustion during the endurance test, the delayed maximal effort perceived exertion, the longer VO_2 half-recuperation time and the absence of any difference between the two groups regarding muscle pain after exercise and the other physiological parameters. The delayed maximal effort perceived exertion observed here could

be related to a better exercise tolerance. Associated with the significant increase of the exercise duration, this result is particularly interesting for athletes.

Firstly, for a given high intensity, the acute intake of Vinitrox® allows to perform longer efforts both during training and competition with a similar amount of fatigue. This result could be interesting for athletes that have to maintain high intensity efforts throughout the exercise or competition. For example, and despite a different type of exercise than tested here, we can suppose that Vinitrox® intake would be beneficial for team sports players to be more efficient until the end of the game (and even during overtime). This could be useful as well for tennis, squash players or high intensive sports lasting half an hour or more. Another similar application could be for long distance and ultra-endurance athletes (biking, running, triathlon...). For them, Vinitrox® would allow to keep a (high) starting speed longer before significant fatigue appearance. Moreover, although there is no direct relation, it can be hypothesized that athletes being able to perform longer exercises at a given intensity might be able to be more efficient at slightly higher intensities. This last aspect needs to be taken with caution since it also depends on appropriate training sessions and athletes' characteristics.

Secondly, the absence of any muscle pain in spite of the longer exercise duration might allow athletes to repeat efforts without any additional muscle discomfort. This issue is important for activities repeating efforts with a short delay for recovery but, more generally speaking, all sport requiring endurance qualities. Stage races or tournament-type sports are examples (e.g., tennis or team-sports where athletes have to repeat high-intensity activities several time a day/week).

Thirdly, on the basis of these results, we can suggest that taking Vinitrox® chronically might allow multiplying high-intensity training more easily. At long term, this would induce greater performance gains (in comparison with athletes not taking Vinitrox®). This hypothesis concerns every sport.

The measured parameters did not allow us to distinguish precisely the origins of the observed gains but it seems that the effect of grape extracts on NO production and of apple extracts on oxidative stress reduction and vasodilation (favouring blood gas exchanges) might be involved. On a physiological point of view, several mechanisms could account for the effects of Vinitrox® on endurance, and this, through its action on NO production (attributable to the stimulation of NO production by NOSe). The first mechanism by which NO might act, is the muscle perfusion increase thanks to a direct vasodilation on vessels smooth muscle cells and to an inhibition of the adrenergic vasoconstriction (Maxwell et al. 1998, Vassilakopoulos et al. 2003). This hyperemia results in an increased oxygen availability to muscle cells allowing a greater and longer aerobic utilisation of glycogen.

Moreover, the present results showed a longer VO_2 half-recuperation time which can be considered surprising or even disappointing (if our aim is to speed recovery). However, this greater oxygen debt (the quantity of O_2 in excess during the recovery period) can be explained by the increased exercise duration in the Vinitrox® group. Indeed, it has been extensively shown in the literature that the O_2 debt duration was directly associated with the exercise duration (Chad and Wenger 1988). Although this is not the first aim of the targeted athletes' population, this result is particularly interesting for people wanting to lose weight since a longer VO_2 recovery induces an increased energy cost following exercise. Lipids are therefore used in priority during this recovery period in order to regenerate energetic stocks (Borsheim and Bahr, 2003).

Conclusion and practical applications

As a conclusion, the results of the present study showed significant beneficial effects with Vinitrox® for athletes looking for performance:

① Vinitrox® enhances sport capacities thanks to the beneficial effect on endurance (i.e., capacity to maintain an intense effort) for almost all sports.

② For our exercise (pedaling), endurance improvement (time to exhaustion and maximal perceived exertion delay) was not associated with additional muscle pain.

③ The longer half-recuperation time (associated to the exercise duration) could also be beneficial to increase energy expenses and so for weight loss protocols.

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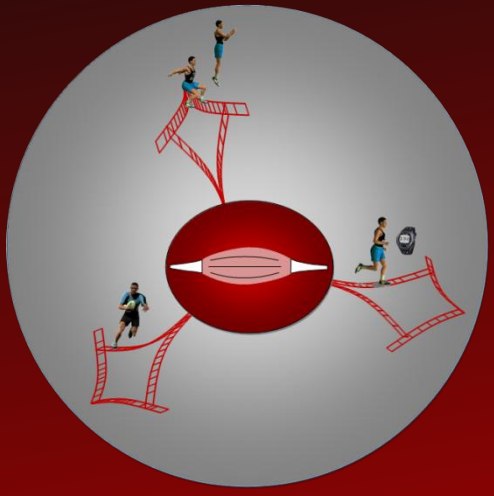
Centre d'Expertise de la Performance

Gilles Cometti

3ème journée Gilles Cometti

LA PREPARATION PHYSIQUE:

du laboratoire au terrain



12 et 13 avril 2013

à la Faculté des Sciences du Sport de Dijon

conference@cepcometti.com

3ème journée "Gilles Cometti"
LA PREPARATION PHYSIQUE : du laboratoire au terrain

12 et 13 Avril 2013 à la Faculté des Sciences du Sport de Dijon

PRESENTATION DU COLLOQUE.

Pour cette troisième édition, nous nous attacherons à mettre en évidence le lien fort qui existe entre connaissance scientifique et préparation physique. Nous chercherons à mieux comprendre les principes physiologiques se cachant derrière la fatigue, les méthodes d'entraînement et la récupération, paramètres essentiels de la performance. Pour cela, les données scientifiques les plus récentes seront présentées. Le lien entre ces données et leur application sur le terrain sera illustré au travers d'exemples concrets.

ORGANISATION DU COLLOQUE

Lieu.

UFR STAPS de Dijon - Campus Montmuzard de l'Université de Bourgogne (uB).

Organisation des conférences.

Huit sessions plénières seront réparties sur la durée du colloque. Ces sessions seront animées par les conférenciers suivants :

J. DUCHATEAU – *« Entraînement de la force explosive »*
Jacques Duchateau est Professeur à l'Université Libre de Bruxelles. Ses travaux, menés depuis des années, font de lui un spécialiste de l'étude des mécanismes d'adaptation neuromusculaire à l'activité physique. Plus particulièrement, ses recherches s'intéressent à l'entraînement, au vieillissement, à la fatigue et au déconditionnement/reconditionnement.

R. ENOKA – *« Fatigue and performance »* ☞
« Fatigue et performance »
Roger Enoka nous vient de l'Université de Boulder (Colorado) aux Etats-Unis où il enseigne la neurophysiologie. Il est également le directeur du laboratoire de neurophysiologie du mouvement de l'Université du Colorado. Soucieux de diffuser ses connaissances et travaux de recherche, portant essentiellement sur la fatigue, il est l'auteur de nombreuses présentations en congrès et de nombreux articles scientifiques. Le plus connu d'entre eux étant, bien entendu, « Neurobiology of muscle fatigue » (1992).

F. GRAPPE – *« Gestion de la récupération dans les activités d'endurance : l'exemple du Tour de France »*
Frédéric Grappe est Maître de Conférences à l'Université de Besançon et ancien Conseiller Scientifique de la Fédération Française de Cyclisme (de 1998 à 2008). En sa qualité de Conseiller technique - Entraîneur de l'équipe cycliste Professionnelle "La Française Des Jeux" mais également de professeur en physiologie de l'entraînement, Frédéric nous apportera un éclairage à la fois théorique et pratique sur la gestion de la récupération lors de courses à étapes, telles que le Tour de France.

C.Y. GUEZENNEC – *« Gestion de la récupération en sport collectif : l'exemple d'un tournoi de rugby »*
Professeur agrégé, physiologiste et nutritionniste, Charles-Yannick est, aujourd'hui, conseiller pour le développement du centre d'entraînement en altitude de *Font Romeu*. Avant cela, il a été médecin du Centre National de Rugby (Marcoussis) pendant plusieurs années et s'est toujours intéressé de près à la thématique de la récupération. Il nous fera ainsi part de son expérience concernant la gestion de cette phase importante lors d'un tournoi où les matchs s'enchaînent avec des délais de récupération parfois très courts.

V. MARTIN – *« La récupération : aspects théoriques et pratiques »*
Après une formation à l'UFR STAPS de Dijon et de nombreux stages en France et à l'étranger, Vincent Martin a rejoint l'UFR STAPS de Clermont-Ferrand où il enseigne la physiologie de l'exercice. Passionné de sport, Vincent s'est toujours intéressé à la fatigue, que ce soit lors d'exercices brefs ou de très longue durée et à l'optimisation de la récupération.

G. MILLET – ***« L'entraînement en altitude pour les sports collectifs »***
Grégoire Millet est professeur d'université et directeur-adjoint de l'Institut des Sciences du Sport de l'Université de Lausanne, spécialiste de l'entraînement intermittent intense en altitude et de la comparaison entre hypoxie normobarique et hypobarique. Il a également été entraîneur et conseiller de niveau national et international (triathlon, VTT, football (coupe du Monde 2010). Il est également conseiller scientifique pour plusieurs athlètes internationaux.

A. MINETTI – *« Biological and technological movements: a comparison between muscles and engines »* 🏳
"Les mouvements biologiques et technologiques: une comparaison entre muscles et moteurs"
Alberto Minetti est professeur de physiologie à la Faculté de Médecine de Milan (Italie). Ses travaux concernent la biomécanique et les biomathématiques, ce qui a donné lieu à de nombreuses publications dans de grandes revues internationales. Depuis quelques années, il s'intéresse tout particulièrement à l'étude de la relation entre les paramètres énergétiques et mécaniques lors de la locomotion. Il nous présentera donc ici une approche intéressante de la physiologie musculaire et de la fatigue, en assimilant le muscle à un moteur.

S. RATEL – *« La préparation physique chez l'enfant »*
Sébastien Ratel, Maître de Conférences à l'Université de Clermont-Ferrand, est sans doute l'expert le plus au point en terme de physiologie de l'effort chez l'enfant en France et reconnu dans le monde pour la qualité de ses travaux. Son intervention s'intéressera aujourd'hui à la préparation physique chez l'enfant.

🏳 Intervention en anglais. Pour le confort de tous, une traduction simultanée de ces présentations sera proposée.

Attention, les titres des interventions ne sont pas définitifs et sont donc susceptibles d'être légèrement modifiés.

PROGRAMME du VENDREDI 12 AVRIL 2013

14h00 – 14h15 : Introduction du colloque

14h15 - 15h45 : Conférences plénières sur le thème de l'Entraînement

« Entraînement en altitude et sports collectifs » – ***G. Millet***

« Entraînement de la force explosive » - ***J. Duchateau***

« La préparation physique chez l'enfant » - ***S. Ratel***

15h45 – 16h15 : Table ronde

16h15 – 17h : Pause café avec visite des stands/présentation des partenaires

17h – 18h30 : Communications orales *(cf Appel à communications)*

PROGRAMME du SAMEDI 13 AVRIL 2013

9h00 – 10h00 : Conférences plénières sur le thème de la Fatigue *

« Fatigue et performance» – ***R. Enoka***

« Biological and technological movements: a comparison between muscles and engines » - ***A. Minetti***

* Traduction simultanée « français/anglais », « anglais/français » à disposition

10h – 10h30 : Table ronde

10h30 – 11h : Pause café et 1ère session poster *(cf Appel à communications)*

11h – 12h00 : Communications orales

Pause repas et visite du CEP

14h00 – 15h30 : Conférences plénières sur le thème de la Récupération

« La récupération : aspects théoriques et pratiques » - ***V. Martin***

« Récupération lors d'un tournoi de rugby » – ***C.Y. Guézennec***

« Récupération lors d'une course à étape : l'exemple du Tour de France » - ***F. Grappe***

15h30 – 16h : Table ronde

16h – 16h45 : Pause café avec Stands et 2ème session Posters

16h45 – 18h00 : Communications orales

APPEL A COMMUNICATIONS

Des sessions de présentation de communications orales (10min de présentation + 5min de questions) et affichées (posters A0 + 2min de présentation) sont prévues avec comme objectif principal de faire un état des lieux des connaissances issues du laboratoire et/ou du terrain.

