SCIENCE PACK

sinetrol®

Proven fat burning solution

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SINETROL®
Proven fat-burning solution

Increasing from year to year, the number of adults who currently have excess body fat is estimated to be 1.9 billion, including 600 million obese (WHO). Excess fat mass is the most common chronic health problem worldwide and one of the greatest public health challenges of the 21st century. The spread of obesity is already responsible for a substantial part of health care costs in industrial countries. Beyond causing various physical disabilities, overweight and obesity drastically increase a person's risk of developing health concerns affecting life expectancy. Nevertheless, overweight and its consequences are preventable.

Recognized as a cultural heritage by UNESCO, the Mediterranean diet is a modern nutritional lifestyle inspired by the traditional dietary patterns of Greece, Southern France, Italy & Spain. The same diet serves as the basis for the WHO's recommendation of consuming at least 400 g of fruit & vegetables daily. Numerous known bioactive substances in this diet improve health and weight management. Giving up the paradigm of the miracle molecule, one of the challenges of innovation within the food supplement industry is now to combine each natural component and optimize positive effects by synergetic partners.

The issue of weight gain resulted from the non-control of 2 complementary parameters: excessive caloric intake and/or inadequate energy expenditure. To address the issue, FYTEXIA® designed SINETROL® as a unique, Mediterranean weight-loss ingredient; the patented combination of natural citrus fruits extracts of orange (*Citrus sinensis* L.) and grapefruit (*Citrus paradisi* Macfad.), in association with an extract of guarana (*Paullinia cupana* Kunth) provides clinically proven enhancement of lipolysis, helping in body weight management.

The results of 3 published clinical studies in a total of 115 adults demonstrate the benefits of SINETROL® for improving the shape of the silhouette and significantly lowering excess body fat. The results also strengthen the evidence for the efficacy of the product in the prevention of metabolic disorders associated with overweight and obesity. Moreover, the last ex vivo study reveals the real synergy generated by the unique selection of each compound of SINETROL® to enhance lipolytic activity and antioxidant protection. The demonstrated synergetic benefits of these polyphenol-rich ingredients provide increased evidences of their potential in weight management.

SINETROL®

Proven fat burning benefits

WHITE PAPER

Excessive body weight is currently the most common chronic health issue worldwide and one of the greatest public health challenges of the 21st century. The prevalence of unbalanced body weight has almost doubled worldwide since the 1980s, and the number of those affected continues to rise at an alarming rate. In 2014, more than 1.9 billion adults were overweight[1]. Of those, over 641 million in 2014 are obese (BMI ≥ 30), which corresponds to 6 times the level reported in 1975(2). The average person had become 1.5kg heavier with each passing decade, and if this trend continues, scientists predict 18% of men and 21% of women worldwide will be obese by the year 2025[2].

Overweight and obesity are regrettably implicated with the leading risks for global deaths[3]. This exclusively includes non-communicable diseases (NCDs): ischaemic heart diseases, strokes, diabetes mellitus, hypertensive heart diseases, and various cancers. At least 2.8 million adults die each year as a result of being overweight or obese. As an example, in different parts of Europe, obesity is already responsible for 2–8% of health costs and 10–13% of deaths[3]. As a primary focus of current worldwide efforts to tackle the increasing epidemic of NCDs, obesity and its consequences are preventable.

What are overweight and obesity?

The body mass index (BMI) is a simple index of weight-for-height and is commonly used to classify overweight and obesity in adults. Since BMI applies to both genders and for all ages of adults, it should provide the most useful population-level measurement of overweight and obesity. Nevertheless, overweight and obesity are beforehand better explained as abnormal or excessive fat accumulation that may impair health, especially when too much fat is accumulated in the abdominal area[4]. Accordingly, a direct measure of abdominal adiposity appears possibly more relevant to classify individuals and the Index of Central Obesity (ICO), which represents waist-for-height ratio, offers a very simple criteria for classification[5]. In addition, a better understanding of the metabolism of cells that store fat in the abdominal area might be considered a key feature of success in developing new strategies for overweight and obesity management (OOM). Obesity and overweight are too frequently associated with decreased high density lipoproteins (HDLs) and increased low density lipoproteins (LDLs) and triglycerides (TGs) — all risk factors for CVDs[6]. Furthermore, obesity and overweight are also associated with chronic low-grade inflammation characterized by abnormal production of markers such as CRP (C-reactive protein) and fibrinogen[7]. All these molecules are involved in many clinical manifestations of NCDs[8-10]. Finally, fat accumulation is also correlated with elevated

markers of oxidative stress, which also play critical roles in the development of NCDs[11-12]. It follows that reducing abdominal fat mass and concomitant oxidative stress and inflammation is a crucial end-goal for the prevention of obesity-related NCDs[13].

How can overweight and obesity be reduced?

The abdominal area is the region of the body where the metabolism generally stores most fat. Reducing abdominal circumference is therefore a question of reducing the ratio of abdominal fat or losing abdominal fat mass. There are two co-existing strategies of achieving OOM and they each go in opposite directions: either decreased caloric intakes or increased caloric depletion. In the context of food supplementation, the former corresponds to a reduction of calorie absorption, generally due to enzyme inhibitors or to satiety enhancers; however, this approach should underline possible nutritional issues because the action is generally based on significant restriction of energy absorption without distinguishing nutritional quality of nutrients within the diet. In turn, this could worsen the absorption of "healthy fats" such as Omega-3 and -6. The other strategy, that of increased caloric depletion, corresponds to a release of stored fat and contributes to a beneficial reduction of abdominal adiposity while individuals can sustain a normal calorie-balanced diet.

How can we enhance a reduction in overweight and obesity?

Recognized as an intangible cultural heritage by UNESCO, the Mediterranean diet is a modern nutritional lifestyle inspired by the traditional dietary patterns of Greece, Southern France, Italy, and Spain. The Mediterranean diet is the basis of the WHO's recommendation for consuming at least 400 g of fruit and vegetables daily, as reported in the WHO Global Strategy on Diet, Physical Activity and Health. The concept is nowadays widely supported by national public health programs in various modern countries.

The philosophy of FYTEXIA® is to bring the benefits of this diet to the greater world by providing unique, scientifically-designed blends of bioactive polyphenols contained in Mediterranean fruit and vegetables. Functioning within a synergetic action, these different polyphenols provide the foundation for an improved metabolism associated with decreased overall NCDs and risk factors for mortality.

SINETROL® Xpur: Mediterranean Diet & Polyphenol Synergy Overlap to create an innovative ingredient to manage body weight

Bioactive polyphenols constitute a widely present organic family of phytochemicals in the plant kingdom. Several phenolic groups are associated in displaying more or less complex structures of various molecular weights. Among them, flavonoids represent the most important class of polyphenolic compounds. The flavonoids are divided into subclasses based on the degree of saturation, oxidation (hydroxyls and methoxyls), glycosylation, and polymerisation. The widespread structural multiplicities of these bioactives contribute to a large number of possibilities for enhancement in regard to their beneficial effects on health. Thus, understanding flavonoids is a topic of increasing importance, especially in the prevention of NCDs[14-21] .

In this context, SINETROL® XPur is a unique citrus fruit-based ingredient made from the juice, peels, and seeds of fruit prepared by physical treatment (crushing, cold-pressure, extraction, centrifugation, filtration, and spray-drying) of specific varieties of sweet and blood oranges (*Citrus sinensis* L.),.), grapefruit (*Citrus paradisi* Macfad.), and guarana (*Paullinia cupana* Kunth). SINETROL® XPur provides a total synergistic polyphenol content of 90% and offers the best of the Mediterranean diet.

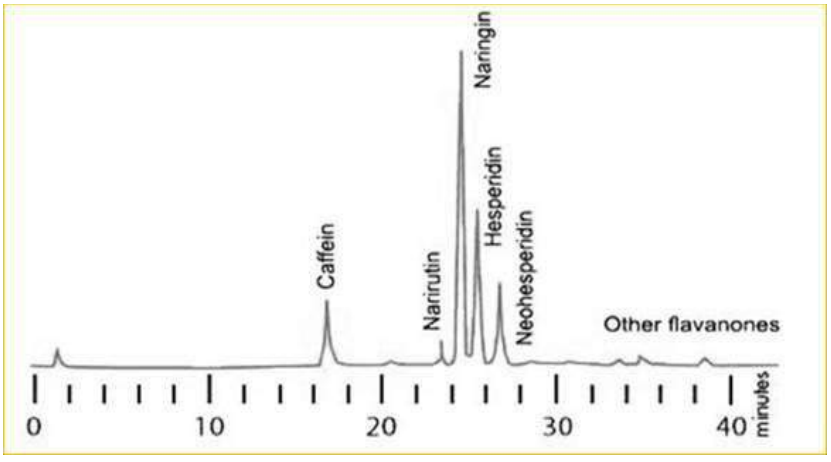

Figure 1: SINETROL® XPur unique HPLC fingerprint at 280 nm

SINETROL®-XPur: main bioactive compounds

SINETROL® XPur's bioactive polyphenols have been identified with the most advanced technologies based on high-performance liquid chromatography (Figure 1). Naringin (Figure 2), a flavanone glycoside derived from naringinin and mainly supplied by grapefruit, represents the most important polyphenol found in SINETROL® XPur. Neohesperidin (Figure 2), the other leading bioactive compound of SINETROL® XPur, is a glycoside derived from hesperidin. Due to their glycoside fractions, the bioavailability of naringin and neohesperidin is enhanced.

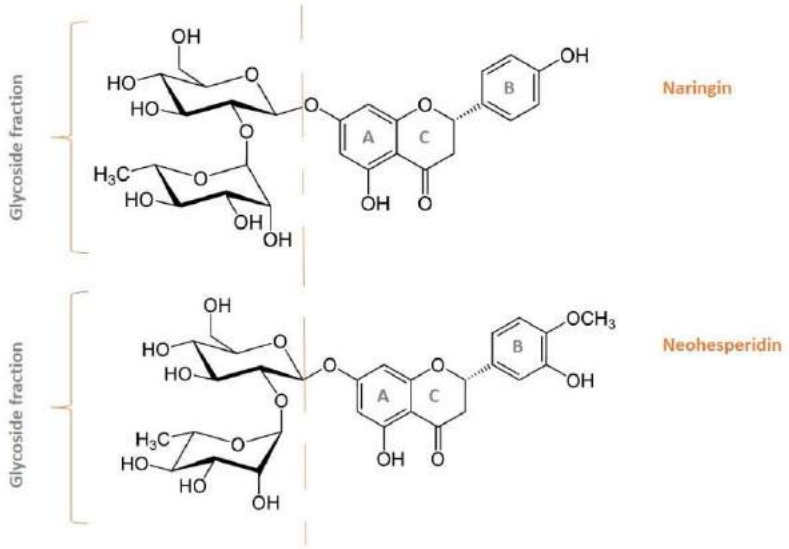

Figure 2: SINETROL® XPur bioactive polyphenols:
Structures of naringin and neohesperidin

SINETROL®-XPur: Mechanism of action

To explain the mechanism of action involved in the decrease of body fat leading to weight loss and reduced waist and hip size, previous *in vitro* studies have shown that certain flavonoids, and especially those found in SINETROL® XPur, possess lipolytic activity[22-23] Lipolysis is a catabolic process leading to the breakdown of TGs stored in fat cells (adipocytes) and the subsequent release of free fatty acids (FFAs) and glycerol[24]

For example, naringin, a flavonoid highly present in grapefruit and currently extracted in SINETROL® XPur, has been reported to induce the expression of fatty-acid oxidation genes, CYP4A11, ACOX, UCP1, and ApoA1[25], every one of which enhance the body's use of FFAs to produce energy. Fatty acids are an important oxidative fuel for the liver, kidney, myocardium, and skeletal muscle. Adipose tissue lipolysis is the major regulator of the body's lipid energy supply because it controls the release of FFAs into plasma, where they circulate interconnected to albumin[26].

The first step of this lipolytic process in adipocytes is regulated by a variety of hormones such as epinephrine, norepinephrine, glucagons, and adrenocorticotropic hormone (ACTH)[27], the production of which is enhanced by the consumption of guarana. The mechanisms of action of these lipolytic hormones are believed to be mediated by the cyclic adenosine monophosphate (cAMP) cascade. Lipolytic hormones activate adenylate cyclase, resulting in increased synthesis of cAMP, leading to activation of cAMP-dependent protein kinase and activation of hormone-sensitive lipase (HSL); so- called because of its responsiveness to insulin and catecholamines[28]. Activation of HSL results in the hydrolysis of stored TGs into FFAs and glycerol. The lipolytic process is stimulated by beta adrenergic agonists[29-30] with high sympathomimetic activity, but also by the inhibition of 2 enzymes: (i) catechol-O-methyl transferase, which degrades norepinephrine[31], and (ii), c-AMP-dependent phosphodiesterase (PDE)[32], which degrades cyclic cAMP and consequently inhibits the activation of HSL (Figure 3).

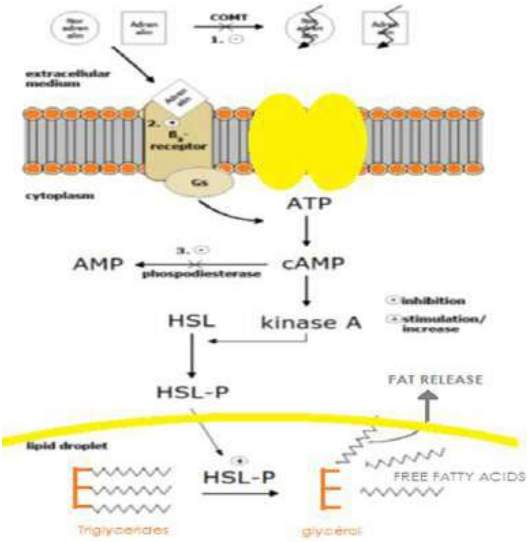

Figure 3: Lipolytic mechanism of action involved in adipocytes by SINETROL® XPur

SINETROL® Xpur: Synergetic Power of Polyphenols for OOM

There are increasing evidences for positive correlations between oxidative stress in fat tissue and adipocyte dysfunction in overweight and obesity[33]. Moreover, a hypothesis emerged that excessive nutritional overload triggers redox changes in adipocyte mitochondria, which impair local and systemic insulin sensitivity, a central metabolic consequence of excessive body fat[33].

Understanding oxidative stress is accordingly fundamental for a better comprehension of overweight and obesity. Thus, efficacy of SINETROL® Xpur has been comparatively studied with the Theoretical version of SINETROL® Xpur in the KRL assay developed to evaluate the capacity of an ingredient to protect red blood cells (RBCs) from oxidative stress (**Figure 4**). In this ex-vivo study, the Theoretical version of SINETROL® Xpur has been calculated from the weighted efficacy of individual ingredients constituting the formulation of SINETROL® Xpur.

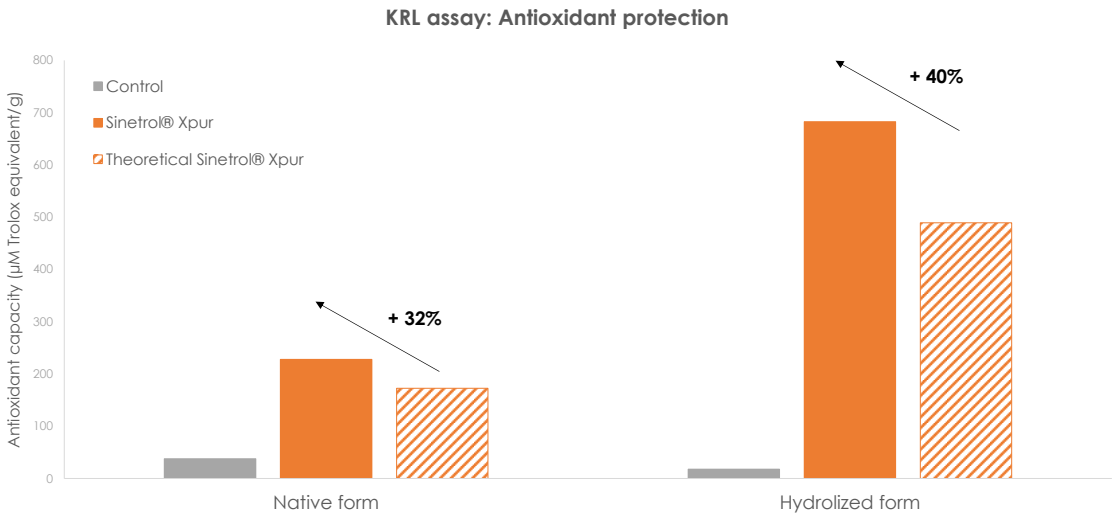

Figure 4: Synergetic efficacy of SINETROL® Xpur *vs* Theoretical SINETROL® Xpur for the protection of red blood cells from induced oxidative stress in the KRL assay.

