sinetrol®

Fat Shredding Technology®

SCIENCE PACK

Fytexia

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 individuals (WHO). Excess fat mass is the most common chronic health problem worldwide and one of the greatest public health challenges of the 21st century.

Recognized as a cultural heritage by UNESCO, the Mediterranean diet is a modern nutritional lifestyle inspired by the traditional dietary patterns of Greece, Italy, Spain and southern France. 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.

Fytexia designed Sinetrol® as a unique Mediterranean weight-loss ingredient. Sold in more than 30 countries, the polyphenol-rich patented formula is a leading ingredient in the weight management area.

Sinetrol® acts by way of Fat Shredding Technology®, an exclusive mechanism of action enhancing the rate of lipolysis. With weight-loss benefits demonstrated in two previous clinical studies, Fytexia has deepened the scientific evidence for Sinetrol® with a third clinical study. Altogether, Sinetrol® has been clinically shown to help with body-fat loss, leading to a healthier body composition, in more than 190 subjects.

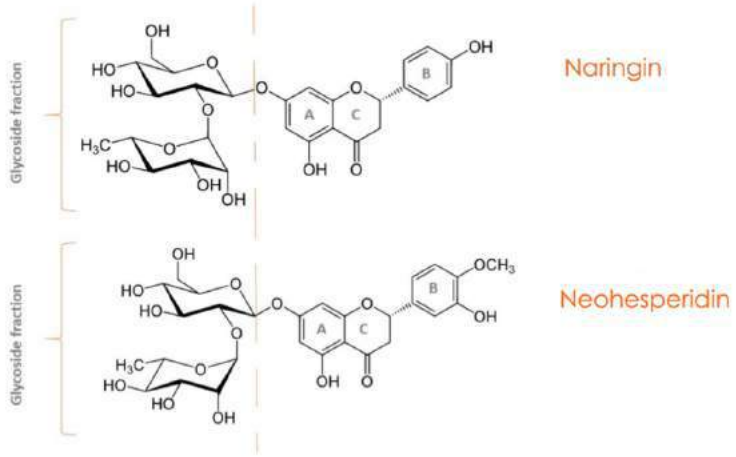
SUMMARY

1- MEDITERRANEAN DIET & POLYPHENOL SYNERGY OVERLAP TO CREATE AN INNOVATIVE INGREDIENT TO MANAGE BODY WEIGHT: SINETROL®

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 polymerization.

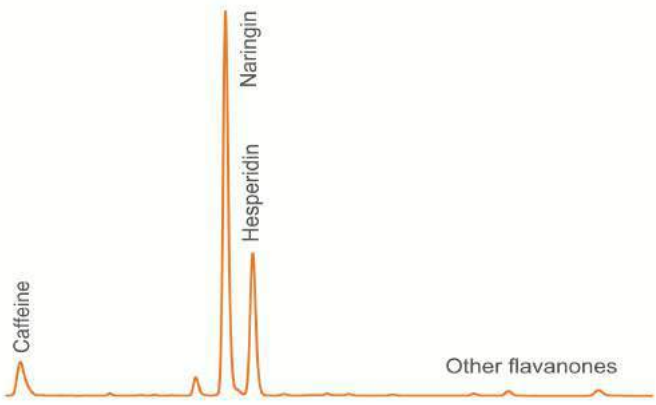
The widespread structural multiplicities of these bioactives contribute to a large number of possibilities for enhancement in regard to their beneficial effects on health. Thus, understanding flavonoids is a topic of increasing importance, especially in the prevention of NCDs.

In this context, Sinetrol® 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® provides a total synergistic polyphenol content of 90% and offers the best of the Mediterranean diet.