Les propositions de communications scientifiques ou professionnelles doivent s'inscrire dans l'un de ces trois thèmes :

* Entraînement
* Fatigue
* Récupération

Les propositions de communication, une page maximum, devront nous être **envoyées au plus tard le 18 janvier 2013** par courrier électronique à : conference@cepcometti.com.

Elles devront respecter la mise en forme du colloque pour publication dans les actes du colloque après acceptation par le comité scientifique. *(Voir exemple sur notre site)*

TARIFS ET INSCRIPTIONS

- Etudiants (sur justificatif) : 60 €
- Autres : 120 €
- Groupe étudiants (à partir de 6) 40 €

Ces tarifs incluent pour chaque participant :

- Conférences et sessions posters
- Pochette et actes du congrès
- Pauses café
- Repas du samedi midi

Dans un souci d'organisation (repas du midi), les inscriptions au colloque devront se faire avant le **vendredi 15 mars 2013**. Pour cela, **renvoyez-nous le bulletin d'inscription dûment complété, accompagné d'un chèque libellé à l'ordre du régisseur de l'UFR STAPS de Dijon** à l'adresse suivante (virement bancaire possible) :

Centre d'Expertise de la Performance
Journée Gilles Cometti
Faculté des Sciences du Sport,
Université de Bourgogne,
BP 27877
21078 Dijon Cedex, France.

Les chèques encaissés ne pourront être remboursés.

Centre d'Expertise de la Performance
"Gilles Cometti"

3ème journée Gilles Cometti
LA PREPARATION PHYSIQUE : du laboratoire au terrain

Vendredi 12, Samedi 13 avril 2013

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❑ Tarif étudiant [1] 60 €

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❑ Tarif normal [1] 120 €

❑ Tarif groupe étudiants *(à partir de 6)* [1] 40 €

[1] *Repas du samedi midi compris*

(Les chèques sont à libeller à l'ordre du « Régisseur de l'UFR STAPS de Dijon »)

Bulletin d'inscription et chèque **à renvoyer avant le 15 mars 2013** :
Centre d'Expertise de la Performance
Journée "Gilles Cometti"
Faculté des Sciences du Sport - UFR STAPS de Dijon
Campus Universitaire Montmuzard
BP 27877 - 21078 DIJON Cedex

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Pour tout renseignement : conference@cepcometti.com ou 03 80 39 67 88

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The effects of a single intake of Vinitrox® on exercise endurance and recovery in healthy subjects: a controlled, randomized, cross-over, double-blind study versus placebo.

Nicolas Babault, Gaëlle Deley

Centre d'Expertise de la Performance Gilles Cometti, Dijon, France

Introduction

Exercising involves adaptations of all the systems of the body (pulmonary, cardiac, muscular and vascular) in order to ensure optimal oxygen furniture and metabolites removal. This requires an increase in peripheral blood flow and a vasodilation. Among the various mediator of this vasodilation, nitric oxide (NO) is one of the most important (Bescos et al. 2012). Indeed, it is now well known that NO plays an important role in many functions in the body regulating vasodilation, blood flow, mitochondrial respiration and platelet function (Shen et al. 1995). Moreover, NO beneficial effects have been demonstrated on muscle strength and endurance (Folland et al. 2000) but also during recovery following an effort (Bloomer 2010). For all these reasons, NO has extensively been studied and it has been shown that synthesis and bioavailability of NO were influenced by arginine, Nitric Oxide Synthase (NOS) and superoxide anion (Drexler 1999).

Supplements such as polyphenols are, therefore, thought to be an interesting ergogenic aid (Petroczi and Naughton 2010). Polyphenols, abundant in human diet, have protective effects on the cardiovascular system (Erdman et al. 2008) but also activate endothelial Nitric Oxide Synthase (eNOS) which will increase synthesis and bioavaibility of NO (Engler et al. 2004). For example, previous works (Muller et al. 2009, Nexira 2008a) showed that Vinitrox®, composed of grape and apple extracts, activate NOSe and increases NO production by 24% (Nexira 2008b).

To date, most of the studies regarding the effects of polyphenols investigated several weeks supplementation and vascular or blood parameters (blood pressure, NO concentration, oxidative stress markers) but only few of them investigated the effects on immediate performance and recovery capacity (Morillas-Ruiz et al. 2005, Lafay et al. 2009).

The present work therefore aimed to study the effects of an acute intake of polyphenol (grape and apple extracts; Vinitrox®) on physical performances. More specifically, subjects, in the present study, had to perform a high intensity all-out exercise until exhaustion revealing their capacity to maintain a constant strong effort hereafter named endurance (notably illustrated by the time to exhaustion).

Methods

48 men (31.0 ± 6.0 years), regularly involved in a physical activity (3.9 ± 1.0 hour per week) were included in this study. It was composed of three experimental sessions interspersed with at least seven days. All were informed about the experimental procedure and signed a written consent form. The protocol and nutritional components used during this study were validated by the local committee of human research and AFSSAPS. Subjects were requested to refrain from any alcohol consumption and exhaustive exercise at least 24 hours before each experimental session.

Experimental setup. During the first experimental session, subjects performed a maximal test on an ergocycle to determine their maximal aerobic power. The ergocycle setup (handlebars and saddle height and distance) was noticed and reused during the two other test sessions. During this test, pedaling rate was imposed at 80 revolutions per minute (rpm).

During the two other testing sessions (respectively noted phase I and II), subjects realized an endurance all-out test at 70% of the maximal power determined during the first session. Subjects were requested to pedal until exhaustion, i.e., until they were unable to maintain the power with the requested pedaling rate. During phase I, the pedaling rate was free but within a 80-95 rpm range. During phase II, phase I pedaling rate was rigorously reproduced. Two hours before each test, subjects took a standardized breakfast (80g whole-wheat bread, 20g butter, 20g marmalade and 25cL orange juice). Moreover, the preceding evening and one hour before the endurance test, volunteers had to absorb either two capsules with 250mg Vinitrox® each or two placebo capsules according to randomization: subjects that had Vinitrox® during the phase I took the placebo during the phase II and inversely. Both the subjects and the experimenters were blinded from the randomization.

Data analyses. The main parameter tested was the time to exhaustion measured during the endurance test. During this test, were also evaluated the maximal and mean heart rate, maximal blood pressure, maximal and mean VO_2 and maximal and mean ventilation.

Blood pressure, heart rate, oxygen saturation, VO_2 and ventilation were measured at exercise stop and during the recovery at 2 min, 3 min and 5 min. Half-recuperation time for VO_2 and heart rate (i.e., the time necessary to obtain half the value measured at exercise end) were also calculated. Every four minutes during

the all-out test, Borg scale was used to determine subjects' perceived exertion. Muscle pain was finally evaluated 48 hours after each experimental session using a numerical 7-points scale.

Statistical analysis. All parameters were analyzed using the cross-over method with treatment effect, time effect and period effect. Mean values ± standard deviation are presented. $P < 0.05$ was taken as significative level for all condition.

Results

Time to exhaustion. In comparison with the placebo, the present study revealed a significant 2.5 min increase of the maximal duration of the endurance all-out test with Vinitrox® ($P < 0.05$) corresponding to a 9.7 ± 6.0 % increase.

Parameters registered during cycling. No significant differences were obtained for maximal and mean heart rate, maximal blood pressure, maximal and mean VO_2, maximal and mean ventilation and oxygen saturation when comparing Vinitrox® with placebo. In contradiction, the maximal perceived exertion was reached 2.7 min later (+12.8 % ± 6.8, $P < 0.05$) with Vinitrox® than with placebo

Recovery. At the end of the exercise, a 8.5 ± 11.4 seconds VO_2 half-recuperation time lengthening was observed with Vinitrox® (+16.2 % ± 7.5%, $P < 0.05$). No other differences were noticed between Vinitrox® and placebo for the parameters registered during the recovery period after the end of the test.

Muscle pain perception, evaluated 48 h after each experimental session, was not different between conditions.

Discussion/interpretation

The main results of the present study are the increased time to exhaustion during the endurance test, the delayed maximal effort perceived exertion, the longer VO_2 half-recuperation time and the absence of any difference between the two groups regarding muscle pain after exercise and the other physiological parameters. The delayed maximal effort perceived exertion observed here could be related to a better exercise tolerance. Associated with the significant increase of the exercise duration, this result is particularly interesting for athletes.

Firstly, for a given high intensity, the acute intake of Vinitrox® allows to perform longer efforts both during training and competition with a similar amount of fatigue. This result could be interesting for athletes that have to maintain high intensity efforts throughout the exercise or competition. For example, and despite a different type of exercise than tested here, we can suppose that Vinitrox® intake would be beneficial for team sports players to be more efficient until the end of the game (and even during overtime). This could be useful as well for tennis, squash players or high intensive sports lasting half an hour or more.

Another similar application could be for long distance and ultra-endurance athletes (biking, running, triathlon...). For them, Vinitrox® would allow to keep a (high) starting speed longer before significant fatigue appearance. Moreover, although there is no direct relation, it can be hypothesized that athletes being able to perform longer exercises at a given intensity might be able to be more efficient at slightly higher intensities. This last aspect needs to be taken with caution since it also depends on appropriate training sessions and athletes' characteristics.

Secondly, the absence of any muscle pain in spite of the longer exercise duration might allow athletes to repeat efforts without any additional muscle discomfort. This issue is important for activities repeating efforts with a short delay for recovery but, more generally speaking, all sport requiring endurance qualities. Stage races or tournament-type sports are examples (e.g., tennis or team-sports where athletes have to repeat high-intensity activities several time a day/week).

Thirdly, on the basis of these results, we can suggest that taking Vinitrox® chronically might allow multiplying high-intensity training more easily. At long term, this would induce greater performance gains (in comparison with athletes not taking Vinitrox®). This hypothesis concerns every sport.

The measured parameters did not allow us to distinguish precisely the origins of the observed gains but it seems that the effect of grape extracts on NO production and of apple extracts on oxidative stress reduction and vasodilation (favouring blood gas exchanges) might be involved. On a physiological point of view, several mechanisms could account for the effects of Vinitrox® on endurance, and this, through its action on NO production (attributable to the stimulation of NO production by NOSe). The first mechanism by which NO might act, is the muscle perfusion increase thanks to a direct vasodilation on vessels smooth muscle cells and to an inhibition of the adrenergic vasoconstriction (Maxwell et al. 1998, Vassilakopoulos et al. 2003). This hyperemia results in an increased oxygen availability to muscle cells allowing a greater and longer aerobic utilisation of glycogen.

Moreover, the present results showed a longer VO_2 half-recuperation time which can be considered surprising or even disappointing (if our aim is to speed recovery). However, this greater oxygen debt (the quantity of O_2 in excess during the recovery period) can be explained by the increased exercise duration in

the Vinitrox® group. Indeed, it has been extensively shown in the literature that the O_2 debt duration was directly associated with the exercise duration (Chad and Wenger 1988). Although this is not the first aim of the targeted athletes' population, this result is particularly interesting for people wanting to lose weight since a longer VO_2 recovery induces an increased energy cost following exercise. Lipids are therefore used in priority during this recovery period in order to regenerate energetic stocks (Borsheim and Bahr, 2003).

Conclusion and practical applications

As a conclusion, the results of the present study showed significant beneficial effects with Vinitrox® for athletes looking for performance:

① Vinitrox® enhances sport capacities thanks to the beneficial effect on endurance (i.e., capacity to maintain an intense effort) for almost all sports.

② For our exercise (pedaling), endurance improvement (time to exhaustion and maximal perceived exertion delay) was not associated with additional muscle pain.

③ The longer half-recuperation time (associated to the exercise duration) could also be beneficial to increase energy expenses and so for weight loss protocols.