Figure 4 shows that when acting altogether, polyphenols and other bioactives from SINETROL® Xpur are capable of providing an enhanced protection of RBCs to oxidative stress (+32%). Because in physiological conditions one must reckon with the biological activity of digestive enzymes, the efficacy of SINETROL® Xpur *vs* Theoretical SINETROL® Xpur has been studied after total enzymatic hydrolysis of both SINETROL® Xpur and its individual ingredients. Here again, the synergetic efficacy of polyphenols is confirmed for SINETROL® Xpur *vs* Theoretical SINETROL® Xpur with a similar, significantly

enhanced protection against oxidative stress (+40%). In addition, it is also relevant to highlight the importance of digestive enzymes in their efficacy of releasing a superior protective ability of polyphenols, both from SINETROL® Xpur and its Theoretical version (respectively, ≅ +200% and ≅ +184%).

The comprehension of adipocyte metabolism toward oxidative stress is fundamental to understanding either fat storage or depletion. Despite the fact that it is now confirmed that redox status plays a significant role in fat-release, it is nonetheless essential to remind that in the end, only lipolytic activity remains as the predominant metabolic pathway that leads towards abdominal fat loss.

Results from **Figure 5** demonstrate that when cultivated in physiological conditions in a 3D-model with 10 µM of SINETROL® Xpur in hydrolysed form, adipocytes obtained from the abdominal area of overweight to obese donors show higher lipolytic activity, as assessed with glycerol production kinetics, within the period of a whole day (24h) when compared to a Control group corresponding to basal endogenous lipolytic activity or to the Theoretical version of SINETROL® Xpur. These results establish for the first time the direct efficacy of SINETROL® Xpur on fat-release from adipocytes and confirm the synergetic activity of polyphenols and other bioactives previously demonstrated to act against oxidative stress. These results are moreover emphasized with the measure of accumulated glycerol production (**Figure 6**), the main biomarker of lipolysis.

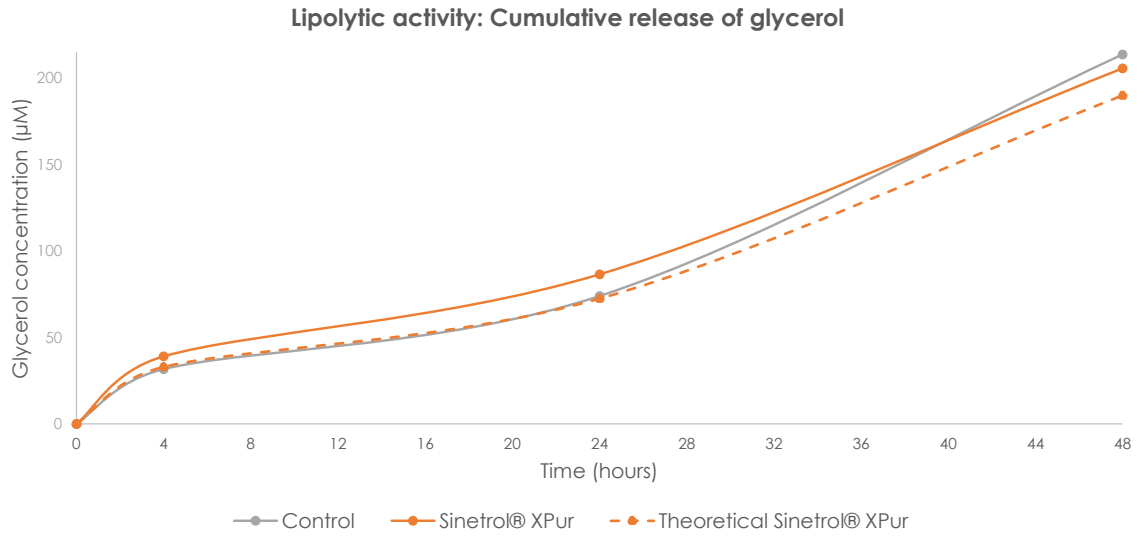

Figure 5: Lipolytic activity of SINETROL® Xpur *vs* Theoretical SINETROL® Xpur and comparison to a Control group within the period of a whole day (24h) and beyond.

Indeed, during the first period of 0–4h after addition of the studied products 10 µM, and when compared to the Control group considered the base with a value of 100%, SINETROL® Xpur is capable of contributing a significantly higher rate of lipolysis than that of the Control group and even surpasses that of the Theoretical SINETROL® Xpur version, for which the rate is not significantly different compared to the rate of the Control group. In all groups during the period 4–24h, levels of polyphenols and other bioactives have clearly started to become depleted. Yet, despite diminishment, their impact on the relative over-production of glycerol is clearly significantly higher than for both the Control group and the Theoretical version of SINETROL® Xpur. After 24h, polyphenols and other bioactives have probably been fully metabolized and SINETROL® Xpur no longer provides more efficiency relative to the Control group. During this last period, the Theoretical version of SINETROL® Xpur even produced a small but significant decrease in lipolysis when compared to the Control group, highlighting a possible anti-lipolytic activity in long-term periods of incubation once polyphenols and other bioactives have all been metabolized and not cleared out of the medium, and prior to and without having been replenished.

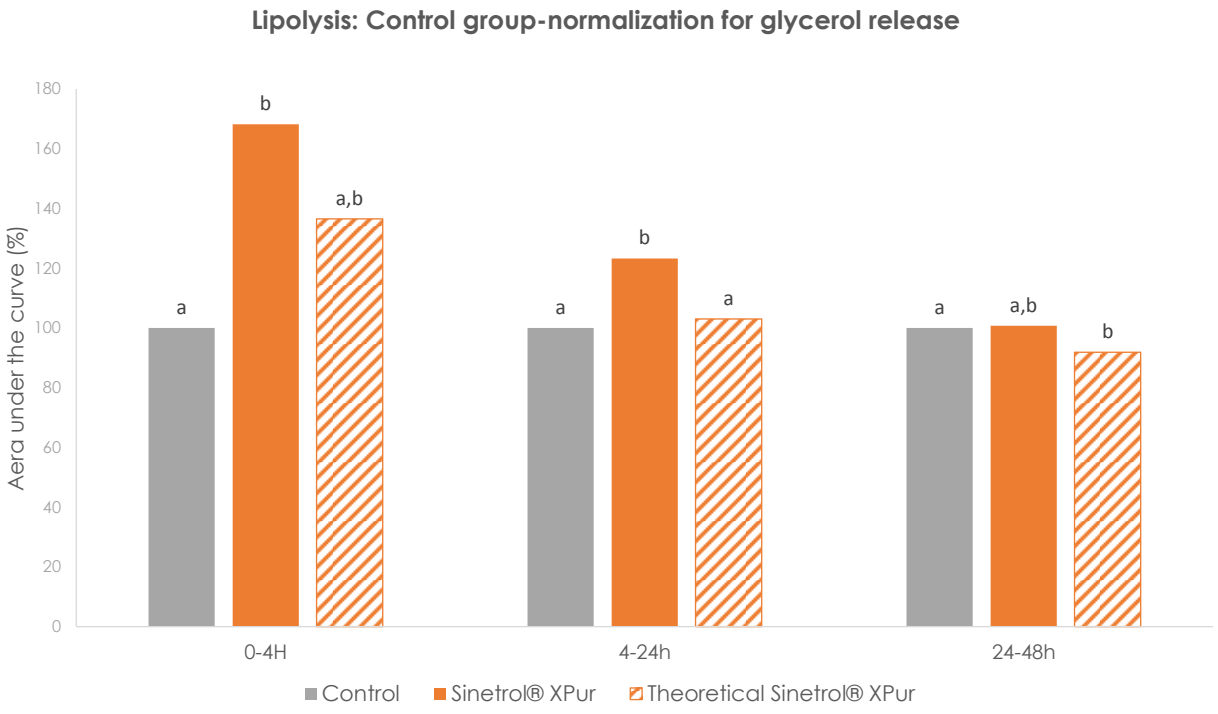

Figure 6: Cumulated production of glycerol in 3 different periods after the addition of SINETROL® Xpur, Theoretical SINETROL® Xpur, and in the Control group.

Accordingly, **Figure 7,** which shows the lipolytic rate as a function of time of incubation, clearly emphasizes that once the first 24h-period is over, the lipolytic rate significantly decreases until anti-lipolytic activity when compared to the baseline of the Control group. For sustained, enhanced lipolysis, this highlights the need of the adipocyte to be perfused again with a new dose of SINETROL® Xpur. This is also the case when, in order to maintain a long-term efficient efficacy to support endogenous lipolysis, individuals supplemented with SINETROL® Xpur renew their supplementation everyday.

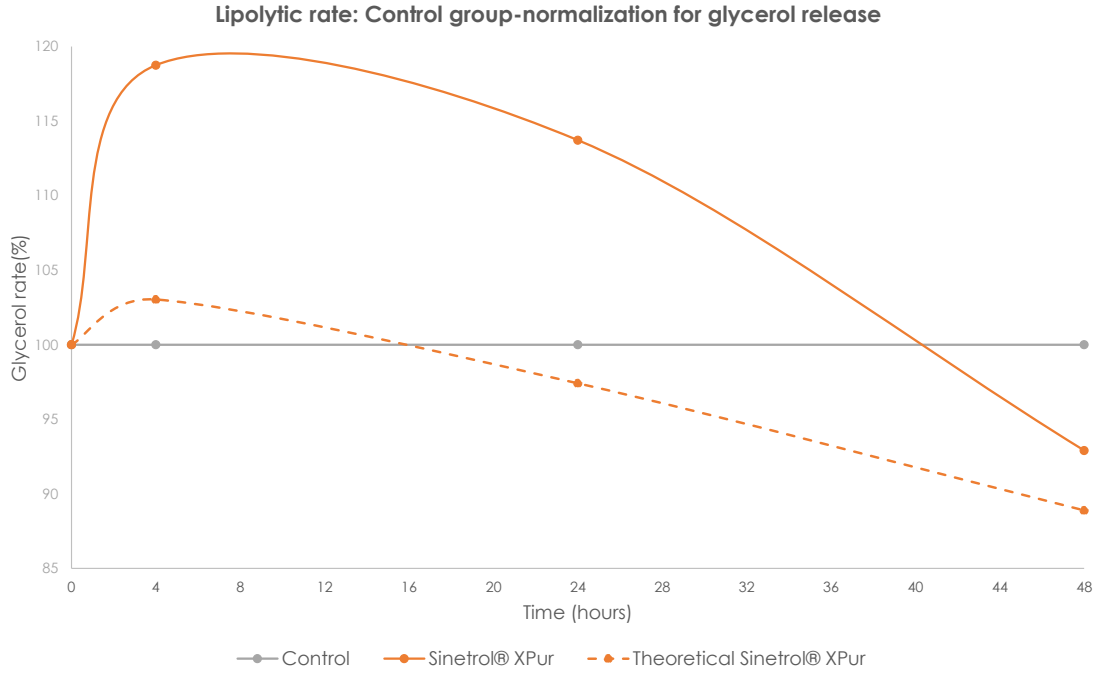

Figure 7: Rate of lipolysis as a function of time of incubation for SINETROL® Xpur and SINETROL® Xpur compared to the Control group with a value of 100%.

These innovative results demonstrate that SINETROL® Xpur is a food supplement based on the synergetic power of polyphenols acting altogether for an enhanced solution providing excellent results at various levels of the metabolism, either toward oxidative stress or to support basal endogenous lipolysis; two different metabolic pathways that seem to be cross-linked for a more efficient management of body fat.

SINETROL® Xpur is designed to enhance basal endogenous lipolytic activity. The previously demonstrated mechanism of action is the maintainence of cAMP levels through the inhibition of phosphodiesterase (PDE).

Human intervention study

The efficacy and safety of SINETROL® XPur in body weight management, improvement of metabolic disorders, and alleviation of inflammatory and oxidative status has been assessed in a 12-week, randomized, double-blind, placebo-controlled human intervention study. SINETROL® XPur was given to overweight subjects twice daily with meals, and the results in the test group (N=47) were compared to those in the placebo group (N=48).

Silhouette shaping

Based on the findings of the intervention study, dietary supplementation with SINETROL® XPur presents a promising strategy to counteract overweight and associated fatness. Body weight, fat mass, and waist and hip circumferences continuously decreased during the study period and reached significant outcomes after 12 weeks of treatment (Figures 8 and 9). Whereas body weight was significantly reduced by 2.61 kg in the SINETROL® XPur group compared to the placebo group ($p<0.0001$), abdominal body fat significantly decreased by 9.73% in the test group, which was 6.55% more than in the placebo group ($p<0.0001$).

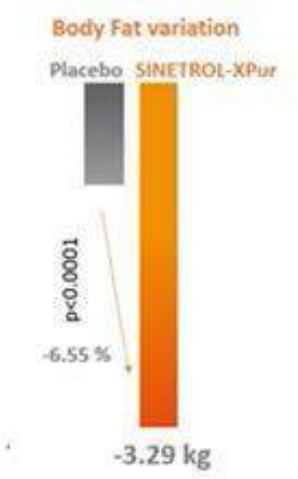

Figure 8: SINETROL® XPur induces a significant decrease in body fat

The benefits of SINETROL® XPur were evident in the global thinning of the silhouette. Waist and hip measurements were considerably reduced by 5.15 cm and 5.17 cm, respectively, corresponding to 3.73 cm and 3.74 cm more ($p<0.0001$) compared to the placebo group.

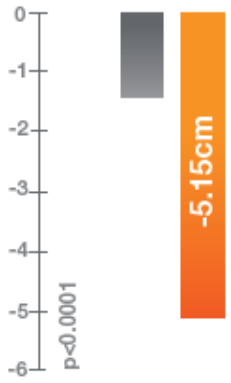

Figure 9: SINETROL® XPur induces a significant reduction in waist size and hip circumference

SINETROL® XPur lipolytic effect

In the SINETROL® XPur human intervention study, the lipolytic mechanism of action involved in the reduction of body fat and previously demonstrated *in vitro* and *ex vivo*[23]was confirmed as measured by FFA release in the plasma at significantly higher levels ($p<0.0001$) in the SINETROL® XPur group with an increase of more than 300% compared to placebo, in which the increase of around 30% was only slight. This remarkable difference demonstrates that the combination of *Citrus* fruits and guarana in the extract formulation contains an array of potent bioactive compounds that can seriously generate weight loss through increased fat loss (Figure 10).

Figure 10: Lipolytic effect of SINETROL® XPur on FFA release from adipocytes

Improvement of inflammatory status

As previously noted, obesity- and fat mass-related complications increase susceptibility to enhanced states of chronic and low-grade inflammation and tend to increase oxidative stress, which is directly linked to the occurrence of increased risk of NCDs in overweight and obese people[12,33]

In regards to the SINETROL® XPur interventional study, while there was no difference between groups at baseline, inflammatory markers (as expressed by both CRP and fibrinogen) showed significant differences between the SINETROL® XPur and placebo groups. Chronic and low-grade inflammation, as assessed with CRP analysis, revealed a 22.87% decrease with SINETROL® XPur compared to a significant increase of 61.79% in the placebo group. Statistically, the difference between the two groups was highly significant ($p<0.0001$). In addition, a significant decrease in fibrinogen of 19.91% was observed after 12 weeks of supplementation with SINETROL® XPur compared to no notable changes in the placebo population, thereby strengthening the evidence for therapeutic effects of SINETROL® XPur in lowering markers of chronic and low-grade inflammation.

Amelioration of oxidative stress

Because overweight and chronic and low-grade inflammation are well correlated with elevated markers of oxidative stress, the effects of SINETROL® XPur on oxidative status were evaluated. At baseline, levels were within normal range with no significant difference between the 2 groups. After 12 weeks of treatment, SINETROL® XPur significantly increased ($p<0.01$) the production of two major protectant antioxidants, super oxide dismutase (SOD) and glutathione (GSH), when compared to the placebo group. This led to a significant decrease ($p<0.0001$) in oxidative stress as assessed by levels of malondialdehyde (MDA), a highly reactive end product of lipid oxidation. While SOD and GSH were respectively raised by 15.19% and 6.99% more in the SINETROL® XPur population versus placebo, MDA levels decreased by 16.79% more in the active treatment group than in the placebo group.