CHARACTERIZATION OF THE BIOACTIVE COMPOUNDS

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



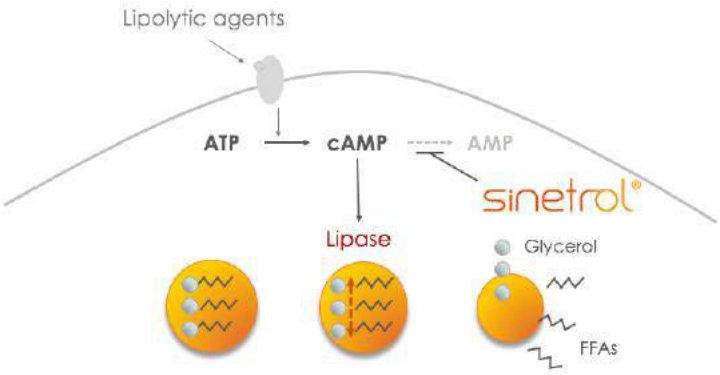
Example of Sinetrol® HPLC fingerprint at 280 nm

sinetrol®

Fat Shredding Technology®

2- MECHANISM OF ACTION

Sinetrol® is a natural combination of polyphenols extracted from citrus and guarana targeting **body weight management**. **Sinetrol®** works as a safe and non-thermogenic fat burner to reduce the excess of fat mass.

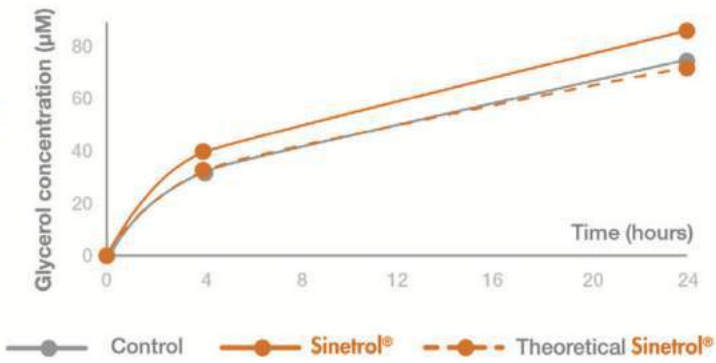


Fat Shredding
Technology®

Lipolysis is a catabolic process leading to the breakdown of triglycerides stored in fat cells (adipocytes), releasing free fatty acids (FFAs) and glycerol.
Sinetrol® acts by way of the exclusive mechanism of Fat Shredding Technology® (FST). **Sinetrol®** facilitates lipolysis through the inhibition of phosphodiesterase-4, the enzyme that catalyzes the hydrolysis of cyclic adenosine monophosphate (cAMP). Higher cAMP levels lead to an increased rate of triglyceride breakdown.

As highlighted in an *ex vivo* study, **Sinetrol®**, by way of the Fat Shredding Technology®, enhances the release of FFAs and glycerol, which results in a reduction in the volume of adipocytes. Acting together, polyphenols and caffeine in the patented formula of **Sinetrol®** provide higher lipolytic potency than when tested separately (theoretical **Sinetrol®**).

Lipolytic activity



sinetrol®
Fat Shredding Technology®

3- CLINICALLY PROVEN EFFICACY

Two double-blind randomized, *placebo*-controlled studies
900 mg/day of Sinetrol® in 2 capsules

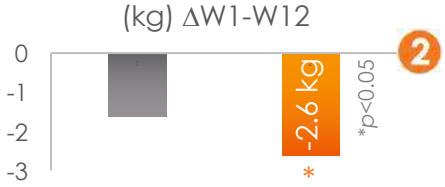
	1	2
	Paris, France American Hospital	Paris, France American Hospital
	12 weeks	12 weeks
	20 subjects BMI: 27-33 Age: 22-55 years	95 subjects BMI: 26-29.9 Age: 22-45 years
	No diet	Normo-caloric diet *(Harris and Benedict)*
	Physical activity < 30 min/week	Physical activity < 30 min/week
	Impedance bioelectrical scale	VISCAN By Tanita®

WEIGHT LOSS

Body weight loss
(kg) ΔW1-W12

OVERWEIGHT & OBESE SUBJECTS

Body weight loss
(kg) ΔW1-W12



OVERWEIGHT SUBJECTS

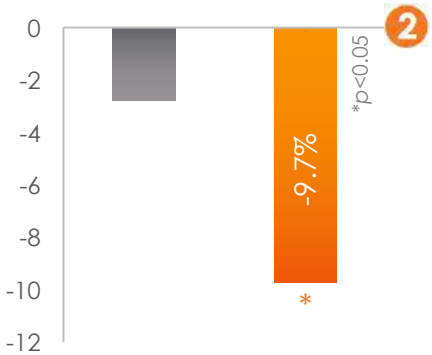
Placebo
Sinetrol®

The two first clinical studies were conducted in a healthy population of overweight or obese men and women. **These studies demonstrated that the polyphenol-rich ingredient Sinetrol® is a safe solution for reducing excess of body weight:**