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Inflammation in atherosclerosis: Imaging, biomarkers and novel therapeutic opportunities

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Chapter 11

Reduction of MCP-1 and MIF by a polyphenol- rich extract in subjects with clustered cardiometabolic risk factors

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ABSTRACT

Inflammation is a hallmark of the metabolic syndrome, which also contributes to a pro-atherogenic state. NF-κB activation, a critical step in regulating inflammatory reactions, can be inhibited by polyphenol (PF) extracts, at least *in vitro*. In the present study we set out to study whether a polyphenol-rich extract could attenuate the chronic inflammatory state and/or an acute immune response *in vivo* in subjects with clustered metabolic risk factors.

A commercially available, polyphenol (PF)-rich extract (Frutologic/Vinitrox™ 500mg daily) or placebo was administered for 4 weeks to 34 subjects with 2 or more metabolic risk factors using a randomized, double-blind cross-over design. During the final study visit, an acute inflammatory challenge (Lipopolysaccharide (LPS) 1 ng/kg bodyweight) was administered to a random subgroup of subjects (n=12 PF-rich extract and n=12 placebo). The PF- rich extract modestly reduced the inflammatory chemokines MCP-1 and MIF (MCP-1 -6.5 % (PF 116 pg/ml [97-136] vs. placebo.124 pg/ml [105-153]; $p<0.05$, median, [interquartile range]); MIF -10.8 % (PF 2512 pg/ml [1898-3972] vs. placebo 2814.5 [2296-3852]; $p<0.05$), however other measured markers of inflammation and cardiometabolic disease remained unaffected (CRP, IL-6, HDL-c, adiponectin and oxidized LDL all n.s.). Following the LPS challenge we found a statistically significant 48% reduction of MCP-1 production in the PF-rich extract group (n=12) versus placebo (n=12) over 6 hours (PF 766 ± 155 ng/ml vs. placebo 1466 ± 989 ng/ml; $p<0.05$, area under the curve (AUC)).

In conclusion, short-term oral administration of PF-rich extract caused a modest anti-inflammatory effect in subjects with clustered metabolic risk factors. Further dose-ranging studies are needed to evaluate whether and to what extent PF-rich extracts can be used to reduce the pro-inflammatory state in subjects with metabolic diseases at increased cardiovascular risk.

INTRODUCTION

Inflammation plays a critical role during all stages of the atherogenic process, ranging from the initiation of endothelial dysfunction to the onset of a cardiovascular event.[1] In agreement with this, elevated levels of pro-inflammatory chemo- and cytokines have been associated with increased cardiovascular risk[2-5]. Novel interventions which attenuate inflammation are being evaluated as add-on strategy in patients at increased cardiovascular risk[6]. The metabolic syndrome, a major risk factor for cardiovascular disease, is characterized by a chronic low-grade inflammatory state[7-9]. The latter has been attributed to inappropriate adipocyte enlargement accompanied by increased stress in the endoplasmatic reticulum of the adipocytes, leading to activation of NF-κB and subsequent inflammatory activation as attested by an increased production of interleukin 6 (IL-6), tumour necrosis factor α (TNF-α), macrophage migration inhibitory factor (MIF) and monocyte chemoattractant protein (MCP)-1[10,11]. These pro-inflammatory cytokines modulate the paracrine function of adipocytes, further contributing to systemic inflammation and insulin resistance in patients with obesity[10, 12].

Polyphenols

Polyphenols (PF) are chemical substances found in plants containing two or more phenol groups. In vitro, PF have been shown to inhibit Toll-like receptor (TLR)-mediated inflammatory responses via the MyD88-independent TLR3- and TLR4-signalling pathways[13], diminish NF-κB activation and reduce the production of downstream chemo- and cytokines[13, 14]. Anti-oxidative and anti-thrombotic effects have also been reported. Consistent with the anti-atherogenic effects in vitro, PF administration has been shown to attenuate atherosclerotic lesion formation in a variety of animal models[15-18]. In humans, however, the results of PF administration are heterogeneous[18-22]. Whereas a single study did report a beneficial effect of PF on the carotid intima-media thickness in patients at increased cardiovascular risk[23], the true impact of PF ingestion for cardiovascular disease needs confirmation in larger clinical trials. To date, the bulk of studies observing an effect of PF ingestion is based on epidemiological studies with very few randomized, placebo controlled trials[e.g. 24-26]. In the present randomized, placebo-controlled, double-blind study, we evaluated the impact of daily oral intake of 500 mg of PF-rich extract (Frutologic/Vinitrox®, Bio Serae) for 4 weeks on inflammatory markers in 34 subjecs with a clustering of cardiometabolic risk factors. In addition, we challenged a random sub-group (n=24) with low dose endotoxin to also evaluate the effect of PF-rich extract on the acute inflammatory response.

METHODS

Participants

The 34 participants of the study were recruited from the outpatient clinic of the Academic Medical Center (Amsterdam, the Netherlands) and through poster advertisement. The subgroup for the endotoxin challenge consisted of 24 participants who were randomly selected and asked for consent at the first study visit. Subjects were eligible to participate if 2 or more of the following criteria were present: a waist circumference of ≥ 102 cm for men or ≥ 88 cm for women, triglyceride levels ≥ 1.69 mmol/L, HDL-C ≤ 1.03 mmol/L for men or ≤ 1.29 mmol/L for women, a systolic blood pressure ≥ 130 mmHg, a diastolic blood pressure ≥ 85 mmHg and a glucose level ≥ 6.1 mmol/L, according to the ATP III criteria (Adult Treatment Panel III, attributed to NCEP/NHLBI).[27] During the study period, participants were instructed to refrain from wine and grape containing beverages, large amounts of tea, fruit juice, and dark chocolate. The use of lipid-lowering medication or antioxidants was not allowed throughout the study. Subjects were excluded if they reported coronary heart disease (CHD), stroke, malignancies, or chronic inflammatory diseases in their medical history. Subjects who were current smokers or known with alcohol abuse were excluded from the study.

Design

The study was designed as a double-blind, placebo controlled, randomized cross-over trial. During the screening visit, two weeks prior to the first study visit, blood was withdrawn for measurement of baseline parameters. Participants were randomized to daily 500 mg PF-rich extract obtained from grapes and apples (Frutologic, also known as VinitroxTM, BioSerae) for 4 weeks or placebo. The PF-rich extract contains a total of 36 (poly) phenolic compounds, including flavan-3-ols (monomers and oligomers up to a degree of polymerisation of 10), flavonols (mainly quercetin derivatives and myricetin), chlorogenic acids (5-caffeoylquinic and 4-*p*-coumaroylquinic acids), stilbenes (*trans*resveratrol), dihydrochalcones (phloretin and derivatives) and anthocyanins (delphinidin, cyanidin, petunidin, peonidin and malvidin derivatives). The total amount of (poly)phenolic compounds in the extract is 507 ± 4 mg/g of power. A detailed analysis section of the PF-extract is available online as Supplemental Material.

Following a 4 week washout period, participants were switched to placebo or polyphenol rich extract, respectively. The daily amount of polyphenol rich extract and placebo was dosed in capsules of 250mg. Participants were asked to take two capsules a day in the morning. At the end of each treatment period, blood was drawn for laboratory testing, including total cholesterol, LDL, HDL, glucose, HbA1C, CRP, Haemoglobin and Creatinine. Blood pressure was measured and endothelial function (flow mediated dilatation (FMD) by ultrasound) was assessed (see below). All measurements were performed after an overnight fast. At the end of the second treatment period, a subgroup of 24 participants (15 men and 9 women), who consented to additional investigation, was challenged with a low dose of endotoxin derived from Escherichia coli at a dose of 1 ng/ kg of bodyweight of endotoxin (Escherichia coli lipopolysaccharide (O113;H10), 10.00EU/ vial, PDS #67801, Lot number 67466 (production date 4.1.97), Cape Cod incorporated/

National Institutes of Health, Bethesda, Maryland, United States)[28-30]. Subsequently, blood was withdrawn from the contralateral antecubital vein at 1, 3, 4, 6 and 8 hours. Vital signs were measured at regular intervals, every 15 minutes in the first 3 hours. Incidence, time and severity of symptoms were recorded by the study physician. FMD measurements were performed at baseline and 4 hours after LPS challenge. Approval for this study was obtained from the internal review board of the Academic Medical Center, Amsterdam, the Netherlands. The study was carried out in accordance with the principles of the Helsinki Declaration. All participants gave written informed consent.

Laboratory measurements

Baseline serum concentrations of total cholesterol, HDL cholesterol and triglycerides were measured in fresh serum samples by standard enzymatic methods (Roche Diagnostics, Basel, Switzerland). LDL cholesterol concentrations were calculated using the Friedewald formula. Glucose was assessed using the hexokinase method (Gluco-quant, Hitachi 917; Hitachi). HbA1C was measured by HPLC (Reagens Bio-Rad Laboratories, Veenendaal, the Netherlands) on a Variant II (Bio-Rad Laboratories). Plasma aliquots were snap-frozen and stored at -80°C for shipment to the Biomarker Laboratory at the University of Ulm, Germany. Plasma C-reactive protein (CRP) levels were measured with a high sensitivity latex-enhanced nephelometric assay on a BN II analyzer (Dade Behring, Marburg, Germany). Tumor necrosis factor-α (TNF-α), Interleukin-6 (IL6), Adiponectin, Oxidized LDL, Monocyte chemoattractant protein-1 (MCP-1) and macrophage Migration Inhibitory Factor (MIF) levels were measured using commercially available ELISA kits (R&D Systems, Abingdon, UK). The intra- and inter-assay coefficients of variation of quality control test sera were <10% and <20% respectively.

Flow mediated dilatation

Each patient underwent measurement of FMD of the left brachial artery by B-mode ultrasound imaging using an Acuson Aspen (Siemens, Mountain View USA) ultrasound system with a L7, 5-10 MHz linear array broadband transducer. The FMD of all 34 participants was measured after each treatment period. The FMD of the 24 participants receiving an additional LPS challenge was also measured 4 hours after the LPS challenge. Patients were instructed to refrain from food, alcohol and any other drink except water prior to the measurements. A blood pressure cuff was placed on the left forearm from the medial epicondyle downwards. The scan of the left brachial artery started with 1 minute of continuous baseline recording, followed by 5 minutes of forearm ischemia, induced by inflating a vascular pressure cuff to 250 mmHg. Hyperemic blood flow was induced by deflating the cuff, after which 3 minutes of continuous ultrasound recording followed. Semi-automated image analysis of scans was performed off-line by an experienced image analyst using dedicated software (Brachial Analyzer, MIA vascular tools, Coralville USA). Images were blinded for treatment and visit. The average baseline diameter and the maximum post-cuff deflation diameter were used to calculate the percentage flow-mediated vasodilation (%FMD). %FMD was defined as: %FMD = 100 (maximum post cuff deflation diameter - baseline diameter) / baseline diameter).

Statistical analysis

Data are presented as arithmetic mean ± standard deviation when normally distributed and as median with corresponding interquartile range for values with a skewed distribution. For the cross-over study we used a repeated measures mixed model with time and treatment as fixed effects. An interaction term of time and treatment was used to test for possible carry-over effects. For the LPS intervention we calculated the production of circulating mediators after LPS by the area under the curve (AUC) from the time of the infusion (t=0) until 6 hours after the start of the challenge (t=6). A paired samples t-test was used for normally distributed values, whereas a Wilcoxon rank test for paired measurements was employed to test for skewed variables. Analyses were performed with SPSS version 16.0 (Chicago, IL, USA). A p-value <0.05 was defined as statistically significant.

RESULTS

Baseline characteristics of the participants

Baseline characteristics of the study participants are listed in Table 1. Of the 34 participants, 44% had two, whereas 56% had 3 ATP-III factors (Adult Treatment Panel III, attributed to NCEP/NHLBI) [27] of the metabolic syndrome. The prevalence of risk factors was distributed as follows: increased waist circumference 88%, hypertriglyceridemia 50%, low HDL-C levels 27%, elevated blood pressure 88% and elevated plasma glucose levels 29%. The average age was 58.3 ± 7.9 years. Baseline characteristics of the participants were: BMI 31.9 ± 4.8 (kg/m$^{2)}$, systolic blood pressure 147 ± 18.3 (mmHg), diastolic blood pressure 89 ± 9.3 (mmHg), total cholesterol 5.6 ± 1.1 (mmol/L), LDL-C 3.3 ± 0.9 (mmol/L), HDL-C 1.3 ± 0.4 (mmol/L), triglycerides 1.76 [1.38-3.23] (mmol/L), glucose 5.6 [5.1-6.1] (mmol/L), HbA1c 5.8 ± 0.5 (%) and CRP 2.1 [1.1-2.8] (mg/L).