Effects of 12 weeks of SINETROL®-XPur treatment on global benefits.

These results demonstrate the benefits of a 12-week regular consumption of a unique and innovative *Citrus*-based polyphenolic dietary supplement, SINETROL® XPur, to improve the silhouette while significantly lowering excess body fat. The proven synergy of the polyphenols contained in SINETROL® XPur could be a useful strategy in weight loss programs in order to boost the benefits of losing fat and reducing risk factors and complications associated with excess weight.

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Lipolytic effect of a polyphenolic citrus dry extract of red orange, grapefruit, orange (SINETROL) in human body fat adipocytes. Mechanism of action by inhibition of cAMP-phosphodiesterase (PDE)

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Abstract

The present study investigated the lipolytic (break of fat stored) effect of a citrus-based polyphenolic dietary supplement (SINETROL) at human adipocytes (*ex vivo*), body fat (clinical) and biochemical levels (inhibition of phosphodiesterase). Free fatty acids (FFA) release was used as indicator of human adipocyte lipolysis and SINETROL activity has been compared with known lipolytic products (isoproterenol, theopylline and caffeine). SINETROL stimulated significantly the lipolytic activity in a range of 6 fold greater than the control. Moreover, SINETROL has 2.1 greater activity than guarana 12% caffeine while its content in caffeine is 3 times lower.

Clinically, two groups of 10 volunteers with BMI relevant of overweight were compared during 4 and 12 weeks with 1.4 g/day SINETROL and placebo supplementation. In the SINETROL Group the body fat (%) decreased with a significant difference of 5.53% and 15.6% after 4 and 12 weeks, respectively, while the body weight (kg) decreased with a significant difference of 2.2 and 5.2 kg after 4 and 12 weeks, respectively.

These observed effects are linked to SINETROL polyphenolic composition and its resulting synergistic activity. SINETROL is a potent inhibitor of cAMP-phosphodiesterase (PDE) (97%) compared to other purified compounds (cyanidin-3 glycoside, narangin, caffeine). These results suggest that SINETROL has a strong lipolytic effect mediated by cAMP-PDE inhibition. SINETROL may serve to prevent obesity by decreasing BMI.

Keywords: Lipolysis; Citrus; Adipocytes; Phosphodiesterase; Body fat; Free fatty acids (FFA)

Introduction

People are becoming fatter in all parts of the world. Recent studies show that excess body fat weight is pandemic, with one-half to two-thirds of the overall study population (men and women in 65 countries) being overweight or obese in 2006. People who are overweight have a higher risk of heart diseases, type II diabetes and other diseases including some cancers (Balkau et al., 2007).

In this context, it seems interesting to consider a food supplement based on polyphenols that could contribute to the loss of body fat weight without any secondary effect.

SINETROL is a polyphenolic mixture of flavonoids such as anthocyanins and flavanones. It is a citrus-based

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fruits (juice, peels, seeds) extracted by physical treatment (crushing of fruits, cold pressure of juice, extraction, centrifugation, filtration, spray drying) of a specific varieties of red orange (*Citrus sinensis L. Osbeck* (*Blood group*)) sweet orange (*Citrus aurantium L. var. sinensis*), bitter orange (*Citrus aurantium L. var.amara*), grapefruit (*citrus paradise*) and guarana (*Paulinia cupanna*).

Polyphenols constitutes a widely present organic family of phytochemicals molecules in the vegetal kingdom. They are characterized by the presence of two aromatic rings (A and B) which are linked via an oxygenated heretocycle (ring C). Several phenolic groups are associated in more or less complex structures generally of high molecular weight.

The most important class of polyphenolic compounds is flavonoids. The flavonoids are divided in sub-classes based on the position of the B and C rings as well as the degree of saturation, oxidation and hydroxylation of the C ring. The number of these conjugates contributes to the large number of flavonoids, estimated at more than 5000 compounds.

The flavonoid sub-classes are most commonly known *as anthocyanins* (malvidin, cyanidin, petunidin) red pigments found in the red fruits (red orange, blueberries, red grapes and wine), as *flavanones* (naringin, hesperidin, narirutin, naringenin, etc.) found in citrus fruits (orange, lemons grapefruit), as *flavan-3-ols* (catechins, epigallocatechin, etc.) found in green tea apples, red wine, and as *flavonols* (quercetin, kaempherol) found in onions, apples, broccoli.

Flavonoids take an increasing importance, notably regarding their beneficial effects on health. Indeed, their role of natural antioxidant arouse interest for the prevention and treatment of cancer (Chen et al., 2004), inflammatory diseases (Laughton et al., 1991), cardiovascular diseases (Frankel et al., 1993) and neurodegenerative diseases (Orgogozo et al., 1997). Several studies have shown that flavonoids possess lipolytic activity via inhibition of cAMP-phosphodiesterase and maintaining lipolysis-inducing cAMP levels (Kuppusamy and Das, 1992).

Lipolysis is a catabolic process leading to the breakdown of triglycerides (TG) stored in fat cells (adipocytes) and the release of free fatty acids (FFA) and glycerol (Renold and Cahill, 1965). Fatty acids are important oxidative fuel for liver, kidney, skeletal muscle and myocardium. Adipose tissue lipolysis is the major regulator of the body supply of lipid energy because it controls the release of fatty acids into plasma, where they circulate as FFA complexed to albumin (Spector, 1975).

The first step of this lipolytic process in adipocytes is regulated by a variety of hormones such as epinephrine, norepinephrine, glucagons and adrenocorticotropic hormone (ACTH) (Robidoux et al., 2006). The mechanisms of action of these lipolytic hormones are believed to be mediated by the cAMP cascade. Lipolytic hormones activate adenylate cyclase, resulting in increased synthesis of cAMP, leading to activation of cAMP-dependant protein kinase and activation of hormone-sensitive lipase (HSL), so named because of its responsiveness to insulin and catecholamines (Steinberg and Khoo, 1977). Activation of hormone-sensitive lipase results in the hydrolysis of stored triglycerides into FFA and glycerol.

The lipolytic process is stimulated by beta adrenergic agonists (Mochizuki and Hasegawa, 2004a, b) with high sympathomimetic activity, but also by the inhibition of 2 enzymes: (i) catechol-*O*-methyl transferase, which degrades norepinephrine (Shixian et al., 2006), and (ii) c-AMP-dependent phosphodiesterase (PDE) (Girotti et al., 2005), which degrades cyclic cAMP and consequently inhibits the activation of HSL.

In the present study we firstly investigated the lipolytic effect of SINETROL in human adipocytes by measuring free fatty acid (FFA) release and secondly the potential of a daily intake of 1.4 g/SINETROL in decreasing the body weight fat and the body mass index (BMI) in human healthy subjects. In a third step, SINETROL was tested for its ability to inhibit cAMP-PDE.

Materials and methods

Reagents

SINETROL and the extract of guarana 12% caffeine were supplied by Fytexia (Beziers, France). SINETROL composition of active ingredients was total polyphenols (expressed as catechin): 60%; total flavanones (expressed as naringin): 16,7%; total anthocyanins (expressed as cyanidin-3-glycoside): 2%; and caffeine: 3.6%.

Guarana (paulinia cupana) 12% is a fruit extract standardised naturally in caffeine (12%).

Purified cyanidin-3-glucoside (96% by HPLC) and naringin (70% by HPLC) were supplied by Extrasynthese (Lyon, France).

Purified theopylline (99%), isoproterenol (99%) and caffeine (99%) were purchased from Sigma-Aldrich (St Quentin Fallavier, France).

SINETROL polyphenols analysis

Total polyphenols analysis was performed by the UV-method using a spectrophotometer SHIMADSU 1601 with a detection at 280 nm wavelength (as described by Dallas and Laureano, 1994a, b). SINETROL sample for analysis was obtained by dissolution of 20 mg in 50 ml of distilled water. A volume of 1 ml of this solution was

removed and completed to 50 ml with distilled water and absorbance was measured at 280 nm. An external standard (catechin) (Extrasynthese, France) was used to quantify the total polyphenols. A standard curve was prepared by using catechin from 4 to 16 mg/l and related absorbance was measured (A_{280}).

SINETROL flavanones analysis

Flavanones HPLC-UV analysis was performed using a using Thermo Electron (UV 600) system, equipped with an analytical column (PLRP-S; 1000 Å; 8 mm). The mobile phase was composed of acetonitrile (A)/water (B)/acetic acid 0.5% (C). A linear gradient was run from 10%(A)/80%(B)/10%(C) to 30%(A)/50%(B)/20%(C) during 40 min. Flow rate was 1 ml/min and detection was made at 280 nm (Fig. 1A). Flavanones were identified by using previously Narirutin, Naringin, Hesperedin and Neohesperedin as external standard obtained from (Extrasynthese, France) and quantified by using Naringin (Extrasynthese, France) as external standard.

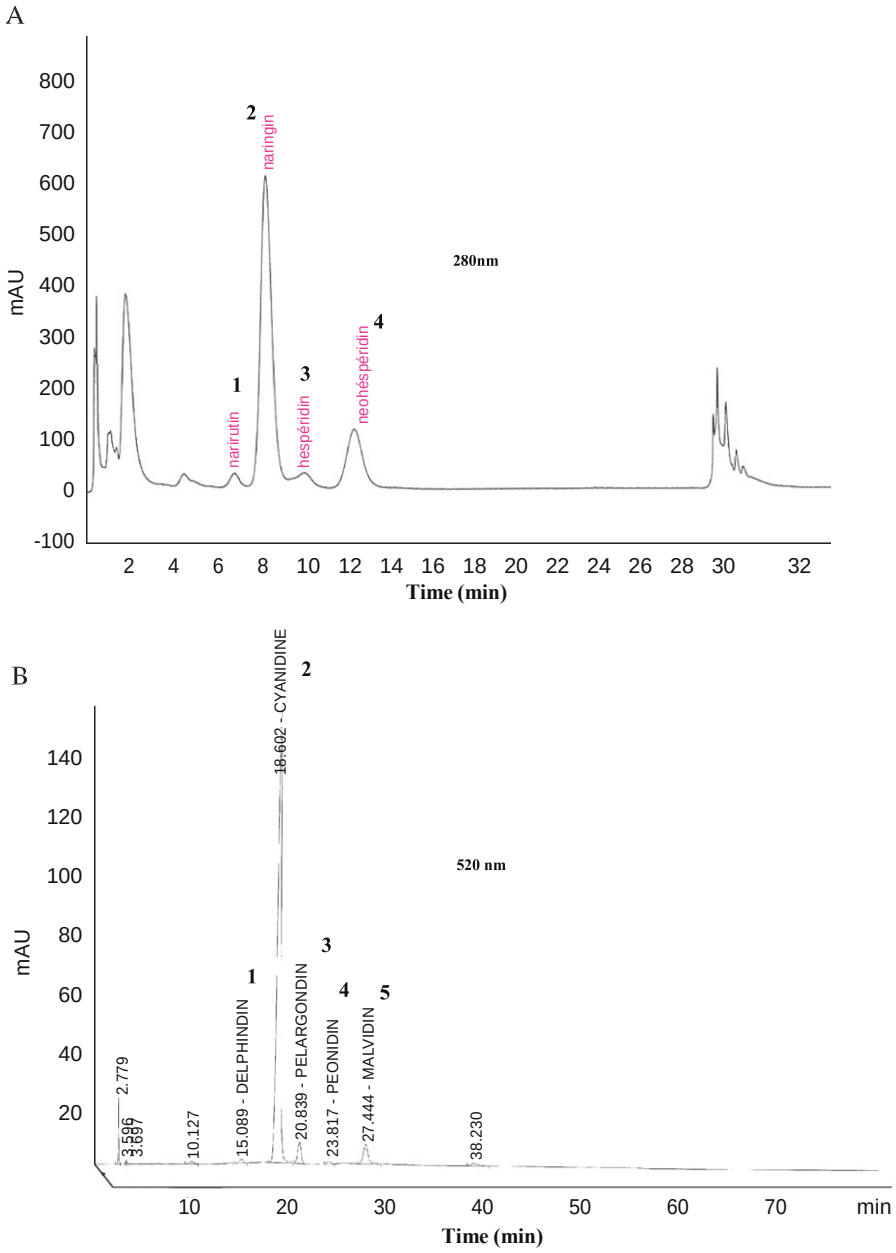

Fig. 1. (A) A typical flavanones HPLC Chromatagram of SINETROL recorded at 280 nm. (1) Narirutin; (2) naringin; (3) hesperidin; (4) neohesperidin. (B) A typical Anthocyanin HPLC Chromatagram of SINETROL recorded at 520 nm. (1) Delphinidin-3-glucoside; (2) cyanidin-3-glucoside; (3) pelargonidin-3-glycoside; (4) peonidin-3-glucoside; (5) malvidin-3-glucoside.

SINETROL anthocyanins analysis

Anthocyanins HPLC-UV analysis was performed using a PERKIN ELMER system, equipped with a reversed phase column Superpher 100, C18 (Merck, Germany) (as described by Dallas and Laureano, 1994a, b; Dallas et al., 1995, 1996a, b). The solvent was 40% formic acid (A)/acetonitrile (B)/water (C). The initial conditions were 25%(A)/6%(B)/69%(C) for 15 min followed by a linear gradient to 25%(A)/25,5%(B)/49,5%(C) during 70 min. Flow rate was 0.7 ml/min and detector wavelength at 520 nm (Fig. 1B). Anthocyanins in SINETROL were identified by using previously external standard obtained from (Extrasynthese, France) and concentration of monomeric anthocyanins was quantified by using cyanidin-3-glucoside chloride (Extrasynthese, France) as external standard.

SINETROL caffeine analysis

Caffeine HPLC-UV analysis was performed using a Thermo Electron (UV 600) system, equipped with a reversed column C18. A preliminary extraction with aqueous acidified solution was realized. The mobile phase was composed of water/acetic acid/acetonitrile. Flow rate was 1 ml/min and detection was made at 270 nm. Concentration of caffeine in SINETROL was quantified by using caffeine obtained from Extrasynthese, France, as external standard.

Normal human adipocyte isolation and treatments

Normal human adipocytes were freshly isolated from surgical samples of healthy abdominal skin (35-year-old woman) as described (Rodbell, 1964). Briefly, pieces of human adipose tissue were incubated for 30 min at 37 °C with 12,500 CDU/ml of collagenase solution (EG/EC 2325829 Sigma-Aldrich, St Quentin Fallavier, France). Adipocyte suspensions were washed and diluted in minimum essential medium supplemented with 1.87 mg/ml sodium bicarbonate, 50 UI/ml penicillin/streptomycin, 2 mM L-glutamine, 0.5% fatty acid-free bovine albumin. Normal human adipocytes were incubated under gentle shaking for 2 h at 37 °C with or without 20 mg/ml guarana 12% caffeine or 20 mg/ml SINETROL; theopylline (1 mM), isoproterenol (1 μM) and caffeine (0.5 mM) were used as positive controls.

Lipolysis assay

Free fatty acid release was used as the indicator of adipocyte lipolysis and was measured using FFA-C kit (OXOID, Dardilly, France). Results were expressed as micromoles of FFA or percentage of control. The absence of interference of the test substances on the FFA assay was checked (data not shown).

Statistical analysis

The raw data were analysed with PRISM® software (Graph Pad Software, Sigma-Aldrich, St Quentin Fallavier, France). The inter-group comparisons were performed by variance analysis (ANOVA) using the Dunnett's multiple comparison test.

Human clinical study

Subjects- enrolled criteria

A total of 20 volunteers participated in a randomized, placebo, doubled blinded trial protocol. The pre-inclusion of volunteers was made based on

- *inclusion criteria*: to be between 25 and 55 years old, to have a body mass index (BMI) between 27 and 33, to be in full health, not taking any drugs or dietary food supplements.
- *excluding criteria:* pregnancy, smokers, persons with hepatic, cardiovascular, renal dysfunctions, having pathologies on going or active during the last month, having received medical treatment (allopathic or homeopathic) during the previous months, having taken a dietary food supplement or drugs during the last month.

After pre-inclusion, volunteers were screened using our evaluation test and after screening 20 volunteers were used as the subjects for our clinical trial. Participation in the study was based on informed consent.