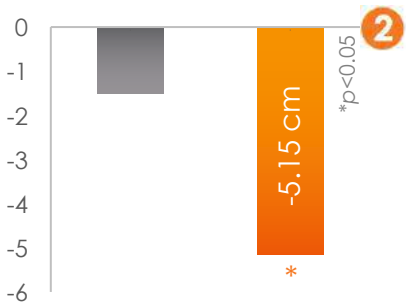
- The Sinetrol® group experienced a **significant and progressive body weight loss during the trials**, especially fat loss in the abdominal area.
- The silhouette of the supplemented group were improved with a **significant reduction in waist and hip size.**

SILHOUETTE SHAPING

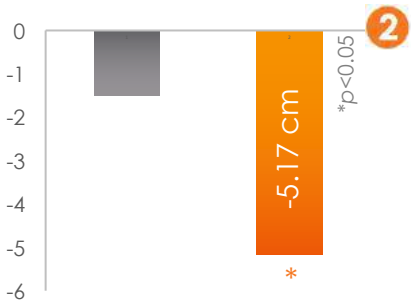
Abdominal fat reduction
(%) Δ W1-W12



Waist size reduction
(cm) ΔW1-W12



Hip size reduction
(cm) ΔW1-W12



NEW

sinetrol®
Fat Shredding Technology®

4- THIRD CLINICAL STUDY

Double-blind randomized, *placebo*-controlled study
900 mg/day of Sinetrol® in 2 capsules

Murcia, Spain
Research center, University of Murcia

77 subjects
BMI: > 25
Age: 29-52 years

16 weeks

Gold Standard: DXA
(Dual energy X-ray Absorptiometry)

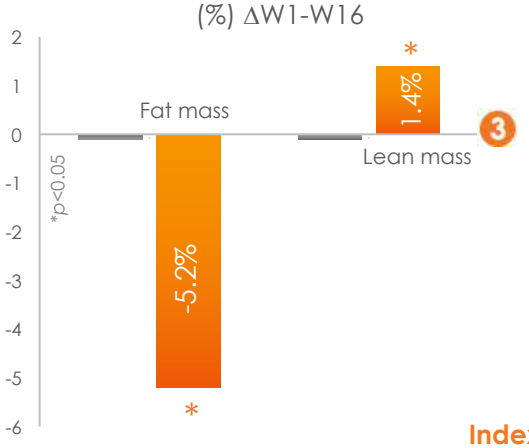
Normo-caloric diet
(Harris and Benedict)

Physical activity
No recommendation

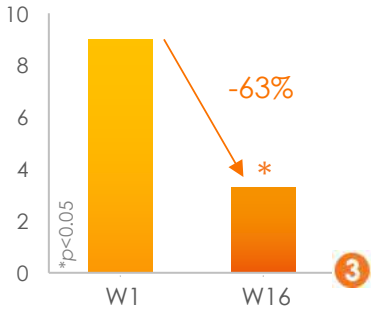
ANTHROPOMETRIC MEASURES

In this third trial, Sinetrol® again induced a significant weight loss. Moreover, the gold standard technology DXA used to measure the changes highlights that supplementing a normo-caloric diet with Sinetrol® led to the support of a **healthy body composition: body fat decreased while lean mass increased.** The supplemented group reduced their excess of fat mass by 63% compared to the theoretical fat mass.