Effect of PF-rich extract on inflammatory parameters

After 4 weeks BMI and blood pressure were unaffected in both the PF-rich extract and the placebo group (Table 1). No significant interaction term was found indicating that no carry-over effects were present in our study. We found a statistically significant reduction of 6.5% for MCP-1 and of 10.8% for MIF levels in the PF-rich extract group versus placebo. No changes were observed for other markers measured (Table 1). FMD was comparable between groups (Figure 1).

Effects of PF-rich extract on LPS response

Twenty-four participants received an inflammatory challenge of LPS (1 ng/kg bodyweight) during the final visit. All participants showed a modest rise in temperature with concomitant side-effects including chills, accelerated heart rate, and headache. These symptoms did not differ between the PF-rich extract and the placebo group..Total MCP-1 production, expressed as AUC up to 6 hours, was reduced in the PF-rich extract group compared to placebo. The ensuing peak increases in other inflammatory parameters measured were not significantly different (not displayed). FMD was significantly reduced 4 hours after LPS challenge (Figure 1) in both groups. This attenuation was comparable in both the PF-rich extract group and the placebo group.

Table 1. Markers for cardiovascular risk and inflammation according to treatment period of all 34 participants

	Placebo	Polyphenols	p-value
Age, years	58.3 ± 7.9	58.3 ± 7.9	ns
BMI, kg/m^2	31.9 ± 4.9	31.6 ± 5.2	ns
SBP, mmHg	142.5 ± 16.8	143.0 ± 14.7	ns
DBP, mmHg	86.1 ± 10.3	87.0 ± 8.9	ns
Total cholesterol, mmol/L	5.57 ± 1.29	5.71 ± 1.19	ns
LDL-C, mmol/L	3.39 ± 1.16	3.56 ± 1.11	<0.05
HDL-C, mmol/L	1.33 ± 0.31	1.32 ± 0.33	ns
Triglycerides, mmol/L	1.76 [1.33-2.39]	1.88 [1.21-2.41]	ns
Glucose, mmol/L	6.0 [5.6-6.8]	6.1 [5.7-6.9]	ns
HbA1C, %	5.8 ± 0.4	5.8 ± 0.4	ns
CRP, mg/L	2.37 [1.36-3.94]	2.43 [1.19-5.03]	ns
Oxidized LDL, U/ml	91.0 [77.1-118]	91.5 [79-116]	ns
IL-6, pg/ml	1.55 [1.21-1.79]	1.38 [1.1-1.83]	ns
MCP-1, pg/ml	124 [105-153]	116 [97-135.8]	<0.05
MIF, pg/ml	2814.5 [2296-3852.3]	2511.5 [1898-3972]	<0.05
Adiponectin, µg/ml	8.0 [6.3-10.8]	8.5 [5.3-11.8]	ns

Data are presented as mean ± standard deviation or median [interquartile range] of 34 patients. BMI = body mass index, SBP = systolic blood pressure, DBP = diastolic blood pressure, LDL-C = low density lipoprotein cholesterol, HDL-C = high density lipoprotein cholesterol, HbA1C = glycated hemoglobin, CRP = C-reactive protein, IL6 = interleukin 6, MCP-1 = monocyte chemoattractant protein 1, MIF = macrophage migration inhibitory factor.

DISCUSSION

In the present study, we show that 4-weeks administration of 500 mg PF-rich extract was associated with a significant, albeit modest reduction in MCP-1 and MIF levels in subjects with clustered metabolic risk factors. The MCP-1 production (AUC over 6 hours) following an LPS challenge was significantly reduced in the PF-group compared to placebo, whereas no differences were observed for other inflammatory cytokines measured. The present findings confirm a modest anti-inflammatory effect of PF-rich extracts in subjects with clustered metabolic risk factors in vivo. Yet, in view of the modest effect on isolated inflammatory parameters, larger studies are needed with prolonged follow-up in order to establish potential clinical relevance of this observation.

PF and inflammatory markers

PF-rich extracts from various food sources, including alcoholic beverages made from grapes, have been shown to exert a variety of beneficial effects, including anti-oxidative, anti-thrombotic and vasodilatory effects[10, 19, 31-38]. In addition, PF-rich extracts have been reported to reduce the

inflammatory response particularly for TLR3- and TLR4-specific ligands. Since the metabolic syndrome has been associated with a low-grade inflammatory state in which diet-related, postprandial increase of endotoxins may play a role[39], we focused on patients with clustered metabolic risk factors to assess a potential anti-inflammatory effect of PF in vivo. Following 4 weeks of PF-rich extract administration, a significant, albeit modest reduction in circulating levels of MCP-1 and MIF was observed. This finding is in correspondence with previous reports corroborating the anti-inflammatory capacity of PF in animal models[40, 41]. Similarly, the phenolic compound resveratrol was shown to inhibit NF-κβ transcription in vitro[13, 42]. In healthy volunteers, consumption of PF-extract in alcoholic beverages has also been reported to reduce NF-kB activation as well as MCP-1 plasma levels during a high fat diet[43]. Both MCP-1 and MIF are considered important pro-inflammatory cytokines associated with increased cardiovascular risk. MCP-1 is an important chemokine, attracting monocytes to sites of endothelial injury, and both MCP-1 serum levels as well as MCP-1 genotype have been associated with CV disease[44]. Mice receiving pharmacologic blockade of MCP-1 and mice deficient in MCP-1 develop less atherosclerosis compared to placebo treated or wild-type mice[45, 46]. Similarly, MIF has been associated with atherogenesis as well as risk factors associated with the development of metabolic disorders such as obesity and insulin resistance[47]. MIF is a pleiotropic cytokine that acts, amongst others, as a pro-inflammatory cytokine[48]. Both MIF and MCP-1 play a crucial role in the chemotaxis of monocytes into the adipose tissue, which is a key step in the progression towards 'dysfunctional' adipose tissue[47, 49, 50], characterized by a dysregulation of adipocyte paracrine function. We did not observe a difference in other markers associated with a proatherogenic state between the PF-rich extract and the placebo group. Based on the present findings, one might speculate whether PF-rich extracts can have a positive effect on 'dysfunctional' fat tissue in patients with the metabolic syndrome after long-term exposure[10].

PF and the inflammatory reaction following LPS challenge

Consistent with the effect of PF-rich extract on the chronic inflammatory state, a challenge with LPS resulted in a reduced MCP-1 area under the curve (AUC) over 6 hours in the PF-rich extract group compared to placebo. Other measured inflammatory markers did not differ. Monagas et al., recently described that pre-treatment of peripheral blood mononuclear cells with certain phenolic metabolites induced a reduction of TNF- α, IL-6 and Il-1β production after LPS stimulation by more than 80% [51]. Another in vitro study observed differential effects between the various phenolic compounds that where used. For instance, a reduction of IL-1β could be detected for addition of oleuropein glycoside, present in olive oil, but not for other PF used. No effect was seen on TNF- α or IL-6[52], the latter finding being more similar to the results of the present study. It has been suggested that not all PF have comparable anti-inflammatory characteristics and that different PF have specific anti-inflammatory properties, which could in part explain the heterogeneous results between previously published studies [53].

Limitations

The results of the present study warrant several comments. First, a treatment period of 4 weeks is a relatively short period and the dose of 500 mg per day may be suboptimal as bio-availability of many polyphenolic substances has been reported to be poor [54]. Thus, large amounts of PF may have to be ingested in order to obtain beneficial effects. It has to be taken in account that this characteristic limits the clinical application of these compounds. Second, Frutologic is a PF-rich mixture of compounds which includes various substances and may even enclose potential non-polyphenolic compounds. This was a first exploratory study to investigate the effect of a PF-rich extract on both acute and chronic inflammatory reactions in obese participants with multiple cardiometabolic risk factors. Markedly, despite the short duration of PF administration as well as the limited number of participants, studied, we were able to detect a significant effect on both basal MCP-1 and MIF plasma levels as well as the acute MCP-1 response following an endotoxin challenge.

Conclusions

The present study shows that oral ingestion of Frutologic, a PF-rich mixture extracted from apples and grapes decreases circulating pro-inflammatory cytokines, both involved in the pathogenesis of adipocyte dysfunction and atherogenesis. Considering the important role for the infiltration of monocytes into fat tissue and the subsequent inflammatory response, PF may have a potentially beneficial effect on the development and/or progression of CVD in patients with clustered metabolic risk factors. However, this needs further confirmation in larger trials investigating the optimal dose and the long-term results of PF administration on the development and progression of cardiovascular disease.

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SUPPLEMENTAL MATERIAL

MATERIALS AND METHODS

Chemicals

Quercetin-3-*O*-glucoside was purchased from Fluka (Sigma Aldrich Co. Ltd, Dorset, UK). (–)-Epicatechin, (+)-catechin, phloretin, phloridzin (phloretin-2 - *O*-glucoside), 5-caffeoylquinic acid and *trans*-resveratrol were obtained from Sigma Aldrich Co. Ltd (Dorset, UK). Quercetin, quercetin-3-*O*-galactoside, quercetin-3-*O*- glucuronide, quercetin-3-*O*-rhamnoside, cyanidin-3-*O*-glucoside, malvidin-3-*O*- glucoside and myricetin-3-*O*-rhamnoside were purchased from Extrasynthese (Lyon, France) and delphinidin-3-*O*-glucoside, petunidin-3-*O*-glucoside, peonidin-3-*O*- glucoside were obtained from PhytoLab Gmbh & Co. KG (Germany). Acetonitrile, acetone and formic acid of HPLC grade were purchased from Fisher Scientific Ltd (Loughborough, Leicestershire, UK), ethanol from Rathburn Chemicals (Walkerburn, Scotland), and acetic acid from WWR International Ltd (Poole, Dorset, UK).

Extract Analysis

Twenty mg of extract were weighted and re-suspended in 10 mL of distilled water:ethanol:formic acid (50:49:1 % v/v). The aqueous extract was vortexed for 1 min and aliquots were stored at -80 C, prior to triplicate 20 µ L volumes being analysed by HPLC-MS^n.

Reversed Phase HPLC-PDA-ESI-MS^n

(Poly)phenolic compounds were analysed using a Surveyor HPLC with a PDA detector and a LCQ Duo ion trap mass spectrometer fitted with an electrospray interface (Thermo Fisher Scientific, San Jose, USA). Separations of (poly)phenolic compounds were performed at 40°C using 4 µ m Synergi 250 x 4.6 mm i.d. reversed phase column (Phenomenex, Macclesfield, UK). Monomeric flavan-3-ols, flavonols, stilbenes and chlorogenic acids were separated

using a 60-min linear gradient of 5-40% acetonitrile in 0.1% formic acid and anthocyanins were separated using a 60-min gradient of 2-25% acetonitrile in 1% formic acid. Injections were carried out with an autosampler maintained at 4°C. The mobile phase was pumped at a flow rate of 1 mL/min. The column eluate initially passed through the PDA detector and was then split, with 0.3 mL/min directed to the mass spectrometer with ESI operating in full scan positive (detection of anthocyanins) or negative ionisation mode (100-1000 *m/z*), data dependant MS^2. The tuning of the mass spectrometer was optimised by infusing diluted grape juice (detection of anthocyanins) or a standard of (–)-epicatechin (detection in negative ionisation mode), dissolved in the initial HPLC mobile phase, into the source at a flow rate of 0.3 mL/min, and collision energy was set at 35 %. Post-HPLC, anthocyanins were

detected and quantified using PDA monitored at 520 nm and selective reaction monitoring (SRM). Mean quantitative data are expressed as mg/g ± SE (n=3) of available standard

equivalents. Other (poly)phenolic compounds were detected and quantified using PDA at 280 nm (monomeric flavan-3-ols), 285 nm (dihydrochalcones and derivatives), 305 nm (*trans*-resveratrol), 325 nm (chlorogenic acids) and 365 nm (flavonols) and identification was confirmed by mass spectrometry using consecutive reaction monitoring (CRM). Mean quantitative data are expressed as mg/g ± SE (n = 3) of available standard equivalents.