Treatment protocol

The subjects were assigned by randomisation into two groups of 10 peoples. The *treatment group* received a dietary supplement of 4 pieces hard capsules per day containing 350 mg of SINETROL and maltodextrin (1.4 g/day) supplied by Fytexia, France, while the *placebo group* received 4 pieces hard capsules per day containing 350 mg of maltodextrin alone. The two tested products (placebo and SINETROL) were administrated twice daily, in the morning and during the main meal.

Hard capsules (red color) were indistinguishable and were administrated in a double blind approach. The subjects were tested 5 times during a visit to the doctor and dietician. The first time was before the supplementation (T0). A test was planned at 1 week (W1) after taking the dietary supplement, at 4 weeks (W4), at 8 weeks (W8) and finally at the end of the trial, 12 weeks (W12). The evaluation tests were filled by the doctor. During the clinical trial, participants maintain their

previous daily physical exercise and eating habits (1500–2000 cal/day) without any particular dietetic program.

Measurement

The International Day for the Evaluation of Obesity (IDEA) study looked at 2 measures of fatness: waist circumference and body mass index (BMI). A BMI (weight in kg divided by square of height in meters) of 18.5–25 is considered healthy. A BMI over 25 is deemed overweight and greater than 30 is obese.

Subjects for our study were monitored for body composition (body fat/body lean) by impedance bioelectrical balance (TANITA) analysis and by anthropometric measures (BMI, body weight, waist circumferences). A global satisfaction test (silhouette, acceptability, efficacy, secondary effects) was monitored at the end of the clinical trial (W12).

The placebo group was 10 overweight persons (9 women, 1 man) with BMI between 27 and 30, age between 22 and 55 years old and mean weight: 73 kg.

The treatment (*SINETROL*) *group* was 10 persons (7 women, 3 men), 4 obese women with a BMI between 29 and 33 and a overweight group (3 women, 3 men) with a BMI between 27 and 30, age between 25 and 55 years old and mean weight 70.50 kg.

Statistical analysis

Results are expressed as mean7SD. A Kolmogornov–Smirnov test for normality and a Bartlett test for homogeneous variance were performed for each group. All the data were analyzed using a nonparametric Kruskal–Wallis test, and differences between groups were tested using the Mann–Whitney U test (po0.05 was considered significant). All analyses were done using the Statview software version 4.51.1 (Abacus Concepts, Berkley, CA, USA).

Phosphodiesterase activity assay

Phosphodiesterase activity was measured by a scintillation proximity assay (SPA)-based method (Amersham Biosciences, Orsay, France). The tested substances guarana 12% caffeine, SINETROL, cyanidin-3 glucoside and naringin were diluted to 0.01% in DMSO. Caffeine diluted to 0.01% in DMSO was used as a positive control and DMSO diluted to 1% (the maximal amount of DMSO in the assay) was used as a negative control. Phosphodiesterase 3^0-5^0-cyclic nucleotide 5^0-nucleotidohydrolase (EC: 3.1.4.17 Sigma-Aldrich, St Quentin Fallavier, France) was incubated for 10 min at +4 1C with or without the tested substances. The reaction was initiated by the addition of 3^05^0-[3 H]cAMP at 0.5 mCi/ml and incubated for 15 min at +30 1C. Yttrium SPA PDE beads (Amersham Biosciences, Orsay, France) were added to the reaction and incubated for 20 min at +30 1C. The 5^0-[3 H]AMP produced by the phosphodiesterase activity specifically binds to SPA yttrium silicate beads and excites the scintillation liquid finally added to tubes. The relative amount of the reaction product was measured by scintillation counting.

Statistical analysis

The raw data were analysed with PRISMs software (Graph Pad Software, Sigma-Aldrich, St Quentin Fallavier, France). The inter-group comparisons were performed by variance analysis (ANOVA) using the Dunnett's multiple comparison test.

Results and discussion

Lipolytic activity on human adipocyte

The lipolytic effect of SINETROL, three purified substances (theopylline, isoproterenol and caffeine) and guarana 12% on human adipocytes is presented in Table 1 and Fig. 2. FFA release was used as indicator of adipocyte lipolysis as described in Material and methods. Isoproterenol stimulated lipolysis via beta adrenergic receptor activation and cAMP-dependent signalling (Robidoux et al., 2006), while caffeine (Jiang et al., 1998) and theopylline (Beavo et al., 1971) induced lipolysis by inhibition of PDE. Moreover, as described in our PDE experiments, caffeine also act with weak

Table 1. FFA assay after treatment of human adipocytes (fat cells) by various lipolytic products

Tested products	Concentrations	FFA (mM)	FFA/control (%)
Control	–	36710[a]	100728[a]
Theophylline	1 mM	38179[b]	1057726[b]
Isoproterenol	1 mM	377711[b]	1048731[b]
Caffein	0.5 mM	33974[b]	941712[b]
Guarana 12% caffeine	20 mg/ml	101711[d]	281730[d]
SINETROL	20 mg/ml	213713[c]	592736[c]

Values are mean7SE, n ¼ 3, for each tested product.
Means within rows followed by the same superscript are not significantly different (po0.05).

affinity as PDE inhibitor and can also stimulate lipolysis in this way.

The analysis of the results (Fig. 2) confirms that theophylin and isoproterenol stimulate significantly (po0.01) the lipolysis in a range of 10 fold greater than control, which represent a liberation of FFA of 36 mM in 2 h. Purified caffeine at 0.5 mM stimulates also the liberation of FFA in a range of 9.5 fold greater than control (po0.05).

In the same order of magnitude, the SINETROL stimulates significantly (po0.05) the lipolytic activity in a range of 6 fold greater than the control (Table 1, Fig. 2). For guarana 12% (standardised naturally in caffeine) the measurement of its lipolytic activity (FFA release) showed an increase in a range of 2.8 fold greater compared to control (po0.05). Moreover, guarana 12% and SINETROL have been tested at the same assay concentration (0.2%) and the results showed that SINETROL has 2.1 greater activity than guarana 12% (po0.05), while SINETROL content in caffeine (3.6%) is 3 times lower.

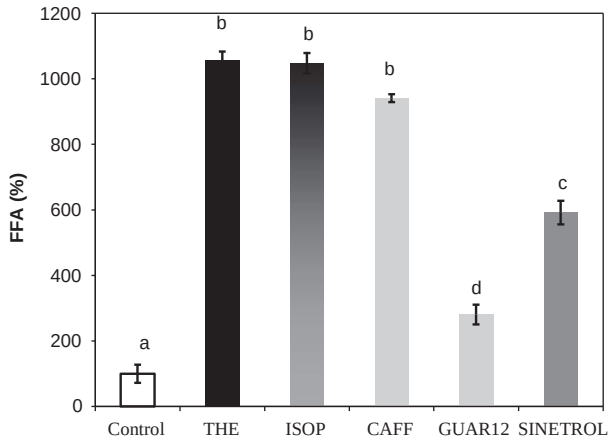

Fig. 2. FFA release after treatment of human adipocytes by various lipolytic products. Fat human adipocytes solution (540 ml) was added to 60 ml solution of tested compounds and each reaction mixture was incubated for 2 h at 37 1C. FFA released from adipocytes were measured as combined FFA-BSA in 30 ml assay medium as described in Materials and methods (method 1). Values are expressed as mean7SE. Bars with different index letters are significantly different (po0.05). *Tested products: THE*: theopylline at 1 mM final concentration; *ISOP*: isoproterenol at 1 mM; *CAFF*: caffeine at 0.5 mM; *GUAR*12: guarana dry extract standardised at 12% of caffeine at final concentration 20 mg/ml; *SINETROL*: citrus-based dry extract standardised at 70% polyphenols at final concentration 20 mg/ml.

These results suggest that SINETROL showed potent lipolytic activity via PDE inhibition. Some dietary supplements (rich in flavonoids) has been related for their lipolytic effect. Pycnogenol, a pin bark extract that contains a mixture of proanthocyanidins, has strong lipolytic activity and effects via stimulation of beta receptor-mediated activity (Mochizuki and Hasegawa, 2004a). Green tea extract, which contains (+)-catechin and (−)- epicgalocatechin-3-gallate (EGCG), has strong lipolytic activity related to EGCG, while catechin did not produce a significant increase ((Mochizuki and Hasegawa, 2004b).

Recently, it has been demonstrated (Tsuda et al., 2005) that anthocyanins have the potency of anti-obesity in mice by the enhancement adipocytokine secretion and adipocyte gene expression in adipocytes. Based on the gene expression profile, up-regulation of hormone-sensitive lipase and enhancement of the lipolytic activity by the treatment of adipocytes with cyanidin 3-glucoside, have been demonstrated.

Human clinical study

The results of supplementation with placebo and SINETROL on body mass index (BMI), body weight and body fat evolution in 20 healthy volunteers during 4 and 12 weeks is presented in Table 2 and Figs. 3 and 4. At the clinical level, intake of SINETROL as compared to placebo revealed a rapid, starting at 4 weeks, and pronounced body weight and fat loss at 12 weeks.

The analysis of the results of the *body weight loss* (Fig. 3) showed that the placebo group have reached a stable level of weight at W0, W4 and W12 (73, 72.2 and 72.6 kg, respectively) and the score stopped to decrease significantly (Table 2).

Table 2. Effect of supplementation with placebo and SINETROL on BMI, body weight and body fat evolution in 20 volunteers after 4 and 12 weeks of treatment

Groups	BMI		Body weight evolution (kg)			Body fat evolution (%)		
	Initial	Variation (%) after 12 weeks	Initial 0 weeks (W0)	After 4 weeks (W4)	After 12 weeks (W12)	Intial 0 weeks (W0)	After 4 weeks (W4)	After 12 weeks (W12)
Placebo	28.570.7[a]	−0.270.5[a]	73.074.8[a]	72.274.7[a]	72.674.5[a]	32.071.0[a]	31.671.0[a]	31.671.0[a]
SINETROL[S]	28.172.45[a]	−2.270.9 [b]	70.576.0[a]	67.575.2[b]	64.974.5[b]	30.771.9[a]	29.070.8[b]	25.971.0[b]

Values are mean7SE, n ¼ 10, for each placebo and SINETROL[S] tested group.
Means within rows followed by the same superscript are not significantly different (po0.05).

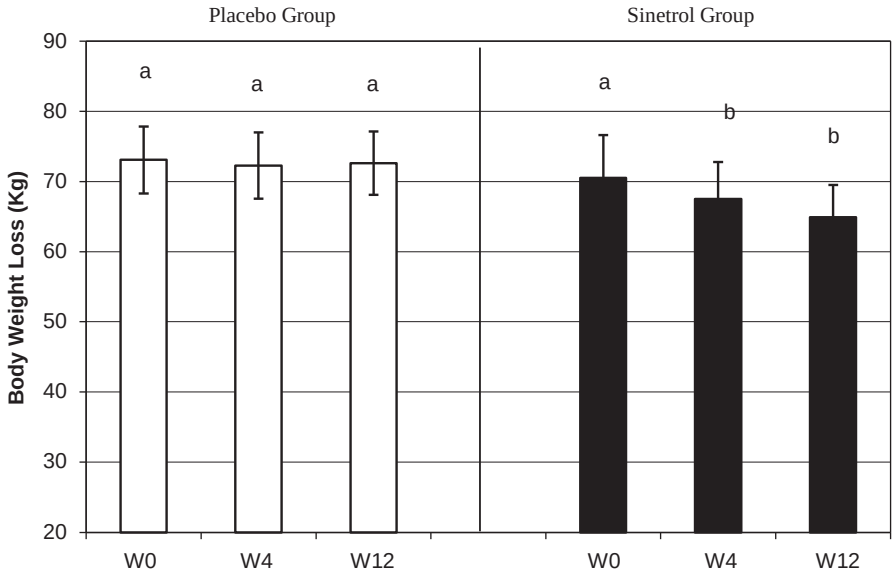

Fig. 3. Effects of supplementation with *placebo* and *SINETROL* on *body weight loss* (kg) in 20 healthy volunteers before (0 weeks), during (4 weeks) and after 12 weeks of treatment. The placebo and *SINETROL* products were administrated as 4 pieces hard capsules containing 350 mg each (1.4 g/day). Two capsules were administrated in the morning and 2 during the main meal. Measurements and treatment protocol were realised as described in Materials and methods (part III). Placebo: maltodextrin; *SINETROL*: citrus extract standardised at 60% of polyphenols and 15% maltodextrin. Values are expressed as mean7SE. Bars with different index letters are significantly different (po0.05).

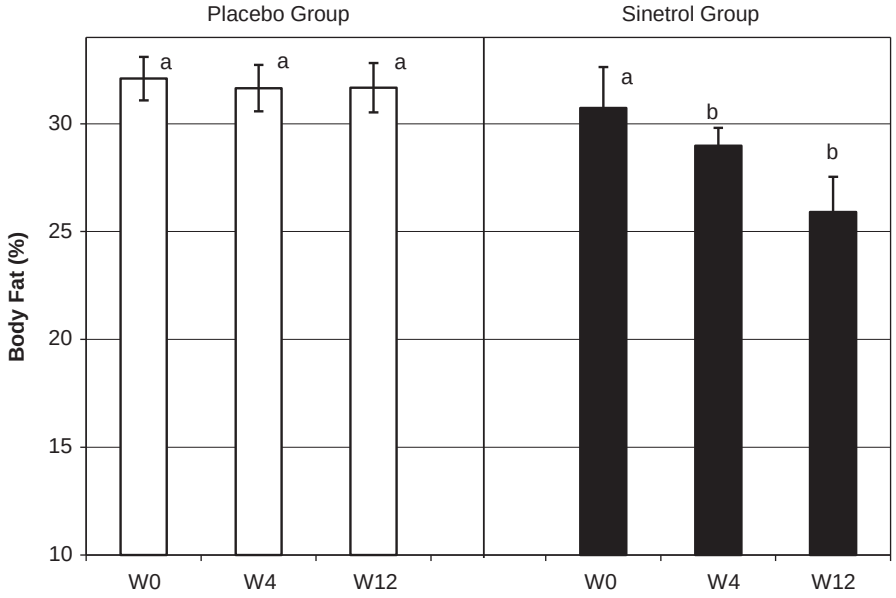

Fig. 4. Effects of supplementation with *placebo* and *SINETROL* on *body fat loss* (%) in 20 healthy volunteers before (0 weeks), during (4 weeks) and after 12 weeks of treatment. The tested products were administrated as 4 pieces hard capsules containing 350 mg each (1.4 g/day) Two capsules were administrated in the morning and 2 during the main meal. Measurements and treatment protocol were realised as described in materials and Methods (part III). Placebo: maltodextrin; *SINETROL*: citrus extract standardised at 60% of polyphenols and 15% maltodextrin. Values are expressed as mean7SE. Bars with different index letters are significantly different (po0.05).

However, in the SINETROL Group the body weight (kg) decreased with a significant difference (po0.05) of 3 kg after W4 and 5.6 kg after W12 weeks compared to W0 SINETROL Group.

The analysis of the results of the *body fat evolution* (Fig. 4) showed that the Placebo group leads to a similar reaction as on the body weight with a stable, non-significant difference (po0.05) in body fat (%) at W0,

W4 and W12 (32%, 31.6% and 31.6%, respectively) (Table 2).

In the SINETROL Group the body fat (%) decreased with a significant difference (p0.05) of 5.53% after W4 and 15.6% after W12 compared to W0 SINETROL Group.

Finally, the medium value of BMI after 12 weeks of treatment with SINETROL (Table 2) decreased significantly by 2.2% compared to the medium BMI (Placebo Group).

Some natural products have been described to have such physiological effect in the literature. Grapefruit capsules or fresh grapefruit groups (obese patients randomized to placebo) lost significantly more weight (Fujioka et al., 2006). A study (Ballard et al., 2006) indicates that consumption of caffeine with naringin in acute dosage does not affect respiratory exchange ratio, oxygen consumption and prevents the increase of resting energy expenditure in adult humans.

Green tea extract relevant of catechin intake is associated with increased weight loss due to diet-induced thermogenesis. This effect is generally attributed to the catechin epigallocatechin gallate to augment and prolong sympathetic stimulation of thermogenesis (Shixian et al., 2006). In a Japanese study (Yoshikawa et al., 2002), a supplementary food consisting of Salacia Reticula has shown a significant lipolytic effect. These antiobesity effects were exerted by mangiferin, (−)-4′-*O*-methylepigallocatechin and maytenfolic through inhibition of fat-metabolizing enzymes and enhanced lipolysis.