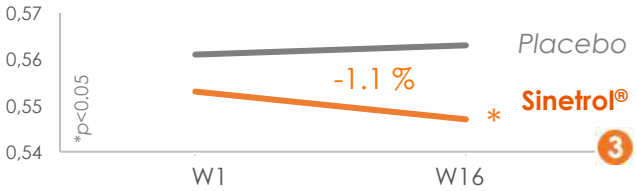
Body-fat & lean mass
(%) ΔW1-W16



Body-fat mass reduction
vs % theoretical fat mass



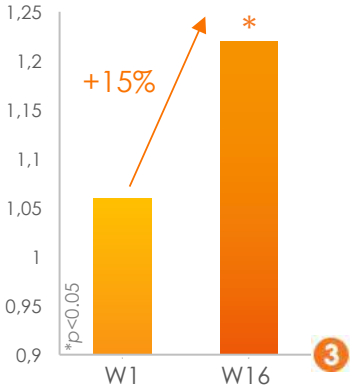
Index of Central Obesity (ICO)



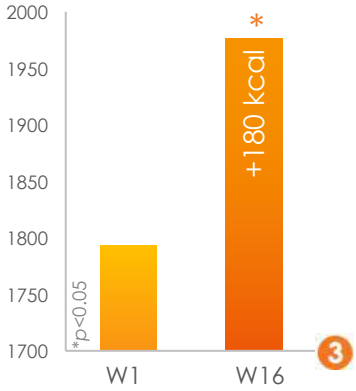
MECHANISTIC MARKERS

Blood measures confirmed the metabolic of action of the **Fat Shredding Technology®**: Sinetrol® was proven to enhance the release of free fatty acids from adipocytes signifying a **higher rate of lipolysis.**

Free fatty acids release
(mmol/L)



Resting energy expenditure
(expended kcalories/day)



THIRD CLINICAL STUDY & FOLLOW-UP

new

Double-blind randomized, *placebo*-controlled study
900 mg/day of Sinetrol® in 2 capsules – Follow-up: cessation of the supplementation

3

Murcia, Spain
Research center, University of Murcia

Sinetrol
900 mg/day

16 weeks

77 subjects
BMI: > 25
Age: 29-52

FOLLOW-UP

Murcia, Spain
Research center, University of Murcia

Cessation of the supplementation

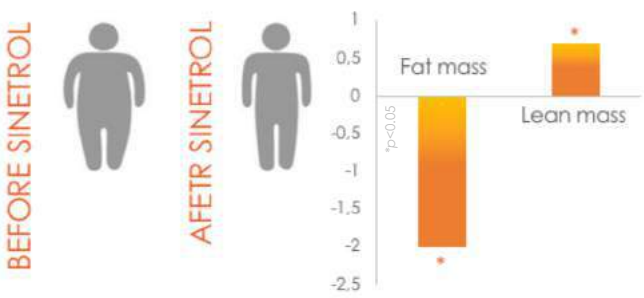
4 additional weeks

47 subjects
31 in Sinetrol group

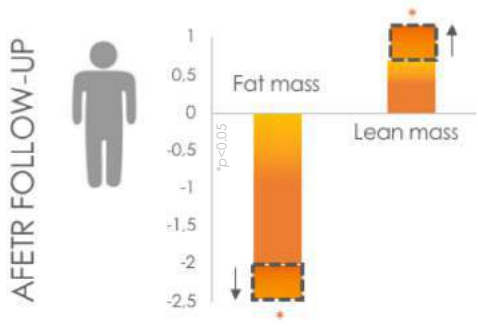
- Weight loss
- Demonstrated Fat Shredding
- Healthier body composition

BODY **COMPOSITION**

Body Fat &Lean mass
(kg) ΔW1-W16



Body Fat &Lean mass
(kg) ΔW1-W16 & after follow-up

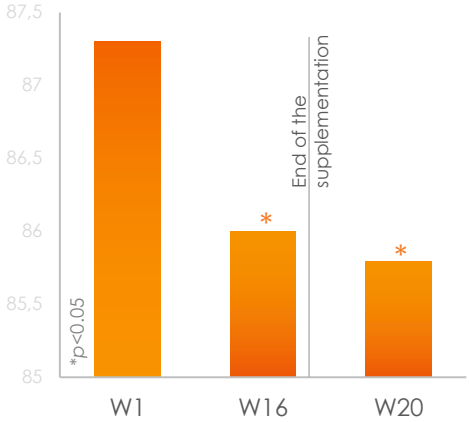


Subjects dropped fat mass significantly while lean mass increased:
The effect persists and amplifies after the supplementation

WEIGHT **LOSS**

Sinetrol group **continues to lose weight** after the supplementation is over while no difference is observed in the placebo group

Body weight loss
(kg) W1-W16 & follow-up (W20)



5 - FIRST CLINICAL STUDY

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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, r e s p e c t i v e l y .