Analysis of Procyanidins

Analysis of the procyanidins in the extract was based on a previously described method (Robbins *et al.* 2009). Briefly, procyanidins in the aqueous extract were extracted in triplicate with 5 mL of an acetone-based solution (acetone:water:acetic acid; 70:29.5:0.5; v/v/v). After vortexing, the samples were sonicated for 5 min at 50 C and centrifuged at 2600 *g* for 10 min. Supernatants were collected and passed through a SPE cartridge, Strata SCX (55 µ m, 70Å, 500 mg/3 mL) (Phenomenex, Cheshire, UK), following pre-conditioning of the cartridge with distilled water. Five µ L of the collected samples were then injected into an HPLC system equipped with a fluorescence detector (FP-920, Jasco (UK) Ltd) and linked to a mass spectrometer. Separation was achieved using a Develosil Diol 100Å (250 x 4.6 mm, 5µ m) (Phenomenex, Cheshire, UK). Mobile phase consisted of acidified acetonitrile and acidified aqueous methanol, and chromatographic conditions were used as previously described (Robbins *et al.* 2009). Following separation, procyanidins with a degree of polymerisation of 2 and above were detected and quantified using a fluorometer (excitation and emission wavelengths at 230 nm and 320 nm), and identification was confirmed by mass spectrometry in full scan negative ionisation (*m/z* 100-2000), data dependant MS. Mean quantitative data are expressed as mg/g ± SE (n = 3) of (–)-epicatechin equivalents.

RESULTS

A total of 36 (poly)phenolic compounds were identified and quantified in the extract. These were flavan-3-ols (monomers and oligomers up to a degree of polymerisation of 10), flavonols (mainly quercetin derivatives and myricetin), chlorogenic acids (5-caffeoylquinic and 4-*p*-coumaroylquinic acids), stilbenes (*trans*- resveratrol), dihydrochalcones (phloretin and derivatives) and anthocyanins (delphinidin, cyanidin, petunidin, peonidin and malvidin derivatives). The total amount of (poly)phenolic compounds in the extract is 507 ± 4 mg/g of powder.

Revered phase HPLC separated and two flavan-3-ol monomers which were identified by co-chromatography and MS^2 as shown in Table 1. (–)-Epicatechin was found in amounts of 19 mg/g and (+)-catechin amounted to 8.2 mg/g. Procyanidins with a degree of polymerisation of >2 do not chromatography satisfactorily on HPLC C18 supports. The procyanidins were, therefore, also analysed by HPLC-MS using a diol support with the partially identified peaks, which separate procyanidins according to their molecular masses, being quantified by fluorometry in (–)-epicatechin equivalents, due to a lack of available standards. The data obtained are

summarised in Table 2 and Fig 2. Compounds with a negative molecular ion, $[M–H]^-$, at *m/z* 865, *m/z* 1153, *m/z* 1441 and *m/z* 1729 were identified as trimeric, tetrameric, pentameric and hexameric procyanidins of (epi)catechin (Es-Safi *et al.* 2006). Procyanidins with a degree of polymerisation of >6 do not ionise, and were therefore identified based on the retention time report by Robbins *et al.* 2009 (Table 2). The main procyanidins in the extract were dimers (99 mg/g), followed by tetramers (72 mg/g) and pentamers (48 mg/g). The total amount of flavan-3-ols accounted for 373 mg/g.

HPLC-PDA-MS^n analysis detected 13 anthocyanins in the extract, accounting for 11% of the total (poly)phenolic content (57 mg/g) (see Table3, Fig. 3). Identification of individual compounds was based on the *m/z* of the positively charged molecular ion ($[M-H]^+$) and the MS^2 fragmentation, retention time of commercially available standards, absorbance spectra and the elution order which depends upon the anthocyanidin aglycone and the attached sugar, as previously described (Giusti *et al.* 1999; Burns *et al.* 2002; Wang *et al.* 2003; Nakajima *et al.* 2004; Tian *et al.* 2005). The anthocyanins consisted of 3-*O*-glucosides ($[M-162]^+$), 3-*O*-(6 -*O*-*p*- acetyl) glucosides ($[M-204]^+$) and 3-*O*-(6 -*O*-*p*-coumaroyl)glucosides ($[M-308]^+$) of delphinidin (*m/z* 303), cyanidin (*m/z* 287), petunidin (*m/z* 317), peonidin (*m/z* 301) and malvidin (*m/z* 331). The main compounds were malvidin-3-*O*-glucoside, malvidin-3-*O*-acetylglycoside and peonidin-3-*O*-glucoside, accounting for 74% of the total anthocyanin content (Table 3). Malvidin-3-*O*-glucoside alone accounted for almost half of the total amount.

Chlorogenic acids were identified based on the co-chromatography with an authentic standard of 5-caffeoylquinic acid, and based on the fragmentation profile of the molecular ion at *m/z* 337 of 4-*p*-coumaroylquinic acid, as previously reported (Clifford *et al.* 2003). The total content of chlorogenic acids in the extract is 53 mg/g. *Trans*-resveratrol was the only stilbene identified in the extract, based on the co-chromatography with an authentic standard, in amounts of 0.2 mg/g. A total of three dihydrochalcone derivatives were identified in the extract (see Table 1, Fig. 1). These were phloretin and phloretin-2 -*O*-glucoside, which co- chromatographed with authentic standards. A third compound was putatively identified as phloretin arabinoglucoside, based on the fragmentation profile of the molecular ion at *m/z* 567, providing fragments at *m/z* 273 (M-H-294). The loss of 294 *amu* was putatively attributed to an arabinoglucoside moiety, based on the fragmentation pattern of a standard of quercetin-3-*O*-arabinoglucoside. The total amount of phloretin derivatives is 19 mg/g, with phloretin-2 -*O*-glucoside accounting for 75% of the dihydrochalcones content. Flavonols in the extract were found in amount of 4.7 mg/g, mainly as quercetin derivatives. The main group of (poly)phenolic compounds in the extract were the flavan-3- ols, accounting for 74% of the total content, followed by the anthocyanins, accounting for 11%, chlorogenic acids (10%), dihydrochalcones (4%), flavonols (0.9%) and stilbenes (0.1%). Table 5 summarises the amount of (poly)phenolic compounds per 500 mg capsules.

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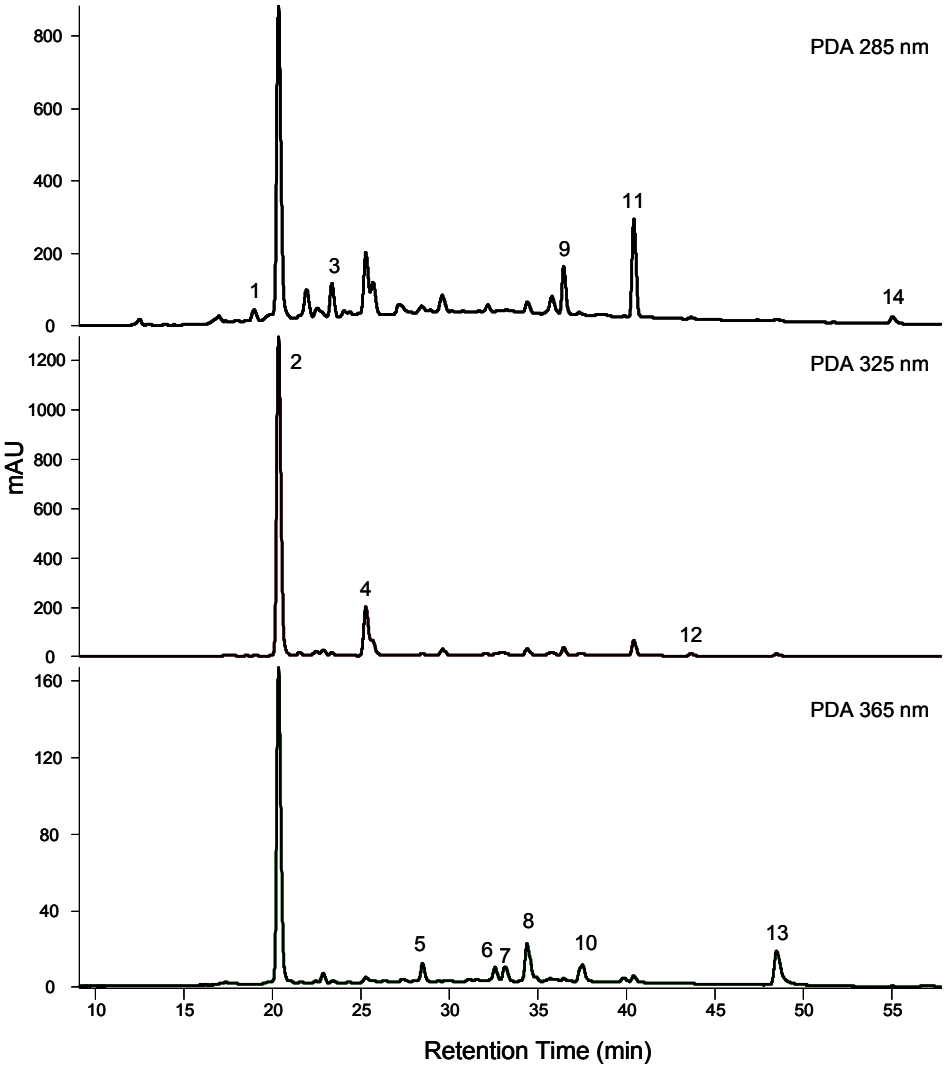

Figure 1. HPLC-PDA traces monitored at 285 nm (dihydrochalcones and flavan-3-ol monomers), 325 nm (chlorogenic acids) and 365 nm (flavonols). For peak numbers, see Table 1.