Mechanism of action by inhibition of PDE activity

The results of inhibition of cAMP-phosphodiesterase (PDE) activity measured by a scintillation assay (SPA) in the presence of different lipolytic products are presented in Table 3 and Fig. 5.

The tested products guarana 12% caffeine, cyanidin-3 glucoside and naringin were selected because of their presence (in smallest concentration) on the polyphenolic composition of SINETROL. Guarana 12% is a natural fruit extract while cyanidin-3 glycoside and naringin are purified polyphenolic pharmaceutical-grade products. Purified caffeine was used as a positive control and DMSO as negative control. All tested substances were diluted to 0.01% in DMSO. With some test products (cyanidin and naringin) quenching could be observed. This reduced the amplitude of scintillation but did not affect the inhibition measured.

The analysis of the results presented in Table 3 showed a decreased efficiency regarding PDE inhibition for the following products: cyanidin ¼ SINETROL 4naringin 4caffeine 4guarana.

SINETROL is a potent inhibitor of PDE product (97% of inhibition; p0.001). The other two purified polyphenolic compounds naringin (flavanones family) and cyanidin-3 glycoside (anthocyanins family) also showed a very strong PDE inhibition (87% and 99%, respectively) (p0.001).

Table 3. Effect of various lipolytic products in vitro PDE inhibition model

Tested products	Concentration	PDE assay	PDE inhibition	
	(%)	Cpm	(%)	Mean (%)
Control	–	2153	−6	075a
		2017	3	
		2016	3	
DMSO control	1	2178	−7	−579a
		2282	−14	
		1986	5	
Caffeine	0.01	1176	56	5675b
		1242	51	
		1093	61	
Cyanidin-3 glucoside	0.01	388	105	9978c
		619	91	
		449	101	
Naringin	0.01	606	91	8776c
		782	80	
		637	89	
Guarana 12% caffeine	0.01	1989	5	773a
		1905	10	
		1943	7	
SINETROL	0.01	625	90	9771c
		344	108	
		595	92	

Values are mean7SE, n ¼ 3, for each tested product.
Means within rows followed by the same superscript are not significantly different (p0.05); Cpm ¼ counts per minute.

These data suggested a strong effect of SINETROL on cAMP-PDE inhibition. These results could be attributed to the synergetic polyphenolic complex of SINETROL. In fact, SINETROL contains approximately 10% of naringin and 2% of cyanidin, while the tested purified products contain 96% cyanidine and 70% naringin.

SINETROL synergetic polyphenolic composition is due to cyanidin and naringin but also due to other identified flavanones (such as naringenin, isonaringin, narirutin, hesperidin) present at 5–10% in SINETROL and some yet non-identified polyphenols as well as due to caffeine (present at 3.6% in SINETROL).

It is important to indicate that natural guarana containing 12% caffeine induced almost no PDE inhibition (7%), while purified caffeine inhibits PDE significantly (56%). These results show that caffeine is an inhibitor of PDE activity but only at high concentrations.

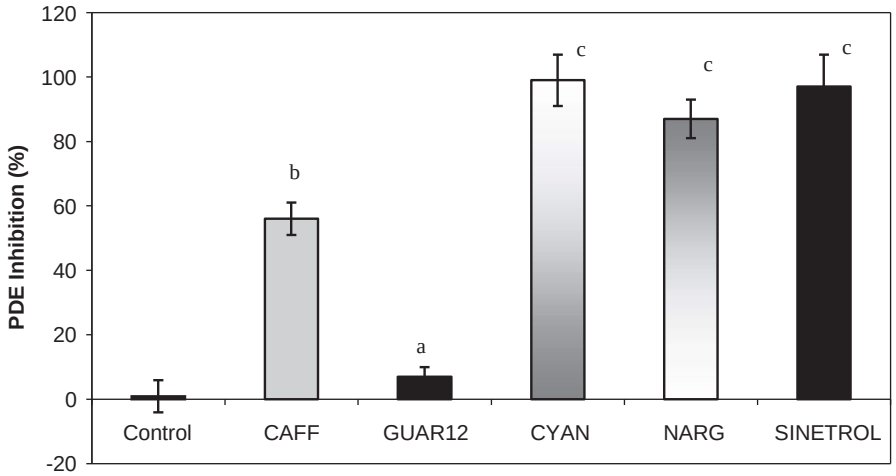

Fig. 5. Effects of various lipolytic products in *in vitro* PDE inhibition assay. PDE (50 mg/ml) were incubated during 10 min at 37 1C in the presence or not of tested products and $3^0,5^0$ cAMP (0.5 mCi/ml). The PDE assay was performed using SPA scintillation beads as described in Materials and methods. The scintillation proximity assay (cpm) was determined by liquid scintillation. Values are expressed as mean7SE. Bars with different index letters are significantly different (po0.05). *Tested products: CAFF*: caffeine at 0.01% (0.5 mM); *GUAR*12: guarana dry extract standardised at 12% of caffeine at final concentration 0.01%; *CYAN*: cyanidin-3-0-glucoside chloride at 0.01%; *NARG*: naringin at 0.01%, *SINETROL*: citrus dry extract standardised at 60% polyphenols at final concentration 0.01%.

For the first time we described the potent phosphodiesterase inhibition property of these 2 polyphenols (i) cyanidin-3-*O*-glucoside (anthocyanin family) and (ii) naringin glycoside (flavanone family).

Both polyphenols have similar squeletal features : C6-C3-C6 with a C3,4 double bond and hydroxyls groups at C5,7,3^0,4^0 for cyanidin and for naringin a keto group at C4 and hydroxyls groups at C5,4^0.

Conclusions

In summary, it has been established that SINETROL has a strong lipolytic activity measured by FFA release. It might be possible that SINETROL lipolytic effect are mediated by cAMP-PDE inhibition and that the subsequent increase in cAMP levels stimulates HSL. Morever, cyanidin-3 glucoside and naringin (two main flavonoids present in SINETROL composition) showed a strong cAMP-PDE inhibition.

These lipolytic results may be attributed to the synergetic polyphenolic complex of SINETROL (anthocyanins, flavonoids and caffeine).

In addition, the results of the clinical study showed that SINETROL may serve to prevent obesity by decreasing BMI and its synergetic polyphenolic composition may help to decrease body weight and body fat.

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Clinical Study to Assess the Efficacy and Safety of a Citrus Polyphenolic Extract of Red Orange, Grapefruit, and Orange (Sinetrol-XPur) on Weight Management and Metabolic Parameters in Healthy Overweight Individuals

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The present study investigated the efficacy and safety effects of Sinetrol-XPur (polyphenolic citrus dry extract) in weight management; metabolic parameters; and inflammatory, glycemic and oxidative status. In a 12-week, randomized, double-blind, placebo-controlled trial, Sinetrol-XPur was given to overweight subjects twice daily with meals in the tested group ($N = 47$) versus a placebo group ($N = 48$). Waist and hip circumference and abdominal fat were decreased in the Sinetrol-XPur group as compared with the placebo group ($p < 0.0001$) (−5.71% vs −1.56% for waist, −4.71% vs −1.35% for hip and −9.73% vs −3.18% for fat). Inflammatory markers were reduced (C-reactive protein: −22.87% vs +61%; fibrinogen: −19.93% vs −1.61%, $p < 0.01$). Oxidative stress was lowered as seen by the reduction of malondialdehyde (−14.03% vs 2.76%) and the increase in superoxide dismutase and glutathione (17.38% vs 2.19% and 4.63% vs −2.36%, respectively, $p < 0.01$). No adverse effects were observed. Kidney, liver, and lipid panels remained unchanged. These results indicated that Sinetrol-XPur supplementation is a viable option for reducing abdominal fat, waist and hip circumference, and body weight and for improving inflammatory, glycemic, and oxidative status in healthy overweight individuals.

Keywords: weight management; citrus extract; polyphenols; overweight; inflammation; oxidative stress.

Abbreviations: Apo, apolipoproteins; BMI, body mass index; CRP, C-reactive protein; CV, cardiovascular; FFA, free fatty acid; GSH, glutathione; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MDA, malondialdehyde; SOD, superoxide dismutase; TG, triglyceride

INTRODUCTION

People are becoming fatter worldwide. Recent data show that excess body fat weight is pandemic, with one-half to two-thirds of the population being overweight or obese in 2006. A greater amount of fat, especially found in the abdominal region, increases the risk of CV diseases and type 2 diabetes (Balkau *et al.*, 2007). Indeed, obesity is associated with decreased HDL and increased LDL and TGs, all risk factors for CV diseases (Kaysen *et al.*, 2009).

Furthermore, obesity is associated with low-grade inflammation and chronic inflammatory response characterized by activation of some pro-inflammatory signaling pathways and abnormal production of markers such as fibrinogen and CRP (Fain, 2010).

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These molecules are implicated in many clinical manifestations of pathologies such as diabetes, arterial hypertension, or CV diseases (Festa *et al.*, 2001; Rodríguez-Rodríguez *et al.*, 2009; Zhang and Zhang, 2010). Fat accumulation is correlated with elevated markers of oxidative stress, which plays critical roles in the development of impaired insulin secretion, diabetes, and atherosclerosis (Furukawa *et al.*, 2004; De Ferranti and Mozaffarian, 2008). Reducing abdominal fat mass and concomitant oxidative stress could be important targets for the prevention of obesity-related diseases (Shen *et al.*, 2009).

Excess body fat is the primary characteristic of obesity. Therefore, a precise measurement of the percentage body fat is considered the reference method for defining obesity. Anthropometric indices such as BMI, waist circumference, and waist-to-hip ratio are the most commonly used indicators for assessing abdominal obesity (Singh *et al.*, 1998; Mushtaq *et al.*, 2011).

Flavonoids constitute the most important class of polyphenolic compounds, such as anthocyanins (malvidin,

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cyanidin, and petunidin), flavanones (naringin, hesperidin, narirutin, naringenin, etc.), flavan-3-ols (catechin, epigallocatechin, etc.), and flavonols (quercetin and kaempferol). Flavonoids have taken an increasing importance with regard to their health benefits in prevention and treatment of cancer (Chen *et al.*, 2004; Moghaddam *et al.*, 2012; Mansoor *et al.*, 2011; Seito *et al.*, 2011; Yang *et al.*, in press), inflammatory diseases (Laughton *et al.*, 1991; Kim *et al.*, 2012; Dai *et al.*, 2012), CV diseases (Frankel *et al.*, 1993; Moon *et al.*, 2012; Vaidya *et al.*, 2012), and neurodegenerative diseases (Orgogozo *et al.*, 1997; Kou *et al.*, 2011; Zhang *et al.*, 2012). Dietary phytochemicals, such as polyphenols, may prevent the risk of obesity-associated chronic diseases such as type 2 diabetes (Dembinska-Kiec *et al.*, 2008; Décordé *et al.*, 2009). *In vitro* studies have shown that flavonoids possess lipolytic activity via inhibition of cyclic adenosine monophosphate (cAMP) phosphodiesterase (PDE) and maintain lipolysis-inducing cAMP levels (Kuppusamy and Das, 1992; Dallas *et al.*, 2008). Naringenin, for example, which is an aglycone of the grapefruit flavonoid naringin, has been reported to induce the expression of fatty acid oxidation genes *CYP4A11*, *ACOX*, *UCP1*, and *ApoA1*. (Goldwasser *et al.*, 2010). These would support the effect observed in overweight subjects on weight and body fat loss after 12 weeks of daily supplementation (Dallas *et al.*, 2008).

Hence, a food supplement rich in polyphenols that would contribute to the reduction of not only body fat but also inflammatory and oxidative stress status would be of great health value.

Therefore, the aim of this study was to demonstrate that a proprietary polyphenolic-rich combination would help reduce body fat, inflammation, and oxidative stress in healthy overweight subjects, safely and without adverse effects.

MATERIALS AND METHODS

Study design. A 12-week, randomized, double-blind, placebo-controlled clinical trial was conducted in overweight individuals with daily supplementation of a citrus polyphenolic extract (Sinetrol-XPur).The study was conducted at four clinical research sites accredited by a joint commission and by the Haute Autorité de Santé: American Hospital in Paris, Centre Medical, Centre Exploitation Vasculaire, and Centre Exploitation Biologique in Paris. The procedures complied with the ethical standards and approved by the Association National de Prévention des Maladies and Biological Research and Collections (clinical trial registration number 2012-A01702-4).

Subjects. Ninety-five healthy overweight volunteers of both sexes (55 women and 40 men) aged 22 to 45 years, with a BMI of 26–29.9 kg/m^2 and comparable socioprofessional status (middle class) and sedentarily living in Ile de France, participated in the study.

Exclusion criteria. Subjects taking weight loss medications or dietary supplements or on weight loss programs in the last 3 months and having a history of weight-reducing surgery or an eating disorder were excluded, together with pregnant or lactating women and postmenopausal women. Individuals having high blood pressure, chronic or allergic metabolic diseases, metabolic syndrome, diabetes, stress diseases, high alcohol consumption, or a known intolerance to one of the components of the tested product were also excluded.

Test compound. Sinetrol-XPur is a proprietary polyphenolic-rich fruit extract (red orange, grapefruit, sweet orange, and guarana). It was standardized to contain at least 90% of total polyphenols (expressed as catechin), at least 20% of total flavanones (expressed as naringin) and between 1% and 3% of natural caffeine.

Total polyphenols, flavanones, and caffeine were measured by high-performance liquid chromatography–ultraviolet (Dallas and Laureano, 1994a, 1994b). The dry extract was packaged in red gelatine capsules (450 mg per capsule). Identical-looking capsules were filled with 450 mg of maltodextrin and used as placebo.

Study protocol. Ninety-five volunteers were randomly assigned into two groups, one receiving placebo (n = 48) and the other group receiving the active compound (Sinetrol-XPur) (n = 47) for 12 weeks. Participants received either 180 placebo capsules (packed in a plastic 100-ml closed box) or 180 Sinetrol-XPur capsules (provided by Fytexia), all labeled and coded in such a way that subjects and staffs were unaware which product each participant was receiving.

Subjects were instructed to take one capsule at breakfast and one capsule at lunch for a total of two capsules per day or 900 mg. Subjects were also instructed to keep the original box closed after each use of the capsules. All participants reported to their corresponding research centers four times during the 12-week intervention: at baseline (W0), at week 4 (W4), at week 8 (W8), and at week 12 (W12).

Diet and exercise. The calorie level was set at 1800–2000 kcal/day for women and between 2000 and 2500 kcal/day for men. A brief diet and physical questionnaire were administrated to determine usual nutrient intakes and detect any significant changes that may have occurred from the recommended diet. All subjects were instructed to have 30 min/week of physical activity (three sessions of 10-min walk).

Primary outcome variables. The primary outcome variables were changes in mean body weight, BMI, body fat, waist and hip circumference, waist-to-hip ratio, and FFA.

Secondary outcome variables (safety). The secondary outcome variables were changes in blood safety parameters such us blood pressure, heart rate, lipid profile (total cholesterol, HDL, LDL, TG, ApoA1, and ApoB), glucose and hemoglobin A1c (HbA1c), kidney function (Na, K, urea, and creatinine), inflammation markers (fibrinogen and CRP), liver function (alanine, alanine amino transaminase, aspartate amino transaminase, gamma-glutamyl transpeptidase, and creatine phosphokinase), and oxidative status (SOD, MDA, and GSH).

Methods of analysis. Body weight (kg) was measured to the nearest 0.1 kg at each visit with subjects wearing light clothing. Height (cm) was measured using a stadiometer

with subjects barefoot; BMI was calculated (weight/height squared) (kg/m^2). Waist circumference (cm) was measured at the narrowest point between the lowest rib and the iliac crest using a nonstretchable tape. Hip circumference (cm) was taken around the maximum circumference of the buttocks. Total abdominal adiposity was measured by the ViSCAN system (Tanita Corporation, Arlington, IL) at baseline and week 12 (Thomas *et al.*, 2010). Systolic and diastolic blood pressures and heart rate were taken in the supine position after 15-min rest at each visit.

Subjects gave blood samples between 8:30 and 9:30 in the morning after an overnight fast at W0 and at W12. Blood samples were prepared and stored appropriately until they were analyzed by using enzymatic and colorimetric methods (Randox reagents, UK) on Hitachi 717 (Japan) for the safety parameters.