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 etal., 2005), which degrades cyclic cAMP and conse- quently 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 quantifiedby using Naringin (Extrasynthese, France) as external standard.

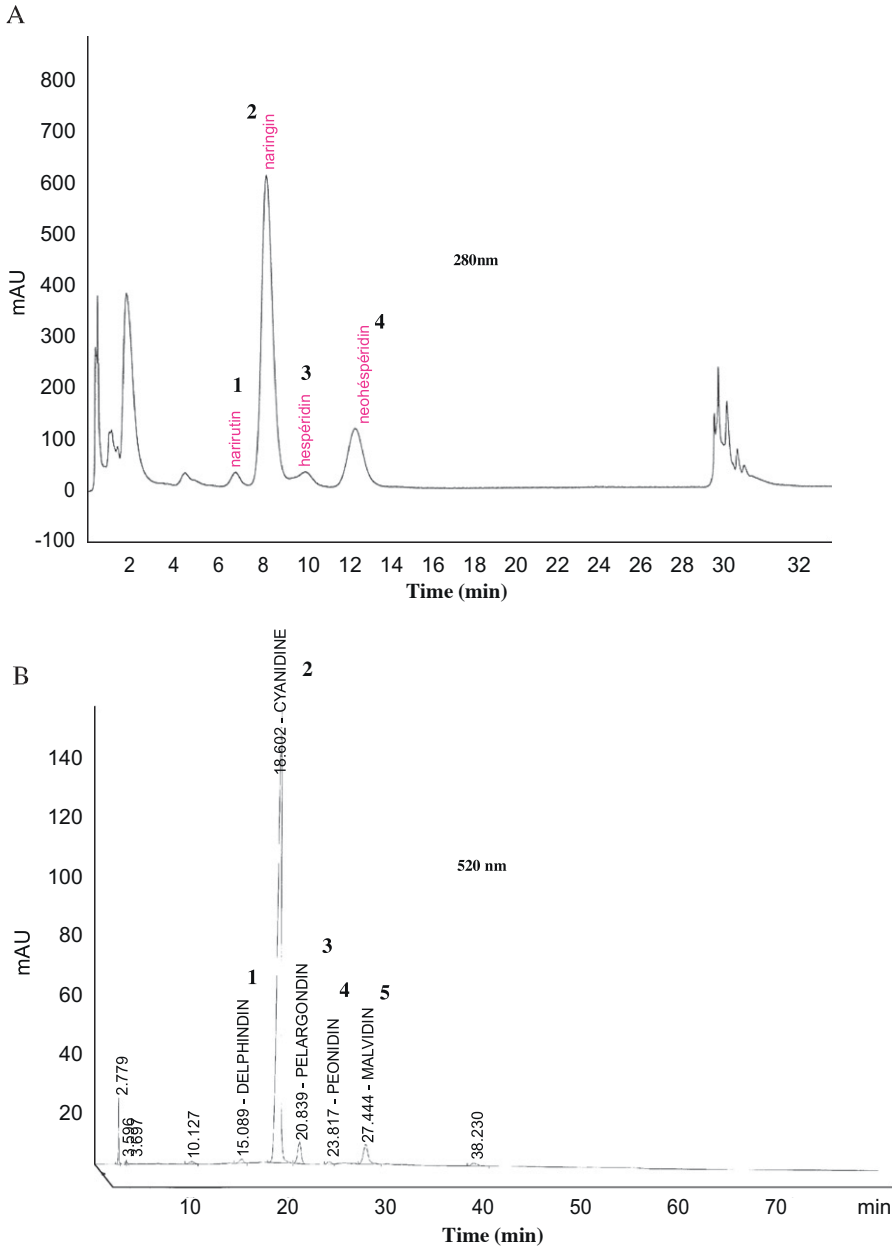

Fig. 1. (A) A typical flavanones HPLC Chromatagram of SINETROL recorded at 280 nm. (1) Narirutin; (2) naringin; (3) hesperidin; (4) neohesperidin. (B) A typical Anthocyanin HPLC Chromatagram of SINETROL recorded at 520 nm. (1) Delphinidin-3-glucoside; (2) cyanidin-3-glucoside; (3) pelargonidin-3-glycoside; (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 1C 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 1C with or without 20 mg/ml guarana 12% caffeine or 20 mg/ml SINETROL; theopylline (1 mM), isoproterenol (1 mM) 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 Kolmogor- nov–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 inTable 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 ($p<0.01$) 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 ($p<0.05$).