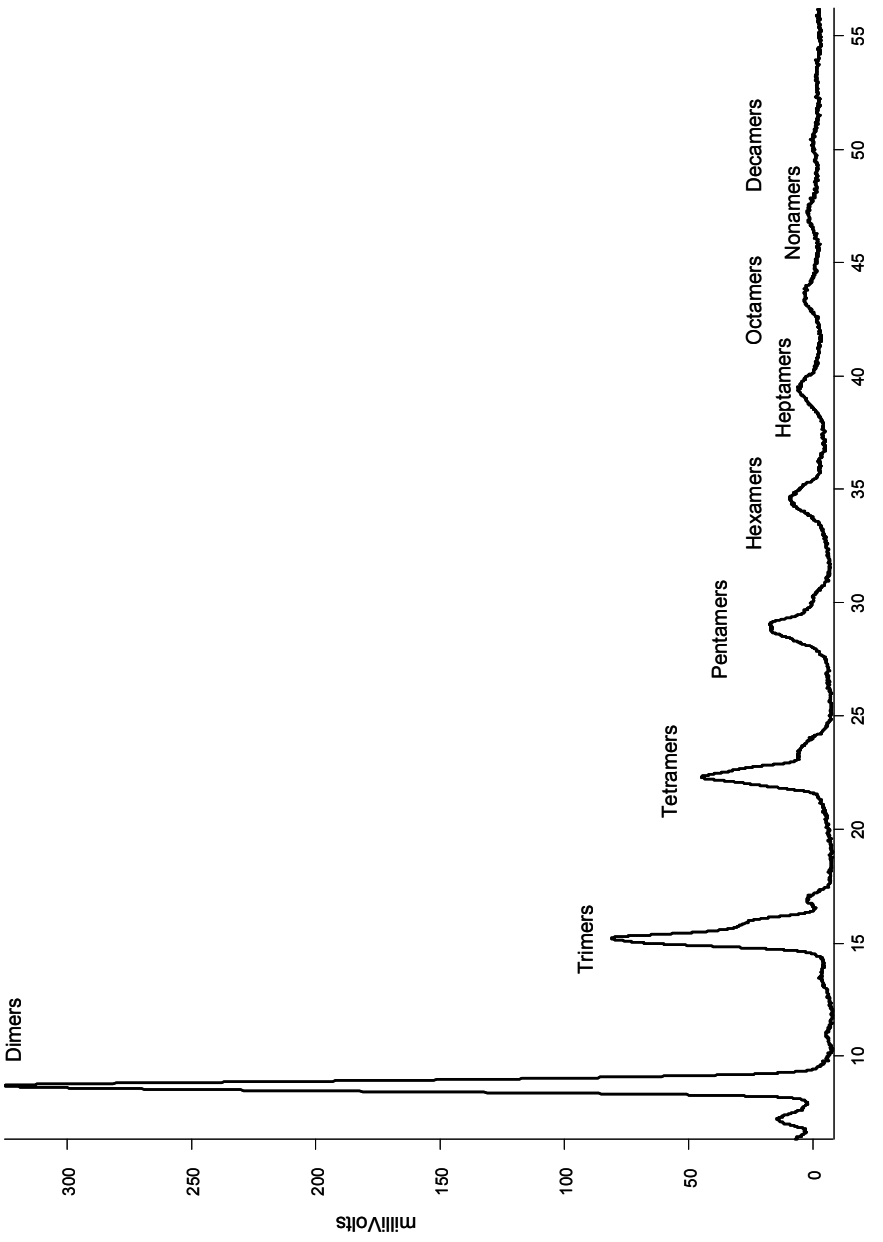

Figure 2. HPLC-diol phase with fluorescence detection of procyanidins identified in an extract of VinitroxTM

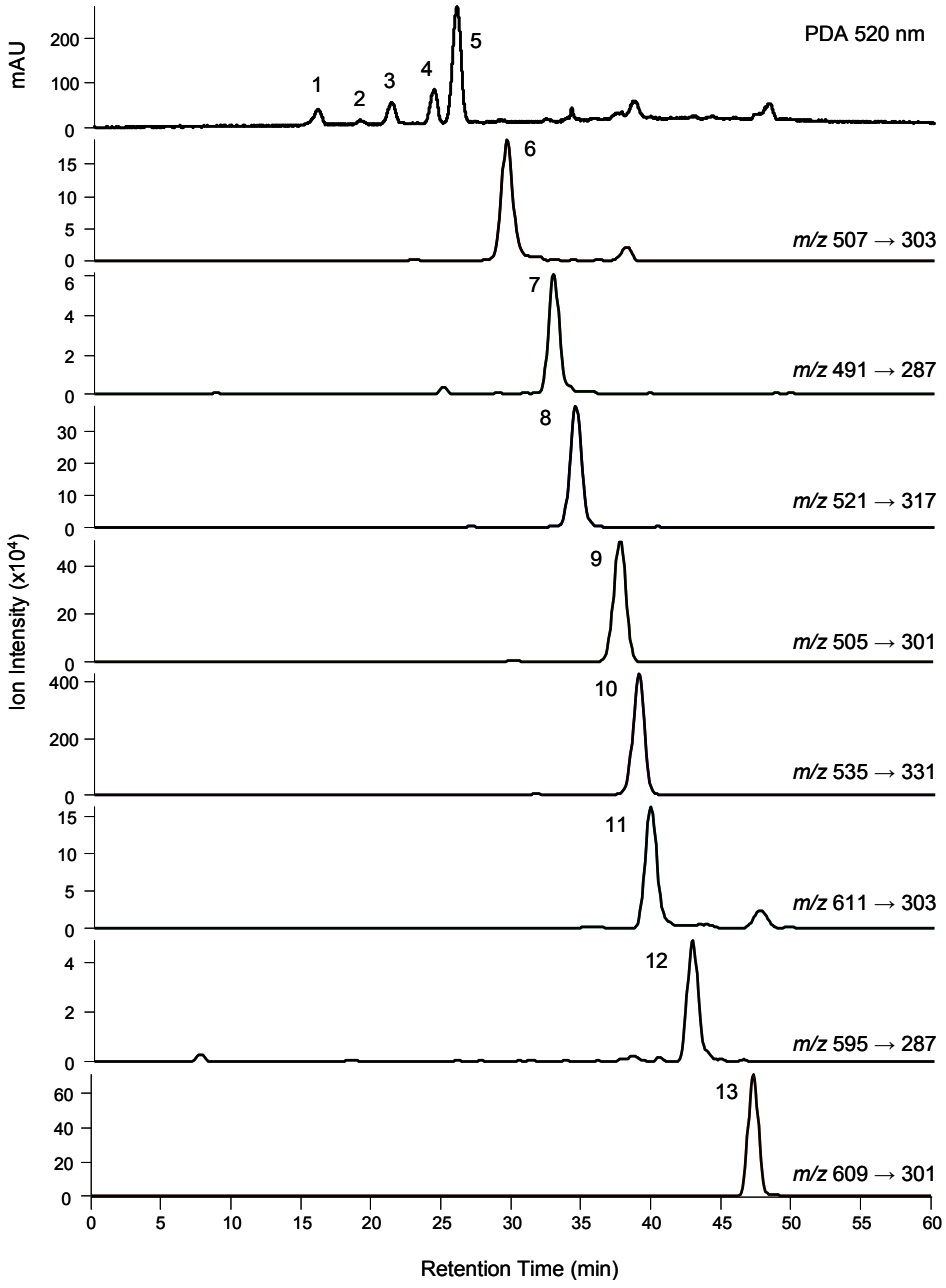

Figure 3. HPLC-SRM traces of anthocyanins identified in an extract of VinitroxTM. PDA monitored at 520 nm. For peak numbers, see Table 3.

Table 1. HPLC-PDA-MSn profiles of (poly)phenolic compounds identified in VinitroxTM [a]

Peak	Compounds	R_t (min)	max (nm)	$[M-H]^-$ *(m/z)*	MS^2 *(m/z)*	MS^3 *(m/z)*	mg per g of extract
1	(+)-Catechin	18.96	280	289	245, 231, 205	-	8.2 ± 0.1
2	5-Caffeoylquinic acid	20.35	325	353	191, 179, 135	-	39.2 ± 1.2
3	(–)-Epicatechin	23.36	280	289	245, 231, 205	-	19.3 ± 0.5
4	4-*p*-Coumaroylquinic acid	25.27	310	337	173, 163, 137	-	13.4 ± 0.2
6	Myricetin-*O*-glucoside	28.75	255, 350	479	316	-	0.3 ± 0.0
5	Quercetin-3-*O*-galactoside	32.58	255, 355	463	301	179, 151	0.5 ± 0.0
7	Quercetin-3-*O*-glucoside	33.13	255, 355	463	301	179, 151	0.8 ± 0.0
8	Quercetin-3-*O*-glucuronide	34.40	255, 355	477	301	179, 151	1.6 ± 0.0
9	Phloretin-*O*-arabinoglucoside	36.46	285	567	273	167	4.0 ± 0.1
10	Quercetin-3-*O*-rhamnoside	37.53	255, 350	447	301	179, 151	0.5 ± 0.0
11	Phloretin-2 -*O*-glucoside	40.43	285	435	273	167	14.2 ± 0.5
12	*trans*-Resveratrol	43.66	305	227	-	-	0.2 ± 0.0
13	Quercetin	48.43	255, 370	301	179, 151	-	1.1 ± 0.0
14	Phloretin	54.67	285	273	167	-	0.8 ± 0.0

[a] For peak numbers, see Fig. 1. Values expressed as mg/g ± standard error (n = 3) of extract. R_t, retention time; max, maximum absorbance (nm); $[M-H]^-$, molecular ion in negative ionisation; MS^2 and MS^3, ion fragments from $[M-H]^-$

Table 2. HPLC-MS identification of oligomeric flavan-3-ols in VinitroxTM using a Diol column and fluorometry detection

Compounds	R_t (min)	$[M-H]^-$ *(m/z)*	MS^2 *(m/z)*	mg per g of extract
Dimers	8.71	577	559, 451, 425, 407, 289	98.9 ± 7.4
Trimers	15.22	865	847, 739, 713, 695, 577, 425, 407	35.8 ± 1.6
Tetramers	22.31	1153	-	72.3 ± 2.8
Pentamers	29.06	1441	-	47.8 ± 1.4
Hexamers	34.66	1729	-	40.7 ± 1.5
Heptamers	39.37	nd	-	20.1 ± 1.6
Octamers	43.31	nd	-	13.7 ± 1.4
Nonamers	47.23	nd	-	11.9 ± 0.9
Decamers	50.43	nd	-	4.2 ± 0.3

Values expressed as mg/g ± standard error (n = 3) of extract. Rt, retention time; $[M-H]^-$, molecular ion in negative ionisation; MS^2, ion fragments from [M-H]-; nd, not detected

Table 3. HPLC-PDA-MSn profiles of anthocyanin compounds identified in VinitroxTM [a]

Peak	Compounds	R_t (min)	$[M-H]^{+-}$ *(m/z)*	MS^2 *(m/z)*	mg per g of extract
1	Delphinidin-3-*O*-glucoside	15.94	465	303	1.9 ± 0.2
2	Cyanidin-3-*O*-glucoside	19.05	449	287	0.8 ± 0.2
3	Petunidin-3-*O*-glucoside	21.16	479	317	4.5 ± 0.1
4	Peonidin-3-*O*-glucoside	24.20	463	301	6.5 ± 0.3
5	Malvidin-3-*O*-glucoside	25.89	493	331	24.5 ± 1.7
6	Delphinidin-3-*O*-acetylglucoside	29.47	507	303	1.4 ± 0.3
7	Cyanidin-3-*O*-acetylglucoside	32.82	491	287	0.3 ± 0.1
8	Petunidin-3-*O*-acetylglucoside	34.38	521	317	2.6 ± 0.1
9	Peonidin-3-*O*-acetylglucoside	37.55	505	301	1.0 ± 0.1
10	Malvidin-3-*O*-acetylglucoside	38.91	535	331	11.3 ± 0.5
11	Delphinidin-3-*O*-*p*-coumaroylglucoside	39.79	611	303	0.9 ± 0.0
12	Cyanidin-3-*O*-*p*-coumaroylglucoside	42.78	595	287	0.2 ± 0.0
13	Peonidin-3-*O*-*p*-coumaroylglucoside	47.34	609	301	1.4 ± 0.0

[a] For peak numbers, see Fig. 3. Values expressed as mg/g ± standard error (n = 3) of extract. R_t, retention time; $[M-H]^-$, molecular ion in positive ionisation; MS^2, ion fragments from $[M- H]^+$

Table 4. Total content of (poly)phenolic compounds analysed in an extract of Vinitrox™ (mg/g)

Compounds	mg per g of extract
Flavan-3-ols	372.9 ± 2.9
Anthocyanins	57.2 ± 2.2
Chlorogenic acids	52.6 ± 0.6
Dihydrochalcones	19.1 ± 0.4
Flavonols	4.7 ± 0.1
Stilbenes	0.2 ± 0.0
Total	**506.6 ± 3.8**

Table 5. Total content of (poly)phenolic compounds in 500 mg of Vinitrox™

Compounds	mg per 500 mg ingested
Flavan-3-ols	186.5 ± 1.4
Anthocyanins	28.6 ± 1.1
Chlorogenic acids	26.3 ± 0.3
Dihydrochalcones	9.5 ± 0.2
Flavonols	2.4 ± 0.1
Stilbenes	0.1 ± 0.0
Total	**253.3 ± 1.9**

nutrients

MDPI

Article

An Acute Dose of Specific Grape and Apple Polyphenols Improves Endurance Performance: A Randomized, Crossover, Double-Blind versus Placebo Controlled Study

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Abstract: Polyphenols are thought to be an interesting ergogenic aid for exercise and recovery. However, most studies regarding the effects of polyphenols investigated several days of supplementations. The present work aimed to study the effects of an acute intake of grape and apple polyphenols on the capacity to maintain intense exercise, here named endurance performance. Forty-eight physically active men (31 ± 6 years) were included in this study. During the two testing sessions, volunteers completed an endurance test at a high percentage of their maximal aerobic power and time to exhaustion was measured. Respiratory and pain parameters were also monitored. The preceding evening and 1 h before testing, volunteers had to absorb either 500 mg of polyphenols or placebo according to randomization. In comparison with the placebo, the mean duration of the maximal endurance test was significantly increased with polyphenols (+9.7% ± 6.0%, $p < 0.05$). The maximal perceived exertion was reached later with polyphenols (+12.8% ± 6.8%, $p < 0.05$). Practically, the present study showed the beneficial effects of grape and apple polyphenols for athletes looking for endurance performance improvements. The specifically designed profile of polyphenols appeared to enhance the capacity to maintain intensive efforts and delay perceived exertion.