The overall compliance in the study was excellent. One hundred thirteen subjects were screened for eligibility, and 18 subjects were excluded (did not meet inclusion criteria). Ninety-five subjects were enrolled and randomized for the study (48 subjects for the placebo group and 47 subjects for the intervention group (Sinetrol-XPur)). All the subjects (95) completed the study. Subjects' compliance was checked at each visit (W0, W4, W8, and W12) to make sure that they all performed the planned program. Compliance to the protocol was checked by measuring the difference between the numbers of unused capsules and the expected number to be taken.

Statistical analysis. Statistical analyses were performed using StatView software version 4.51.1 (Abacus Concepts, Berkeley, CA). The data are expressed as mean ± standard deviation. A Kolmogorov–Smirnov test for normality and a Bartlett test for homogeneous variance were performed for each group at all times. Changes within groups between baseline and week 12 and between groups for the clinical and laboratory parameters were analyzed using unpaired Student *t*-test, with a significance set up at $p < 0.05$. Results of the questionnaire were analyzed with the Wilcoxon rank test. Sample size calculation was based on the results obtained in a previous preliminary clinical study (changes and variation). The new calculation was made with a power of 95% and a risk alpha of 5%.

RESULTS AND DISCUSSION

This performed protocol studied the effect of Sinetrol-XPur on weight management; metabolic parameters; and inflammatory, glycemic, and oxidative status in overweight men and women. At the start of the study, there was no difference between groups with respect to age, BMI, height, body weight, and body fat (Table 1). Weight and waist and hip circumference continuously decreased during the study (data not shown). After 12 weeks of treatment, percent changes in waist and hip circumference, abdominal body fat, and body weight for the Sinetrol-XPur group were statistically lower than those of the placebo group (Table 2). Waist reduction was 5.71% for the Sinetrol-XPur group versus 1.56% for the placebo group ($p < 0.0001$), corresponding to a mean waist reduction of 5.15 versus 1.42 cm, respectively. Hip circumference decreased by 4.71% for Sinetrol-XPur compared with 1.35% for placebo, corresponding to a mean hip reduction of 5.17 and 1.43 cm respectively ($p < 0.001$). The waist-to-hip ratio was 0.809 and 0.808 for the placebo group at baseline and W12, respectively, with the lowest level (0.784) found for the Sinetrol-XPur group after 12 weeks of treatment. The change (%) in this ratio was not significant between the two groups. A 9.73 ± 0.54% reduction of body fat was observed in the Sinetrol-XPur group, whereas only 3.18 ± 0.33% was lost by the placebo group, with a difference between the two groups being highly significant ($p < 0.0001$). Body weight decreased by 3.28 ± 0.24% for Sinetrol-XPur compared with 2.09 ± 0.17 % for placebo ($p < 0.0001$), corresponding to a loss of 2.62 vs 1.6 kg, respectively.

Previously, a small clinical study versus placebo has evaluated the influence of a similar, yet not identical, citrus extract made of a variety of oranges and grapefruit plus guarana fruit on body weight and composition in 20 overweight and obese individuals for 12 weeks (Dallas *et al.*, 2008). Possible mechanisms of action included the result of citrus polyphenols on the inhibition of PDE, thereby prolonging the lipolytic-induced cAMP action. Another one may involve induction of the expression of fatty acid oxidation genes (Goldwasser *et al.*, 2010). This demonstrated that the combination of citrus fruits and guarana contains an array of potent bioactive compounds that can generate weight and fat loss.

A safety study showed that kidney function, liver enzymes, blood pressure, and serum lipid profile (except ApoA) were not statistically different at the beginning of the study and between Sinetrol-XPur and placebo groups after 12 weeks of treatment (Table 3). Heart rate did not change in the placebo group but was slightly higher in the Sinetrol-XPur group by the end of the study (+3.32%), although all values remained within normal limits (74 to 77 rates/min). The increase in cardiac rate corresponds to what would be experienced after consuming three cups of coffee per day related to the content of caffeine (19.8 mg/day).

The FFA significantly increased in both groups (Table 3). However, the rise in the Sinetrol-XPur group (+329.73 ± 14.68%) was significantly greater than that for placebo (+33.16 ± 4.6%) ($p < 0.0001$). Lipolytic activity was clearly demonstrated by the high plasmatic change of FFA (+330%) probably related to the citrus polyphenol-inhibited PDE. The increase in plasma FFAs did not affect lipid profiles, which remained unchanged. Levels of cholesterol, TG, HDL, and LDL remained within normal limits. The HDL/LDL ratio

Table 1. Baseline characteristics of healthy overweight study sample by intervention group.

	Placebo	Sinetrol-XPur
N	48	47
Men, *n* (%)	20 (41.7)	20 (42.5)
Women, *n* (%)	28 (58.3)	27 (57.5)
Age (years)	37.8 ±0.7	37.6 ±0.7
Caucasian, *n* (%)	45 (93.7)	44 (93.6)
Others, *n* (%)	3 (6.3)	3 (6.4)
BMI (kg/m^2)	27.27 ± 0.14	27.58 ± 0.16
Body weight (kg)	77.39 ± 1.23	78.14 ± 1.35
Height (m)	1.69 ±0.01	1.69 ±0.01
Body fat (%)	36.87 ± 1.48	37.97 ± 1.59

Values are means ± standard deviation or *n* (%). Groups did not differ at baseline.

Table 2. Percent change for BMI, weight, body fat, and waist and hip size at baseline and after 12 weeks of treatment with placebo or Sinetrol-XPur in healthy overweight adults

	Placebo			Sinetrol-XPur		
	Baseline	W12	% change	Baseline	W12	% change
BMI (kg/m^2)	27.27±0.14	26.12 ± 0.35[a]	−4.23 ± 1.12	27.58±0.16	26.39 ± 0.33[a]	−4.31 ± 1.02, NS
Body weight (kg)	77.39±1.23	75.78±1.23	−2.09 ± 0.17	78.14±1.35	75.52±1.25	−3.28 ± 0.24***
Body fat (%)	36.87±1.48	35.85±1.51	−3.18 ± 0.33	37.97±1.59	34.36±1.49	−9.73 ± 0.54***
Waist (cm)	88.44±1.09	87.02±1.02	−1.56 ± 0.20	88.68±1.05	83.53±0.87[a]	−5.71 ± 0.35***
Hip (cm)	109.90 ± 0.96	108.47 ± 0.99	−1.35 ± 0.19	110.08 ± 1.21	104.91 ± 1.23[a]	−4.71 ± 0.29***
Waist/hip	0.809 ± 0.113	0.808 ± 0.101	−0.23 ± 1.69	0.813 ± 0.113	0.784 ± 0.155	−1.01 ± 2.28, NS

Values are means±standard deviation, $n = 48$ (placebo) or 47 (Sinetrol-XPur). Group mean did not differ at baseline.
NS, not significant; W12, week 12.
[a]An intragroup difference between baseline and W12 at $p < 0.05$. Intergroup percent change differences:
*$p < 0.05$;
**$p < 0.01$;
***$p < 0.0001$.

Table 3. Percent changes on clinical safety values (kidney, liver, cardiac function, and lipid profile) at baseline and after 12 weeks of treatment with placebo or Sinetrol-XPur in healthy overweight adults

	Placebo			Sinetrol-XPur		
	Baseline	W12	% change	Baseline	W12	% change
Kidney function						
Na (mmol/L)	134 ± 1	133 ± 1	−0.45 ± 0.82	136 ± 1	134 ± 1[a]	−1.53 ± 0.55, NS
K (mmol/L)	4.4 ± 0.1	3.9 ± 0.1[a]	−10.86 ± 1.48	4.5 ± 0.1	4 ± 0.1[a]	−9.43 ± 1.55, NS
Urea (mmol/L)	6.3 ± 0.2	7 ± 0.2[a]	18.87±5.27	6.5 ± 0.3	7.5 ± 0.87[a]	28.93±7.06, NS
Creatinine (mmol/L)	106 ± 2	116 ± 2	12.63 ± 3.60[a]	108 ± 2	113 ± .2	6.57±3.53, NS
Liver function						
ALT (IU/L)	26.17±1.61	19.87 ± 0.53[a]	−18.42 ± 3.25	25.49±0.67	18.85 ± 0.48[a]	−23.13 ± 3.40, NS
AST (IU/L)	26.40±0.84	24.62±0.43	−3.11 ± 3.34	26.60±0.68	23.96 ± 0.49[a]	−6.75 ± 3.66, NS
GGT (IU/L)	40.68±1.51	35.58 ± 0.68[a]	−5.86 ± 4.67	43.04±1.38	34.43 ± 0.71[a]	−16.35 ± 2.37, NS
CPK (IU/L)	142.34±5.9	112.25 ± 3.69[a]	−13.71 ± 4.64	156.83±6.0	112.21 ± 2.91[a]	−23.36 ± 3.60, NS
Cardiac function						
Heart rate (beats)	74.33±0.74	74.64±0.77	−0.51 ± 0.68	74.74±0.90	77.06±0.78	3.32 ± 0.76**
SBP (mmHg)	131.29±1.1	131.90 ± 1.09	0.52±0.46	133.91±1.1	136.08±1.2	1.67 ± 0.47, NS
DBP (mmHg)	74.04±0.69	74.58±0.63	0.97±0.91	74.85±0.68	77.11±0.69	3.12 ± 0.68, NS
Lipids profile						
Chol (mmol/L)	5.96±0.11	5.74±0.74	−2.44 ± 2.05	6.02±0.11	5.59±0.06	−5.83 ± 1.90, NS
TG (mmol/L)	1.29±0.06	1.38±0.03	19.75±6.40	1.33±0.05	1.38±0.03	12.42±5.72, NS
HDL (mmol/L)	1.46±0.04	1.40±0.03	−0.83 ± 3.33	1.49±0.04	1.49±0.03	2.71±3.32, NS
LDL (mmol/L)	3.67±0.08	3.51±0.06	−2.33 ± 2.30	3.61±0.09	3.43±0.05	−2.61 ± 2.37, NS
ApoA (mmol/L)	50.95±1.18	46.75±0.38	−5.89 ± 2.39	50.76±1.21	51.85±0.53	5.38 ± 3.02*
ApoB (mmol/L)	2.26±0.07	2.75±0.03	27.30±4.78	2.21±0.06	2.50±0.03	17.65±4.19, NS
FFA (mmol/L)	152.1±4.05	197.93±6.3[a]	33.16±4.6	151.15 ± 2.96	638.63 ± 17.11[a]	329.73±14.68***

Values are means±standard deviation, $n = 48$ (placebo) or 47 (Sinetrol-XPur). Group mean did not differ at baseline.
ALT, alanine amino transaminase; Apo, apolipoprotein; AST, aspartate amino transaminase; Chol, cholesterol; CPK, creatinine phosphokinase; DBP, diastolic blood pressure; FFA, free fatty acid; GGT, gamma-glutamyl transpeptidase; HDL, high-density lipoprotein; IU, international units; LDL, low-density lipoprotein; NS, not significant; SBP, systolic blood pressure; TG, triglyceride; W12, week 12.
[a]An intragroup difference between baseline and W12 at $p < 0.05$. Intergroup percent change differences:
*$p < 0.05$;
**$p < 0.01$;
***$p < 0.0001$.

was also within normal limits (between 0.39 and 0.43). A recent epidemiologic and experimental study (Green *et al.,* 1985) suggested that the HDL/LDL ratio may adequately represent the joint contribution of the lipoproteins to heart disease. Alone, ApoA increased in the Sinetrol-XPur group by 5.38 ± 3.02% compared with a decrease of 5.89 ± 2.38% in the placebo group, with a statistically significant difference ($p < 0.05$). Previous studies have shown that citrus flavonoids such as naringenin are effective plasma lipid-lowering agents

on laboratory animals, especially those fed with a high-cholesterol diet (Gorinstein *et al.*, 2005; Mulvihill *et al.*, 2009). Both citrus flavonoids and palm tocotrienols or pomelo–grapefruit hybrid fruit juice reduce cholesterol levels in hypercholesterolemic patients (Gorinstein *et al.*, 2003; Roza *et al.*, 2007). We speculated that this lack of effect in our study suggests a different flavanone profile in Sinetrol-XPur than the ones used in the studies quoted earlier.

Another key link between increasing fat mass and obesity-related complications is a chronic low-grade inflammatory state and an increased oxidative stress. Previous studies have shown the direct link between a high level of inflammatory biomarkers (such as CRP and fibrinogen) and obesity-related diseases such as diabetes, hypertension, and CV diseases in overweight and obese people (de Ferranti and Mozaffarian, 2008; Nguyen *et al.*, 2009). In our study, at baseline, there was no difference between groups with respect to those parameters (Table 4). No subject displayed any sign of infection throughout the study (data not shown). Inflammatory markers (as expressed by CRP and fibrinogen) showed significant differences between the Sinetrol-XPur and placebo groups. CRP decreased by 22.87 ± 7.30% with Sinetrol-XPur, whereas they increased by 61.79 ± 14.44% with the placebo, and the difference between the two groups was highly significant ($p < 0.0001$). Fibrinogen levels decreased by 19.91 ± 2.04% with Sinetrol-XPur, whereas they remained the same for placebo. The difference between the two groups was significant ($p < 0.0001$).

The related effect of Sinetrol-XPur on oxidative status was evaluated by measuring plasma MDA, SOD, and GSH. At baseline, these levels were within normal range with no significant difference between groups. By the end of the study, MDA decreased by 14.03 ± 1.18% in the Sinetrol-XPur group compared with a slight increase in the placebo group (2.76 ± 1.61%) with a highly significant difference between the two groups ($p < 0.0001$). SOD increased in the Sinetrol-XPur group eight times more than in the placebo group (17.38 ± 4.08% vs 2.19 ± 3.66%, $p < 0.01$). GSH levels increased by 4.63 ± 11.62% in the Sinetrol-XPur group, whereas they decreased by 2.36 ± 1.13% in placebo group ($p < 0.01$). We have shown that a 12-week consumption of a citrus polyphenolic dietary supplement had beneficial changes in measures related to inflammation status including a significant decrease of circulating levels of CRP (−23%) and fibrinogen (−20%). In our current study, supplementation with Sinetrol-XPur led to an improvement in oxidative status in overweight healthy subjects. After 12 weeks of treatment, Sinetrol-XPur significantly decreased MDA plasma levels (almost equal to −14%) and increased SOD and GSH levels (+17% and +5%, respectively). Therefore, consumption of anti-inflammatory and antioxidant substances contained in fruits could be a useful strategy to add to weight loss programs to boost the benefits of losing fat and reducing risk factors and complications associated with excess weight (Crujeiras *et al.*, 2006).

Mean fasting blood sugar levels were normal at baseline in each group (Table 4). However, blood sugar further decreased by 9.95 ± 1.87% in the Sinetrol-XPur group, whereas it increased by 5.40 ± 1.90% in the placebo groups with a significant difference between the two groups ($p < 0.0001$). Concurrently, HbA1c rose slightly by 7.15 ± 12.56% in the Sinetrol-XPur group and by a higher level (24.35 ± 2.46%) in the placebo group, although all values remained within normal limits (less than 7%). This difference between fasting blood sugar and HbA1c can be explained by the fact that changes in HbA1c can only be observed after 3 months. We can expect a more relevant decrease of the HbA1c after a longer period of treatment with Sinetrol-XPur (6–9 months).

Grapefruit and grapefruit products that contain naringenin and naringin have been shown to reduce insulin resistance in subjects with metabolic syndrome (Fujioka *et al.*, 2006). An inhibition of intestinal glucose uptake and renal glucose reabsorption by naringenin can

Table 4. Percent changes for inflammatory, oxidative, and glycemic status at baseline and after 12 weeks of treatment with placebo or Sinetrol-XPur in healthy overweight adults.