Keywords: maximal exertion; aerobic; cycling

1. Introduction

Endurance performance during high intensive exercises is mainly determined by the capacity of the aerobic metabolism [1]. It generally induces muscle fatigue, defined as the reversible decline in skeletal muscle contractile function [2]. Fatigue is multifactorial and is often associated with many physiological parameters including reduced neural input and disruptive metabolic changes in skeletal muscles such as lactic acidosis and the production of oxidative free radicals [2]. Moreover, it could lead to oxidative stress as a result of an imbalance between reactive oxygen species (ROS) production and intrinsic antioxidant defense [3].

To alleviate oxidative stress, some ergogenic strategies have been tested. Numerous studies have reported that different types of supplementation such as polyphenols were of interest to protect against these mechanisms [4–6]. Indeed, although some studies demonstrated no or harmful effects [7,8], most studies observed the positive effects of antioxidants on oxidative stress or performance [9–11]. More particularly, polyphenols, have great antioxidant capabilities and protective effects [12,13].

In addition, polyphenols increase the synthesis and bioavailability of nitric oxide (NO) [14,15] which is well known as the most important mediator of vasodilation [16]. NO also plays an important role in many functions such as blood flow, mitochondrial respiration and platelet function [17]. As a consequence, the beneficial effects of NO have been demonstrated on muscle strength [18,19], but also during recovery following intensive efforts [20].

To date, most of the studies exploring the effects of polyphenols investigated several days or weeks of supplementation on vascular, blood parameters (blood pressure, NO concentration, oxidative stress markers) or endurance performance [4,21]. For instance, Trinity et al. [22] observed no alteration in the cycling time-trial after seven days of polyphenol dietary supplementation. However, conflicting results are often obtained [19]. According to a recent review [23], depending on the type of polyphenols, chronic consumption has potentially detrimental to promisingly beneficial effects. Only few studies have investigated the effects of a single intake on immediate performance and recovery capacity [5,10,24–26]. Therefore, the present work aimed to study the effects of an acute intake of a specific profile of polyphenols from grape and apple on physical performance. More specifically, performance in the present study referred to a high-intensity cycling exercise until exhaustion revealing the capacity to maintain a constant strong effort hereafter named endurance. We therefore hypothesized that an acute supplementation of polyphenols would increase the time to exhaustion during a high-intensity cycling exercise.

2. Materials and Methods

2.1. Experimental Overview

The primary objective of this randomized, crossover, double-blind and controlled study was to evaluate the effect of an acute intake of polyphenols supplement versus Placebo (maltodextrin) on endurance measured during a cycling test until exhaustion. Participants were tested on two separate occasions with a constant-load ergocycle. Before test sessions, participants were asked to absorb either two capsules of 250 mg of grape and apple polyphenols or two capsules of placebo according to randomization. The main parameter, i.e., time to exhaustion during each session, was tested using a Student's *t*-test.

2.2. Participants

A total of 48 healthy physically active males (exercising from 3 to 6 h a week) were recruited for the study (Figure 1). Volunteers with more than 6 h training a week, regularly trained in aerobic activities, who were asthmatic, smokers or under medicinal drugs, dietary supplement, sports drink, special dietary food or functional food, of any kind, liable or presented as liable to enhance physical performances, were excluded from the study. Throughout the study, volunteers maintained their usual training routine and diet. All gave their written informed consent after being told about the experimental protocol. The study was conducted in accordance with the Helsinki Declaration, was approved by the local ethics committee (CCP Est I: 2011/57) and was registered at ClinialTrials.gov (NCT03214276). Volunteers' characteristics are presented on Table 1. This sample size was calculated a priori using Nquery Advisor (version 6.01) software based on the primary criterion and allowing for a power of more than 90%.

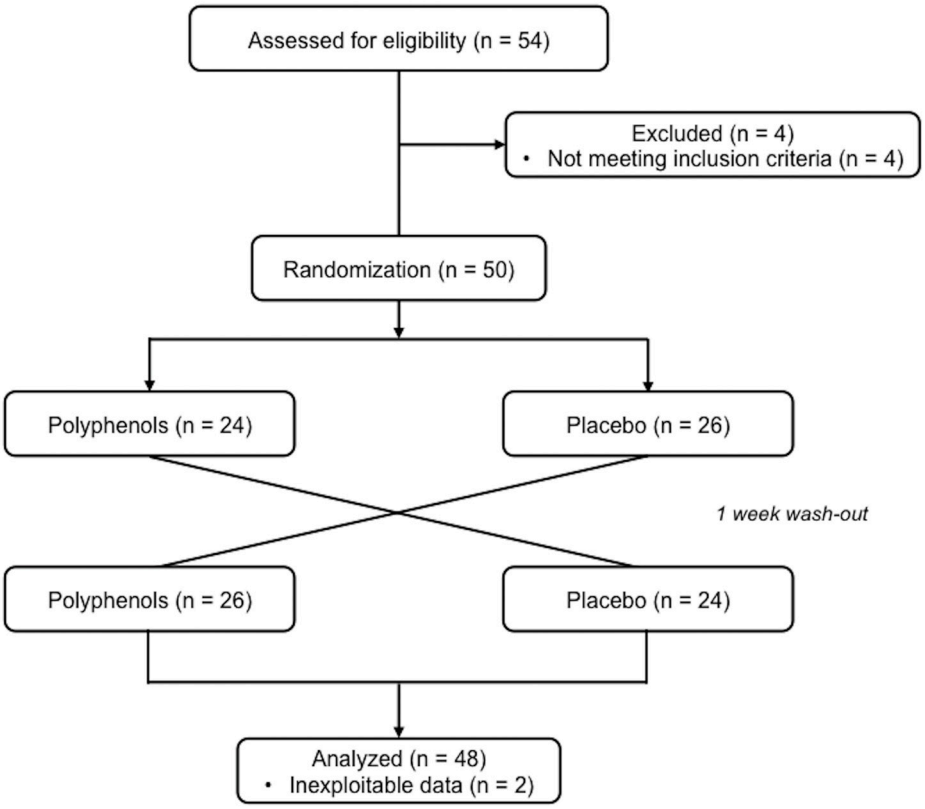

Figure 1. CONSORT flowchart.

Table 1. Participants' characteristics after inclusion.

Characteristics	Mean Values
Age (years)	31.0 ± 6.0
Height (cm)	181.2 ± 6.4
Weight (kg)	77.3 ± 9.3
Weekly activity (h/wk)	3.9 ± 1.0
BMI (kg/m^2)	23.5 ± 2.2
Resting HR (bpm)	62.4 ± 9.3
Resting VO_2(mL/min/kg)	4.6 ± 1.3
Resting SBP (mm Hg)	129.0 ± 8.3
Resting DBP (mm Hg)	75.7 ± 6.7
Maximal aerobic power (Watts)	294.4 ± 52.4

Values are means ± standard deviation. BMI: body mass index, HR: heart rate, VO_2: oxygen consumption, SBP: systolic blood pressure, DBP: diastolic blood pressure.

2.3. Experimental Procedure

After inclusion, participants came to the laboratory for three tests performed on a CycleOps 400 PRO equipped with PowerTap power meters (CycleOps, Madison, WI, USA) that allowed constant power output independently of pedalling rate. During the first visit, maximal aerobic power was determined during an incremental cycling test. Characteristics of the test were determined individually according to the equation of Hansen et al. [27] in order to have a test lasting between 8 and 15 min. Briefly, participants started at an intensity ranging from 45 to 60 W during 180 s followed by increments of 20 to 30 W every 60 s. The test was interrupted when participants were unable to maintain the requested cycling rate and the last power value maintained at least 30 s was considered as the maximal aerobic power. It was used as the reference during the two other test sessions. During this test, participants were asked to remain seated all the time and to keep a constant pedalling rate of

80 revolutions per minute. Saddle and handlebar settings were individually adjusted and used during the other test sessions. Heart rate was measured at rest, during the test and during 3 min after the end of the test (Polar, Polar Electro Oy, Kempele, Finland).

At least two days after the incremental test, participants underwent two constant-load exercises at an intensity corresponding to 70% of their maximal aerobic power until exhaustion. Pedalling rate was kept constant during the whole test and during each session. Also, participants were regularly encouraged by the experimenter using standardized sentences and timing. Participants were blinded for the duration of the test. The main parameter tested was the time to exhaustion, measured in seconds. Heart rate, blood pressure, ventilation, and gas exchanges (Cosmed K4b2, Cosmed, Rome, Italy) were also measured at rest, during the test and during five minutes after the end of the test. Beat by beat heart rate as well as breath by breath ventilation and VO_2 were averaged every five seconds throughout the test. The average values over the last 30 s of the tests were considered as maximal values. Half-recovery times for heart rate and VO_2 (i.e., the time necessary to obtain half the value measured at exercise end) were also calculated. Every 4 min during the all-out test, Borg scale was used to determine participants' perceived exertion [28]. Muscle pain was finally evaluated 48 h after each experimental session using a numerical seven-point scale.

The two endurance tests were separated by at least 7 days (washout period) and were performed in the morning at the same hour, i.e., 2 h after a standardized breakfast composed of 125 mL orange juice, 80 g of wholemeal bread, 20 g of butter and 20 g of jelly. The preceding evening and one hour before the endurance test, participants were asked to absorb either two capsules of 250 mg of polyphenols (Vinitrox™) or two capsules of placebo (maltodextrin) according to the order defined by the randomization (similar appearance and flavour). Because polyphenols are partiallydirectly bioavailable [29] but also later after gut microbiota and liver metabolization [30], two intakes have been imposed (one hour prior exercise andthe evening before, respectively). Participants receiving polyphenols in the first session received placebo in the second and reciprocally. Both the participants and the experimenters were blinded from the randomization. Except the standardised breakfast, diet was not controlled. Nevertheless, participants were instructed to have an almost similar food intake the day before tests.

Vinitrox™, supplied by Nexira (France) is a combination of specific profile polyphenols from grape and apple. Vinitrox™ is a purified extract with low lipids, fibers and proteins content (respectively 0.5%, 1.4% and 5.5%) and more than 60% of total polyphenols (Folin method—expressed as gallic acid equivalent) with antioxidant properties demonstrated by ORAC value (12,000 µMtrolox equivalent per gram of ingredient). The main polyphenol classes are proanthocyanidins (as catechins, B2 dimer), phenolic acids (as chlorogenic acids, gallic acids) and anthocyanins (as malvidin-3-glucoside). The principal polyphenol group is monomeric and oligomeric forms of flavanols (which include proanthocyanidins) with more than 10% (high-performance liquid chromatography). Preliminary unpublished observations performed on endothelial human cells demonstrated statistical significant improvements of NO synthesis by these polyphenols. They allowed polyphenols formulation optimisation to reach the highest synergetic effect on endothelial nitric oxide synthase activation (via serine 1177 phosphorylation). This formulation demonstrated the ROS protection effect via downregulation of peroxinitrite (unpublished observations on in vivo trained hamsters) and NO-dependent vasodilation activation (unpublished observations on ex vivo rings of aorta from rats).

2.4. Statistical Analyses

Quantitative variables were presented as mean values and standard deviation. Qualitative variables were expressed as frequencies and percentages. Means are compared using a Student's *t*-test and percentages using Chi square test. Statistics were conducted using SAS software (Ver. 9.2, SAS institute, Inc., Cary, NC, USA). $p < 0.05$ was taken as the level of statistical significance.

3. Results

All recruited participants performed the entire protocol and no adverse event was reported.

3.1. Endurance Test

In comparison with the placebo, the present study revealed a significant increase (+9.7% ± 6.0%, $p < 0.05$) of the time to exhaustion during the endurance test with polyphenols (Table 2). As shown in Table 2, no significant differences were obtained for the maximal and mean heart rate, the maximal blood pressure, the maximal and mean VO_2, and the maximal and mean ventilation between endurance tests performed with polyphenols or with placebo. In contrast, the maximal perceived exertion showed a significant difference, and was reached 2.7 min later (+12.8% ± 6.8%, $p < 0.05$) with polyphenols than with placebo.

Table 2. Parameters recorded during and after endurance tests in polyphenols and placebo conditions.

	Polyphenols	Placebo	*p* Value
Time to exhaustion (s)	1680.2 ± 779.2	1531.5 ± 643.5	0.032
Mean HR (bpm)	166.9 ± 12.0	166.7 ± 12.9	0.917
Mean VO_2 (mL/min/kg)	40.5 ± 5.2	41.5 ± 7.0	0.251
Mean VE (L/min)	94.0 ± 15.9	95.6 ± 16.6	0.313
Maximal HR (bpm)	178.2 ± 12.0	178.3 ± 12.9	0.952
Maximal VO_2 (mL/min/kg)	42.4 ± 5.3	43.1 ± 5.3	0.236
Maximal VE (L/min)	114.8 ± 22.6	117.7 ± 22.1	0.226
Maximal systolic BP (mm Hg)	143.2 ± 14.3	144.9 ± 17.4	0.489
Maximal diastolic BP (mm Hg)	78.1 ± 6.3	77.8 ± 8.3	0.835
Time to reach maximal perceived exertion (s)	1434 ± 594	1272 ± 540	0.005
Half-recovery time for VO_2 (s)	60.9 ± 21.1	52.4 ± 15.2	0.014
Half-recovery time for HR (s)	194.0 ± 95.6	191.5 ± 99.4	0.888
Muscle pain	1.7 ± 0.9	1.5 ± 0.8	0.753

Values are means ± standard deviation. HR: heart rate, VO_2: oxygen consumption, VE: ventilation.

3.2. Recovery

The VO_2 half-recovery time was significantly longer in the polyphenol condition as compared with the placebo (+8.5 ± 11.4 s, $p < 0.05$). No other differences were noticed for the parameters registered after the end of the tests. Finally, muscle pain perception, evaluated 48 h after each experimental session, was not different between conditions (1.7 ± 0.9 and 1.5 ± 0.8 for polyphenols and placebo, respectively; $p = 0.753$).

4. Discussion

The main aim of the present study was to investigate the effects of an acute intake of a specific formulation of polyphenols from apple and grape on endurance capacity and recovery. The present study demonstrated that the intake of polyphenols prior to an endurance exercise increased the time to exhaustion and lengthened the time to onset of maximal perceived exertion as compared with the placebo. Taken together, these results suggested an increased endurance capacity with the acute intake of polyphenols.

The mean endurance test duration was ~25.5 min and ~28.0 min with the placebo and polyphenols, respectively. Such a duration may appear low when considering the power output (70% of the maximal aerobic power). This short duration could firstly be attributed to the initial test that used 1 min increments which could overestimate the maximal aerobic power. Secondly, volunteers were physically active and not specifically trained for cycling. The inherent variability of this population and of the measurements was counterbalanced by the large sample size tested here and the randomized, crossover design. Indeed, as indicated in a recent review [11], most studies considered small

samples. The 48 participants of this randomized, crossover, double-blind study therefore strengthen our conclusions.

The increased time to exhaustion is concordant with some previously published studies [25]. In this last study, the authors registered significant increases in the running time to exhaustion with the acute intake of polyphenols as compared to the placebo [25]. In addition to some increases in the endurance aerobic performance, other authors revealed some improvements in anaerobic power with caffeine-based products [10]. However, the few studies that investigated the effects of the intake of acute polyphenols are often conflicting. Other authors [22,26] did not register any effect of polyphenols on the cycling time-trial performance in elite or well-trained cyclists. Such discrepancies could be ascribed to the training status (elite vs. amateur athletes), nature and duration of exercise or to the type of polyphenols used [23]. For example, Jowko et al. [24] concluded that an acute intake of green tea polyphenols was not efficient to attenuate exercise-induced oxidative stress while Morillas-Ruiz [5] detected some protective effects of polyphenols (mostly from fruits) against exercise-induced oxidative stress. Also polyphenols from cranberries and grapes increased artery flow-mediated dilation [26].

It is important to note that the increased time to exhaustion with polyphenols, observed here, is obtained with a delayed fatigability (as witnessed by the late rate of perceived exertion), but with similar physiological responses compared to the placebo. Indeed, the heart rate, blood pressure, VO_2 and ventilation rate are similar between conditions. These results are concordant with previous findings [10].

Some studies attributed antioxidants' and more particularly polyphenols' effects to enhanced blood flow [26]. Although not tested here, several mechanisms might explain the present increase in endurance through an action on NO. Indeed, polyphenols (notably from grape sources) have great antioxidant capabilities [12,13] and increase the synthesis and bioavailability of NO [14,15], thus having the potential to delay fatigue. Additionally, polyphenols such as green tea or grape have been associated to improved endothelial function [31]. Based on in vivo and ex vivo preclinical unpublished observations with Vinitrox™, we could speculate that performance benefits might be due to the modulation of NO-dependent vasodilation with NO synthesis increase and protection. Previous studies demonstrated that this antioxidant effect might also contribute to improving NO effects by two main actions. Firstly, its lifespan protects against O_2^- and, secondly, it prevents eNOS uncoupling, leading notably to a lower flow-mediated dilation of arterioles [32,33].

Previous studies demonstrated that the primary mechanism of NO is the increase in muscle perfusion through a direct vasodilator action on vascular smooth muscle cells and an inhibition of adrenergic vasoconstriction [34,35]. This muscular hyperemia might induce an increased oxygen supply to muscle cells, as well as higher nutrient supply and metabolite product removal, which would allow an enhanced aerobic metabolism [25]. Another mechanism may lie in the action of NO on the glucose metabolism of the muscle, in particular through an increased muscle uptake. In addition, the endogenous production of NO on the sarcoplasmic reticulum Ca^{2+} is likely to improve muscle contractile performance [36,37].

The second aim of the present experiment was to assess the effects of polyphenol supplementation on exercise recovery. Indeed, an NO production increase is supposed to enhance oxygen and nutrient delivery to active muscles, thus improving tolerance to physical exercise and recovery mechanisms [16]. Contrarily, our results revealed a significant lengthening of the time of half-recovery of the VO_2 under the polyphenol condition. Therefore, the lengthening of the recovery that could reflect the existence of an oxygen debt might be surprising when considering NO effects. However, it is in agreement with the longer duration of the endurance tests. Indeed, the duration of the O_2 debt has been shown to be directly related to the exercise duration [38]. The longer recovery could therefore be primarily attributed to the longer-endurance exercise (obtained with polyphenols ingestion) rather than physiological mechanisms related to polyphenol ingestion and potential vasodilation effects. However, additional measurements are necessary to verify this speculative statement. Our result might be of great interest,

particularly for people wishing to lose weight since the lengthening of the O_2 debt is associated with increased energy expenditure, and more specifically lipids oxidation [38,39].

Lastly, the absence of any muscle pain two days after exercise in both conditions indicates that this acute polyphenol supplementation allowed participants to perform longer exercises without further adverse effects. This is of particular interest for athletes training regularly since one of the limiting factors of training is often muscular pain resulting from effort.

In conclusion, the present randomized, crossover, double-blind and controlled study demonstrated that the acute supplementation of polyphenols in healthy, physically active males allowed significant increases in endurance performance (hereby the capacity to maintain a strong effort) with greater energy expenditure as demonstrated by the lengthening of time to exhaustion and time to maximal perceived exertion. Also, the main cardiovascular and respiratory measured parameters showed no significant differences between conditions. A similar observation was obtained on muscle pain two days after exercise. These results indicate that performance improvements, as a result of acute polyphenol intake, have been obtained under safe conditions and without additional pain. In contrast with some previous studies, the present conclusions were made on an almost large sample size and reinforce the positive effects of polyphenols on cycling endurance. An interesting perspective of this work would be to control diet during the duration of the experiment and to quantify specific biomarkers in order to better understand the mechanisms behind the present results.

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Author Contributions: G.D., F.A.A. and N.B. were responsible for the study design, data analyses and writing of the manuscript. G.D. and N.B. were involved in the experimental procedure and performed data collection. F.A.A. performed the statistical analysis. D.G. participated in the study design and manuscript preparation. All authors read and approved the final manuscript.

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The effects of a single intake of polyphenols extracts® on exercise endurance and recovery in healthy subjects: a controlled, randomized, cross-over, double-blind study versus placebo

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Introduction and objectives

Exercising requires an increase in peripheral blood flow and a vasodilation. Among the various mediator of this vasodilation, nitric oxide (NO) is one of the most important [1] and supplements such as polyphenols are thought to be an interesting ergogenic aid.[2] To date, most of the studies regarding the effects of polyphenols investigated long term supplementation and vascular or blood parameters only.[3] **The present work therefore aimed to study the effects of an acute intake of polyphenol (Vinitrox®) on physical performances.**

Materials & Methods

48 physically active men (31 6 yrs) were included in this study, composed of **3 experimental sessions** interspersed with at least 7 days.

• *First experimental session:* maximal test on an ergocycle to determine maximal aerobic power.

• *Two other testing sessions*: endurance test at 70% of maximal power.
Time to exhaustion was measured as well as the **perception of the effort difficulty** (Borg scale).

The preceding evening and 1 hour before the endurance test, volunteers had to absorb either **2 capsules with 250mg Vinitrox®** each or **2 placebo capsules** according to randomization.

Results

• As shown in Table 1 and 2, there was **no effect of the testing sessions order**.

• In comparison with the placebo, there was a significant **2.5 min increase of the maximal duration of the endurance test with Vinitrox®** (*P*<0.05) corresponding to a 9.7 6.0% increase.

Table 1. Cross over analysis of the maximal duration of the endurance tests.

Order	Vinitrox®	Placebo	Order effect P value	Product effect (Vinitrox® - Placebo)		P value
1	24.7 ± 7.90 min	26.4 ± 12.8 min	0.2277	2.5 ± 4 min	9.7 ± 6.0 %	0.0324
2	32.3 ± 18.1 min	24.7 ± 8.6 min				

• Maximal perceived exertion was reached **2.7 min later** (+12.8 6.8%, *P*<0.05) with Vinitrox® than with placebo.

Table 2. Cross over analysis of the time to onset of the maximal hardship.

Order	Vinitrox®	Placebo	Order effect P value	Product effect (Vinitrox® - Placebo)		P value
1	21.2 ± 6.90 min	21.0 ± 9.9 min	0.3901	2.7 ± 3.3 min	12.8 ± 6.8 %	0.0056
2	26.6 ± 14.9 min	21.4 ± 8.1 min				

• **No difference** were found **for muscle pain occurring** two days after endurance exercises.

Discussion & Conclusion

Vinitrox® appears to:

- enhance endurance (i.e., capacity to maintain an intense effort)
- delay maximal effort perceived exertion.

➔ **Interesting for athletes looking for performance.**

Several mechanisms, associated with NO production, could account for these effects:

- direct vasodilation on vessels smooth muscle cells
- inhibition of the adrenergic vasoconstriction.[4,5]

➔ **Increased oxygen availability to muscle cells allowing a greater and longer aerobic utilisation of glycogen.**

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