	Placebo			Sinetrol-XPur		
	Baseline	W12	% change	Baseline	W12	% change
Inflammation						
CRP (nmol/L)	26.46±2.09	34.75 ± 1.99[a]	61.79 ± 14.44	33.12±2.95	20.84±1.9[a]	−22.87 ± 7.30***
Fibrinogen (mmol/L)	10.26±0.29	10.14±0.20	−1.61 ± 2.59	10.81±0.29	8.77±0.14[a]	−19.93 ± 2.04***
Oxidative status						
MDA (mmol/l)	2.99±0.5	3.04±0.5	2.76±1.61	2.94±0.06	2.52±0.05[a]	−14.03 ± 1.18***
SOD (IU/Hb)	1339.7 ± 40.6	1330.1 ± 35.7	2.19±3.66	1276.6 ± 37.9	1436.7 ± 33.9[a]	17.38±4.08**
GSH (mmol/l)	878.65 ± 7.91	854.08 ± 4.24	−2.36 ± 1.13[a]	868.92 ± 10.20	898.66 ± 5.93[a]	4.63±1.62**
Glycemic status						
Glycemia (mmol/L)	5.7±0.1	5.9±0.1[a]	5.40±1.90	5.8±0.1	5.2±0.1[a]	−9.95 ± 1.87***
HbA1c (%)	5.55±0.10	6.79±0.05[a]	24.32±2.46	5.64±0.10	5.95±0.08[a]	7.15 ± 2.56***

Values are means ± standard deviation, $n = 48$ (placebo) or 47 (Sinetrol-XPur). Group mean did not differ at baseline.

CRP, C-reactive protein; Hb, hemoglobin; IU, international units; GSH, glutathione; MDA, malondialdehyde; NS, not significant; SOD, superoxide dismutase; W12, week 12.

[a]An intragroup difference between baseline and W12 at $p < 0.05$. Intergroup percent change differences:

*$p < 0.05$;

**$p < 0.01$;

***$p < 0.0001$, NS = not significant.

explain, at least partially, the *in vivo* antihyperglycemic action of naringenin and its derivatives. Naringenin also improves insulin sensitivity and glucose metabolism in metabolic syndrome-prone mice (Mulvihill *et al.*, 2009).

In conclusion, the safety of Sinetrol-XPur supplementation was assessed in our study during 12 weeks on kidney and liver parameters. Sinetrol-XPur had no effect on blood pressure. We suggest that consumption of Sinetrol-XPur produces beneficial changes in body fat composition and improves inflammatory, glycemic, and oxidative status in overweight healthy individuals. When taken twice a day for 12 weeks, Sinetrol-XPur supplement was well tolerated with no adverse effects. However, additional research is warranted to delve deeper into the mechanisms of action and confirm these results over a longer period.

Conflict of Interest

The corresponding author and all the authors have read and approved the final submitted manuscript. The authors declare no conflict of interest.

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RESEARCH ARTICLE

A 12-week randomized double-blind parallel pilot trial of Sinetrol® XPur on body weight, abdominal fat, waist circumference, and muscle metabolism in overweight men

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Abstract
Overweight and obesity are associated to increased risk of developing non-communicable diseases that might dramatically affect life expectancy according World Health Organization. Overweight, obesity and decline in physical activity are correlated to a significant propensity to lose skeletal muscle mass as a result of prolonged inflammation and oxidative stress whereas cohort surveys and clinical investigations have demonstrated health benefits of Citrus-based polyphenols to reverse such regression. Overweight men were included in a double-blind, randomized, parallel pilot trial where they received daily for a 12-week period 900mg of a Citrus-based polyphenol extract, Sinetrol® XPur. Body composition, anthropometric and blood parameters were assessed before and at the end of the intervention period. After 12 weeks, while the silhouette slimmed down, metabolic parameters were significantly improved and skeletal muscle catabolism held back. These data suggest that over a 12-week period, the efficacy of the supplement improve both overweight process and correlated skeletal muscle mass metabolism.

Keywords
Weight loss, polyphenols, obesity, inflammation, insulin resistance, abdominal fat

Introduction

Excessive body weight is currently the most common chronic health problem worldwide and one of the greatest public health challenges of the 21st century. The etiology of overweight is rooted in cumulative habitual concerns, including imbalanced diets and sedentary behaviors. In addition to causing various physical disabilities and psychological problems, overweight and obesity drastically increase a person's risk of developing a number of non-communicable diseases (NCDs) (Aballay et al., 2013, Balkau et al., 2007b, Cardoso-Saldana et al., 2010, Janus et al., 2007, Kaysen et al., 2009, Wadden and Phelan, 2002) including metabolic syndrome (MS), cardiovascular diseases (CVDs) and type 2 diabetes mellitus (T2DM), which dramatically affect average life expectancy, making overweight and obesity the fifth leading risk factor for global death (World Health Organization, 2013). Nevertheless, overweight and obesity and their consequences are preventable.

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Overweight and obesity are defined as abnormal fat accumulation that may impair health, especially when disproportionate fat is stored in the abdominal segment, as it is during the development of MS (Balkau et al., 2007a). Therefore, measuring the abdominal adiposity ratio is considered as the reference method for studying overweight and obesity. Anthropometric measurements such as body mass index (BMI) or waist and hip circumference are generally the most commonly used indicators to assess overweight or obesity (Mushtaq et al., 2011, Singh et al., 1998). However, these markers should be considered as a rough guide because they may not correspond to the same degree of fatness in different individuals. Hence an accurate measurement of abdominal adiposity ratio seems to be more suitable.

In addition to reflecting overweight or obesity, excessive abdominal fat is generally well-associated with surrogate biomarkers involved in chronic and low-grade inflammation (Fain, 2010), oxidative stress, and the

development of insulin resistance, which are all directly linked to an increased incidence of various clinical NCDs (de Ferranti and Mozaffarian, 2008, Festa et al., 2001, Furukawa et al., 2004, Rodriguez-Rodriguez et al., 2009, Zhang and Zhang, 2010). Accordingly, there is no sudden departure from healthiness to illness in the development of NCDs. Following a generally slight transition, starting without biochemical dysfunctions or other clinical signs, cumulative deviations might lead to a diminishment in well-being and lack of vigor, more or less rapidly before illnesses are confirmed (Stewart and Brook, 1983, Wadden & Phelan, 2002, Wadden and Stunkard, 1985). Consequently, reducing abdominal fat mass and associated metabolic disorders appear as clear and crucial targets for the prevention of excess weight-related manifestations of NCDs (Shen et al., 2009). However, during abdominal fat accumulation throughout the progression of overweight or obesity, it was reported that various metabolic effects associated with age-related changes in body composition and a decline in physical activity were involved with a significant propensity to lose skeletal muscle mass (SMM) (Kim et al., 2014). In addition, several authors observed a significant reduction of SMM in response to a modified diet during weight loss intention in overweight populations with excessive abdominal fat (Janssen and Ross 1999, Ross et al., 1996). Preserving SMM consequently appears to be essential when individuals with a medium to long-term history of overweight or obesity decide to start a weight loss program.

Sinetrol® XPur is a food-based ingredient product inspired by the Mediterranean diet and designed to provide a synergistic fingerprint of various naturally occurring bioactive components from Citrus; mainly polyphenols in the family of flavanones. Polyphenols from Sinetrol® XPur have previously been shown to enhance weight loss and decrease the abdominal adiposity ratio through the induction of lipolysis (Dallas et al., 2008, Dallas et al., 2014); a catabolic process leading to the breakdown of triglycerides (TG) into non-esterified fatty acids (NEFAs) inside adipocytes (Renold and Cahill, 1965).

Based on the rationale of inducing lipolysis, the present study endeavored to demonstrate the health benefits of Sinetrol® XPur in supporting overweight men volunteers to lose significant body weight and reduce the abdominal adiposity ratio while preserving the metabolism of their SMM during a 12-week, balanced normo-caloric dietary program.

Materials and methods

Subjects

Twenty five overweight men volunteers with moderate metabolic deviations, but otherwise healthy, were recruited by RDVC Produits Santé, at Le Havre, France, after they agreed to sign a written informed consent form.

Inclusion criteria

Inclusion criteria incorporated overweight men, aged 30 to 45 years, with a body mass index (BMI) within the range of 26-29.9 kg/m2 and the prerequisite criteria for the diagnosis of MS, defined as a waist circumference equal to or greater than 94 cm, according to the International Diabetes Federation (IDF) (Alberti et al., 2005).

Subjects who in the previous 6 months were enrolled in a restricted diet for a weight loss program or took weight loss medications or any dietary supplements were not eligible. Exclusion criteria comprised history of weight reducing surgery, eating disorders, circulation weaknesses or hypertension, chronic allergic or metabolic diseases, stress or anxiety disturbances, and a high rate of either alcohol consumption or smoking. Mean values for central anthropometric characteristics of subjects participating in this study were as follows: waist circumference, 98.6±3.4 cm; hip circumference, 105.1±4.2 cm; body weight, 87.8±5.5 kg; and abdominal adiposity ratio, 26.8±3.3 %.

Experimental design

The study was approved by a French Ethical Committee for Human experimentation and was conducted according the Good Clinical Practice guidelines of the International Conference on Harmonization of technical requirements for registration of pharmaceuticals for Human use in harmony with the Declaration of Helsinki and in accordance with French drug laws.
A 12-week, double-blind, randomized [1:1] and parallel clinical pilot trial was conducted. Once enrolled, subjects were assigned to one of two groups, with one receiving *placebo* (*n*=13) and the other (*n*=12) Sinetrol® XPur. Subjects were instructed to take one capsule in the morning at breakfast and one at lunchtime every day for 12 weeks. Participants reported to the research center 4 times during the 12-week intervention study: at baseline (W0), at week 4 (W4), week 8 (W8), and at the end of the intervention period (W12).

Test treatment

Sinetrol® XPur was obtained by alcohol and/or water extraction from specific varieties of grapefruit (*Citrus paradisi* Macfad.), sweet orange (*Citrus sinensis* L. Osbeck), guarana (*Paullinia cupana* Kunth) and blood orange (*Citrus sinensis* L. Osbeck). Sinetrol® XPur provided polyphenols, mainly flavanones, of which naringin and hesperidin are respectively leading markers of grapefruit and both sweet orange and blood orange. It also supplied caffeine from an extract of guarana. The *placebo* was 100% maltodextrin, which is polyphenol- and caffeine-free. Each pill contained 450 mg of either Sinetrol® XPur or *placebo*.

Diet and exercise

The daily energy intake level was recommended at 110-125% of the basal metabolic rate (BMR) according to the

revised Harris-Benedict equation (Roza and Shizgal, 1984) which corresponds to 2,200 to 2,500 Kcal/d. For the whole duration of the study, all subjects were instructed to have 30 min per week of physical exercise.

Determination of anthropometric, vital, and nutritional parameters

Anthropometrics (body weight, waist and hip circumference), blood pressure and heart rate were monitored at each visit. For body weight (kg) measurements, subjects wore light clothing at each visit. Waist circumference (cm) was measured at the narrowest point between the lowest rib and the iliac crest using a non-stretchable tape. Hip circumference (cm) was taken around the maximum circumference of the buttocks. Total abdominal adiposity ratio (%) was measured by the ViScan™ system (Tanita Corporation) at W0 and at W12 (Cases et al., 2014).

Blood analysis

Subjects were sampled for blood after an overnight fast at W0 and at W12. Blood samples were prepared and stored appropriately prior to analyses by enzymatic and colorimetric methods with reagents (Randox, UK) on a Hitachi 717 Chemistry Analyzer (Japan) for the following parameters in plasma: metabolic parameters - non-esterified fatty acids (NEFAs), apo-lipoprotein A1 (Apo A1) and glucose; catabolic parameters - uric acid and creatinine; inflammatory markers – fibrinogen and high-sensitivity c-reactive protein (hs- CRP); oxidative status - malondialdehyde (MDA); renal function – urea, sodium (Na), and potassium (K).

Well-being questionnaire

An in-house questionnaire was developed to subjectively assess overall satisfaction with regard to the treatment at W12. The questionnaire was based on the rating of 3 items: overall satisfaction; perception of greater energy; and perception of well-being. Subjects were requested to score each item on a 0–10 rating scale with 0 for extremely unsatisfied and 10 for extremely satisfied.

Statistics

Statistical analyses were performed using Statview software version 4.51.1 (Abacus Concepts, Berkeley, CA, USA). The data are expressed as mean ± standard deviation (SD). A Kolmogorov Smirnov test for normality and a Bartlett test for homogeneous variance were performed for each group. Changes within and between groups at W0 and W12 for the clinical and laboratory parameters were analyzed using unpaired Student's *t*-test. Results of the questionnaire were analyzed with the Wilcoxon rank test. A minimum value of $p<0.05$ was selected as the threshold for statistical significance.

Results

Anthropometric results and body composition

Before the onset of the intervention period (W0), body weight, abdominal body fat, and anthropometric parameters were similar in the *placebo* and Sinetrol® XPur groups (Table 1). Percent changes in waist and hip circumference and body weight began to show significant differences between the *placebo* and Sinetrol® XPur groups from week 4 for the former and week 8 for the latter (data not shown).

After 12 weeks of treatment, percent decreases in waist and hip circumference, abdominal body fat, and body weight in the Sinetrol® Xpur group were greater than those of the *placebo* group (Table 1). Waist reduction was 7.5% for the Sinetrol® XPur group *versus* 2.1% for the *placebo* group ($p<0.001$), corresponding to a mean reduction in waist circumference of 7.4 cm *versus* 2.1 cm, respectively. Hip circumference decreased by 5.3% in the Sinetrol® XPur group compared with 1.9% for *placebo*, corresponding to mean reductions of 5.6 cm and 2.0 cm, respectively ($p<0.001$).

The waist-to-hip ratio in the *placebo* group at baseline and W12 was 0.93, and 0.95 and 0.92 in the Sinetrol® XPur group at W0 and W12, respectively. In the *placebo* group, the ratio variation (Δ%) showed no change during the study, whereas it significantly decreased by 2.3% in the Sinetrol® XPur group ($p<0.05$).

At W12, abdominal body fat was decreased by 9.7% in the Sinetrol® Xpur group, whereas the decrease was 4.8% in the *placebo* group, with a highly significant difference between the two groups ($p<0.001$). Body weight decreased by 3.7% in the Sinetrol® XPur group *versus* 1.8% in the *placebo* group ($p<0.001$), corresponding to a loss of 3.4 kg *versus* 1.5 kg, respectively.

Metabolic parameters

Between the *placebo* and Sinetrol® XPur groups, blood NEFAs, Apo A1, and glycaemia levels showed no difference at the beginning of the study (Table 2) and were in the normal range, *i.e.* <5.6 mmol/L (glycaemia), <720 Rmol/L (NEFAs) and 37–77 Rmol/L (Apo A1). Glycaemia was not modified between W0 and W12 in the *placebo* group, whereas it was reduced by 13.6% ($p<0.05$) in the Sinetrol® XPur group. The level of NEFAs increased in both groups ($p<0.05$, Table 2) at W12. However, the increase in the Sinetrol® XPur group (+274%) was significantly greater than that of the *placebo* group (+20%; $p<0.001$). Apo A1 increased in the Sinetrol® Xpur group by 5.4% whereas it decreased by 3.3% in the *placebo* group ($p<0.05$).

Table 1. Weight, abdominal fat, waist size and hip circumference and % change (Δ) at baseline (W0) and after 12 weeks (W12) of treatment with *placebo* or Sinetrol® XPur in healthy overweight male adults.

	Placebo			Sinetrol® XPur		
	W0	W12	Δ (%)	W0	W12	Δ (%)
Body weight (kg)	86.4±4.7	84.9±4.7[a]	-1.76±0.61	89.3±6.1	85.9±5.6[a]	-3.75±0.81**
Abdominal fat (%)	26.7±3.3	25.4±2.8[a]	-4.81±1.74	26.9±3.4	24.3±3.3	-9.74±3.84**
Waist (cm)	98.5±3.6	96.4±3.4[a]	-2.11±0.48	98.8±3.3	91.4±3.5[a]	-7.50±2.00**
Hip (cm)	105.5±4.0	103.5±4.0[a]	-1.89±1.24	104.7±4.5	99.1±4.5[a]	-5.33±1.68**
Waist/hip ratio	0.93±0.02	0.93±0.02	-0.20±1.53	0.95±0.04	0.92±0.04[a]	-2.27±2.42*

Values are means ± SD, *n*=13 (*placebo*) or *n*=12 (Sinetrol® XPur). Δ (%): % difference W12 – W0. [a]an intragroup difference between W0 and W12 at p<0.05. *p<0.05 and **p<0.001 indicate Δ differences between *placebo* and Sinetrol® Xpur.

Renal function and muscle mass metabolism

Kidney function was assessed through plasma K, Na, and urea levels, which were not affected and remained within the normal healthy range throughout the 12 weeks of treatment in both the Sinetrol® XPur and *placebo* groups (Table 3).
The level of creatinine increased by 15.7% in the *placebo* group, reaching the upper healthy limit, whereas no significant change occurred in the Sinetrol® XPur group. The MDA level, within normal range in the *placebo* group at baseline, significantly increased by 14.8% (p<0.05), which was beyond the upper limit of the healthy range, whereas it tended to decrease (p=0.0689) in the Sinetrol® XPur group. Muscle inflammatory markers, such as levels of hs-CRP and fibrinogen, showed no differences between the *placebo* and Sinetrol® XPur groups at W0. At W12, while no changes occurred for fibrinogen in the *placebo* group, hs-CRP significantly increased by 16.7% (p<0.05). The same parameters significantly decreased at W12 in the Sinetrol® XPur group; respectively, by 14.7% (p<0.05) and 46.7% (p<0.05). Similarly to the level of fibrinogen, the level of uric acid decreased by 17.8% (p<0.001) after 12 weeks of treatment with Sinetrol® XPur (Table 3).

Tolerance

During the course of the study, there were no signs of metabolic disturbances among the volunteers, as indicated by preserved renal function (Table 3) and liver enzymes (ALT, ASAT, g-GT) (data not shown). Neither adverse events nor side effects were reported by the investigator. In the in-house subjective questionnaire, all items scored significantly higher values in the Sinetrol® XPur group compared to the *placebo* group (Figure 1).

Table 2. Blood metabolic parameters at baseline (W0) and after 12 weeks (W12) of treatment with *placebo* or Sinetrol® XPur in healthy overweight male adults.

	Placebo		Sinetrol® XPur	
Normal range	W0	W12	W0	W12
Glycemia (mmol/L) <5.6	6.1±0.5	6.1±0.3	5.9±0.6	5.1±0.5[a]*
NEFAs (µmol/L) <720	154.6±25.3	186.2±36.8[a]	155.6±19.3	581.3±115.6[a]*
Apo A1 (µmol/L) 37-77	48.2±8.0	46.6±3.5	50.2±9.4	52.9±3.0*

Values are means ± SD, *n*=13 (*placebo*) or *n*=12 (Sinetrol® XPur). [a]an intragroup difference between W0 and W12 at p<0.05. *p<0.001 indicate a difference between *placebo* and Sinetrol® Xpur. NEFAs: non-esterified fatty acids, Apo A1: apolipoprotein A1.

Table 3. Muscle metabolism and kidney function at baseline (W0) and after 12 weeks (W12) of treatment with *placebo* or Sinetrol® XPur in healthy overweight male adults.

	Placebo		Sinetrol® XPur	
Normal range	W0	W12	W0	W12
Muscle metabolism				
Creatinine (mg/L) 9-14	12.1±2.4	14.0±1.9[a]	12.4±1.7	13.3±2.2
Inflammation				
hs-CRP (mg/L) <5	2.4±1.4	2.8±1.2[a]	3.0±1.7	1.6±0.7[a]
Fibrinogen (g/L) 1.5-3	3.5±0.7	3.5±0.7	3.4±0.8	2.9±0.5[a]*
Oxidative stress				
MDA (µmol/L) <2.8	2.7±0.3	3.1±0.3[a]	3.2±0.4	2.9±0.5
Uric acid (mg/L) 40-60	56.5±10.0	58.8±5.2	58.3±6.4	47.9±4.1[a]**
Kidney function				
Na (mmol/l) 135-145	134.6±3.3	135.4±4.6	136.2±2.4	135.6±2.7
K (mmol/L) 3.6-5.2	4.0±0.3	3.9±0.2	4.3±0.3	4.5±0.4**
Urea (g/L) 0.18-0.45	0.44±0.05	0.45±0.05	0.36±0.12	0.41±0.08

Values are means ± SD, *n*=13 (*placebo*) or *n*=12 (Sinetrol® XPur). [a]an intragroup difference between W0 and W12 at p<0.05. *p<0.05 and **p<0.001 indicate a difference between *placebo* and Sinetrol® Xpur.

Discussion

The present study demonstrates health benefits of a 12-week supplementation with Sinetrol® XPur, a Citrus-based polyphenol extract inspired by the Mediterranean diet, on anthropometric and metabolic parameters of overweight men at risk of developing MS.

MS is the result of a constellation of metabolic deviations that increase an individual's risk for the occurrence of NCDs, mainly CVDs and DMT2. Despite the existence of several official definitions for MS, they all agree that resistance to insulin is a key feature generally resulting from a higher prevalence for individuals with excessive abdominal obesity (Despres et al., 2008). As a consequence, the IDF introduced excessive abdominal obesity as a prerequisite criteria for the diagnosis of MS, defined as waist circumference equal to or greater than 94 cm for Caucasian men (Alberti et al., 2005). Thus, the targeted population of the present study displayed 1 recognized risk factor among a mandatory minimum of 3, according to the IDF definition of MS. Consequently, this population be considered at risk for developing MS, or, as reported by others, a "pre-metabolic syndrome" (de las Fuentes et al., 2007, Stagnaro, 2007).

Anthropometric parameters and body composition

In this study, supplementation with Sinetrol® XPur was able to significantly decrease body weight (-3.75%) and abdominal fat deposit (-9.74%), as well as waist (-7.50%) and hip (-5.33%) circumference. These changes were all significantly higher than for the *placebo* group. It is of further interest to note that after 12 weeks of Sinetrol® XPur, excessive abdominal obesity was sufficiently reversed that it brought volunteers below the limit of risk for MS as defined by the IDF (waist circumference, <94 cm), and that the attending decrease in waist circumference became significant from the fourth week of supplementation (data not shown). Combined with the loss in body weight, highly improved perceptions of well-being, energy-gain and overall satisfaction, as shown by the results of the questionnaire presented to volunteers at the end of the study, serve to emphasize progress towards a greater state of health. The positive outcomes of the present study are supported by a previous clinical trial (Dallas et al., 2014) conducted in 95 overweight men and women in which a daily supplement of Sinetrol® XPur was demonstrated to significantly decrease body fat, waist and hip circumference.

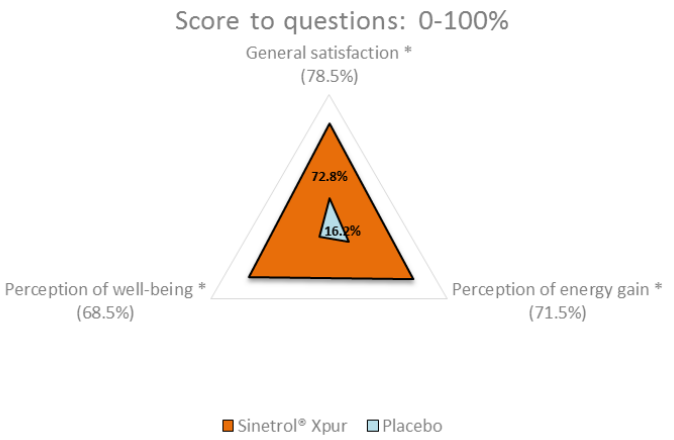

Figure 1. Perception of Sinetrol® Xpur efficacy after 12 weeks of supplementation. Values are means ± SD, *n*=13 (*placebo*) or *n*=12 (Sinetrol® XPur). *p<0.001 indicate a difference between *placebo* and and Sinetrol® Xpur.

Previously, several authors have reported that a decrease in body weight correlated with the consumption of Citrus fruit and/or Citrus fruit-derived polyphenols (Chudnovskiy et al., 2014, Titta et al., 2010). Although the majority of correlating interventions were pre-clinical studies, a clinical investigation (Fujioka et al., 2006) conducted with 91 obese adults clearly demonstrated that consumption of grapefruit, known to be rich in a specific flavanone, naringin, is able to induce significantly higher weight loss (-1.6 kg) than *placebo* after 12 weeks of supplementation.

Metabolic parameters

Anthropometric benefits highlighted in the present study can be readily attributed to the specific polyphenolic combination, mainly naringin and hesperidin, probably acting synergistically together and with other polyphenols in the product. A previous study (Dallas et al., 2008) demonstrated that a Citrus extract (Sinetrol® EXP) of similar polyphenolic content was able to operate as an efficient fat burner through a lipolytic mechanism involving the inhibition of cAMP-phosphodiesterase (PDE), resulting in an induced lipolysis as assessed by a significantly higher release of NEFAs in the plasma of treated volunteers. In the present study, volunteers consuming Sinetrol® XPur also displayed a significant increase of NEFAs in plasma, which reached more than three times the level observed in the *placebo* group. In addition, it should be noted that at baseline, volunteers of both groups failed to exhibit either hypercholesterolemia or hypertriglyceridemia (data not shown), and this lipid profile remained unchanged and within healthy range throughout the study. However, Sinetrol® XPur supplementation resulted in an increase of Apo A1 levels in plasma, which attained a significantly higher level compared to *placebo* after 12 weeks. Apo A1 is the principal protein component of HDL ensuring the removal of excess cholesterol from tissues for which its protective properties on the cardiovascular system are attributed. A significant increase in Apo A1 concentration has formerly been reported with orange juice intake (Asgary et al., 2014) in healthy volunteers after 8 weeks of regular consumption, while HDL levels remained constant. A previously reported correlation between increased Apo A1 and a lipolytic effect could, in the case of Sinetrol® XPur, be elicited through an increased transcriptional regulation of lipid metabolism, particularly through the peroxisome proliferator-activated receptor (PPAR) pathway (Mulvihill and Huff, 2012). PPARs are nuclear receptor transcription factors controlling and regulating the expression of many genes, including lipid metabolism-based genes (Monsalve et al., 2013). Evidence supporting the mechanism was shown in an *in vitro* study (Goldwasser et al., 2010) in which it was demonstrated that naringenin, the aglycone form of naringin, is an agonist of PPARs, enabling their activation and inducing a “fasted-like state” in rat hepatic cells in culture.

Beyond adipose fat deposition in the abdominal segment, another common and central criteria for MS is excessive fasting glycaemia. It is now widely accepted that abdominal obesity is strongly associated with hyperglycaemia-induced resistance to insulin, resulting at term in the development of DMT2. The unhealthy limit, as defined by the IDF, is a fasting glycaemia equal to or greater than 5.6 mmol/L. Regarding the individuals included in the study, although all volunteers might not have been measured below the limit of 5.6 mmol/L, the average fasting glycaemia at baseline, 6.0 mmol/L, can nonetheless be considered a pre-diabetic state. Supplementation with Sinetrol® XPur for 12 weeks was able to reverse this metabolic dysfunction toward a normal range (-13.6 % at 5.1 mmol/L), preventing, as a consequence, one of most important risk factors for MS immediately after excessive abdominal adiposity. Metabolic effects of Citrus fruit on glycaemic homeostasis have been well documented in experimental animal models of diabetes. Thus, *db/db* diabetic mice supplemented with naringin or hesperidin at 200 mg/kg for 5 weeks (Jung et al., 2006) displayed a significant decrease in blood glucose levels (-30% and -20% respectively), which were directly linked to an up-regulation of enzymes involved in the metabolism of glucose. Furthermore, in a study of streptozotocin-induced diabetes in rats (Sharma et al., 2011), oral

administration of naringin for 28 days was able to lower hyperglycaemia and resistance to insulin, as well as the release of inflammatory cytokines, in a dose-dependent manner. Although the mechanism of action of Citrus polyphenols on glucose homeostasis is not fully understood, it appears that antioxidant effects associated with anti-inflammatory properties would play a primary function similar to an insulin-like effect (Mahmoud et al., 2012). Collectively, these effects are apparently emphasized in the present study with Sinetrol® XPur through its ability to help regulate glucose homeostasis and at the same time being effective in significantly decreasing markers of inflammation, fibrinogen and hs-CRP, respectively by 14.7% and 46.7%.

Skeletal muscle catabolism markers

The literature is clear and there are no doubts that markers of inflammation, fibrinogen and hs-CRP, when elevated beyond a normal healthy range, reflect a condition of low grade and most often, chronic inflammation, directly correlated with an excessive deposit of abdominal fat mass (Maury et al., 2010). Hence, weight loss, and particularly a decrease in waist circumference, should be associated with a reduction of inflammatory markers, as others have recently demonstrated (Petelin et al., 2014). Furthermore, it is noteworthy that weight loss interventions have also been associated with an induction of muscle catabolism resulting in a slight but significant loss of SMM (Janssen & Ross, 1999). Among biomarkers for SMM catabolism, urinary excretion of creatinine has been clearly correlated and widely used as the reference marker for assessing SMM variation (Davies et al., 2002). Results obtained in the present study underline a moderate but significant increase of plasma creatinine, despite the fact that renal function appears efficient and unchanged, as observed from values in plasma for K, Na, and urea within the healthy range for the *placebo* group. This can easily be related to an enhanced SMM catabolism. Such an increase of SMM catabolism was also marked, as previously suggested, by the existence of a low grade inflammation in the *placebo* group. The relation between inflammation and SMM catabolism has been highlighted in several clinical trials, mainly in regard to TNF-α, IL-6 or CRP levels (Cesari et al., 2004, Schaap et al., 2006); all inflammatory markers generally recognized to have possible catabolic effects on SMM (Fanzani et al., 2012). While fibrinogen remained constant but beyond the standard healthy range, the *placebo* group displayed an increase in plasma hs-CRP concentration (+16.7% at week 12), thereby lending further evidence for the role of this additional marker as a probable but

decrease of abdominal fat (-9.74%), a profound reduction in waist circumference (-7.50%, corresponding to more than 7.40 cm), and an evident preservation of the SMM during weight loss. The partial inductor of SMM reduction during weight loss. Finally, volunteers supplemented with *placebo* showed an increased MDA level, a typical by-product of lipid peroxidation, pointing out the presence of oxidative stress, probably linked to their overweight condition with excessive deposits of abdominal fat. In an elegant review (Cesari et al., 2012), it was reported that products of oxidative damage were associated with an enhanced SMM catabolism. Sinetrol® XPur-supplemented volunteers, on the other hand, did not appear to display any additional SMM catabolism as assessed by the markers involved. Indeed, creatinine levels were not significantly increased, low-grade inflammation was decreased below the upper limit level, and none of the volunteers in the group exhibited any signs of enhanced oxidative stress. Along the same line, a significant reduction of uricemia was measured within the Sinetrol® XPur group whereas the *placebo* group had a significantly higher level at the end of the investigation, confirming the possible role of Sinetrol® XPur in the reduction of SMM catabolism, as previously observed with alanine on the decrease of SMM catabolism correlated with a decrease in hyperuricemia in an obese population (Genuth, 1973). These beneficial effects can easily be explained by known antioxidant and anti-inflammatory properties of polyphenols derived from Citrus fruits. Supportive evidence is shown in a recent study (Jain and Parmar, 2011) with a robust *in vivo* model of inflammation, *i.e*. the rat air pouch model, in which the effects of naringin and hesperidin on oxidative and inflammatory markers were compared. The authors concluded that both flavanones were able to reverse air pouch-induced inflammation, and they proposed that the individual mechanism of action of polyphenols should not be the same: hesperidin would display superior anti-inflammatory effects as highlighted by a decrease in TNF-α while naringin would contribute to an improvement in health through the reduction of oxidative stress, as observed from a significant decrease in plasma MDA, which is also the case in the present study. Both the level of MDA and inflammation markers were decreased with Sinetrol® XPur, which confirms a synergistic effect of Citrus polyphenols, acting in concert to significantly ameliorate a vicious cycle between the inflammation and oxidative stress arising from excessive deposits of abdominal fat.

Conclusion

In the present study, Sinetrol® XPur clearly appears to be a natural and safe option for overweight and obese populations. Indeed, supplementation with Sinetrol® XPur was associated with a significantly important

results show a genuine benefit of the product in managing overweight and obesity-linked metabolic disturbances, which could deter and prevent the development of MS in individuals at risk. Nevertheless, further investigations of the mechanisms of action of

Citrus polyphenols in relation to their respective bioavailabilities need to be conducted in order to gain a better understanding of their beneficial effects on the modulation of body composition.

Declaration of interest

Fytexia is involved in the research, development, marketing and sales of polyphenolic extracts from various fruit and vegetables regularly consumed within the Mediterranean diet for food and nutraceutical industries. Therefore, Fytexia has a commercial interest in this publication. RDVC was paid by Fytexia to conduct the clinical investigation and perform the clinical and biochemical measurements forming the basis of this publication. UMR 204 NUTRIPASS examined raw data to determine health benefits and hypotheses. Fytexia, RDVC and UMR 204 NUTRIPASS declare that the data in this report represent a true and faithful representation of the work that has been performed. The financial assistance of Fytexia is gratefully acknowledged.

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