In the same order of magnitude, the SINETROL stimulates significantly ($p<0.05$) 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 ($p<0.05$). 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% ($p<0.05$), while SINETROL content in caffeine (3.6%) is 3 times lower.

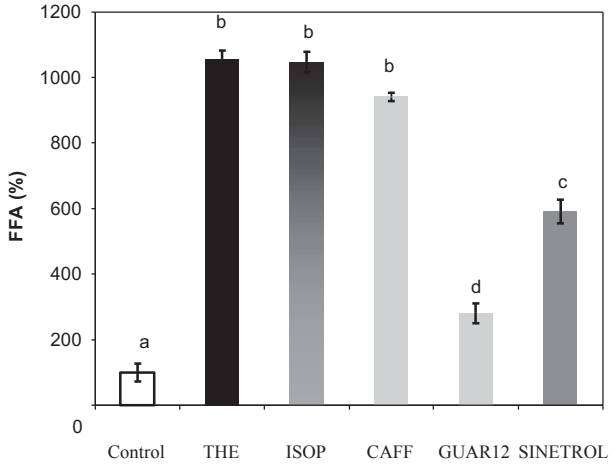

Fig. 2. FFA release after treatment of human adipocytes by various lipolytic products. Fat human adipocytes solution (540 ml) was added to 60 ml solution of tested compounds and each reaction mixture was incubated for 2 h at 37 1C. FFA released from adipocytes were measured as combined FFA- BSA in 30 ml assay medium as described in Materials and methods (method 1). Values are expressed as mean7SE. Bars with different index letters are significantly different ($p<0.05$).
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-obesityin 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, $n = 10$, for each placebo and SINETROL[S] tested group.
Means within rows followed by the same superscript are not significantly different ($p<0.05$).

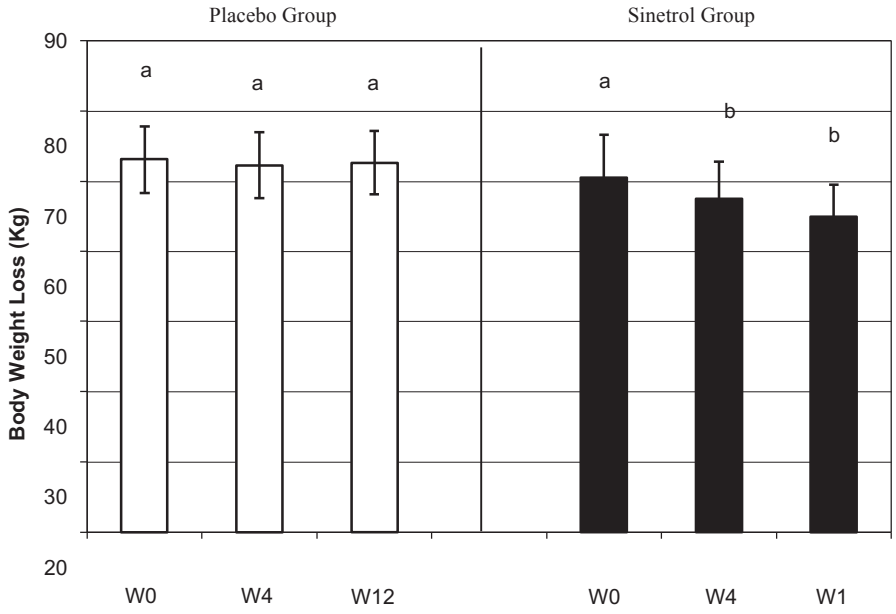

Fig. 3. Effects of supplementation with *placebo* and *SINETROL* on *body weight loss* (kg) in 20 healthy volunteers before (0 weeks), during (4 weeks) and after 12 weeks of treatment. The placebo and *SINETROL* products were administrated as 4 pieces hard capsules containing 350 mg each (1.4 g/day). 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. Barswith different index letters are significantly different (po0.05).

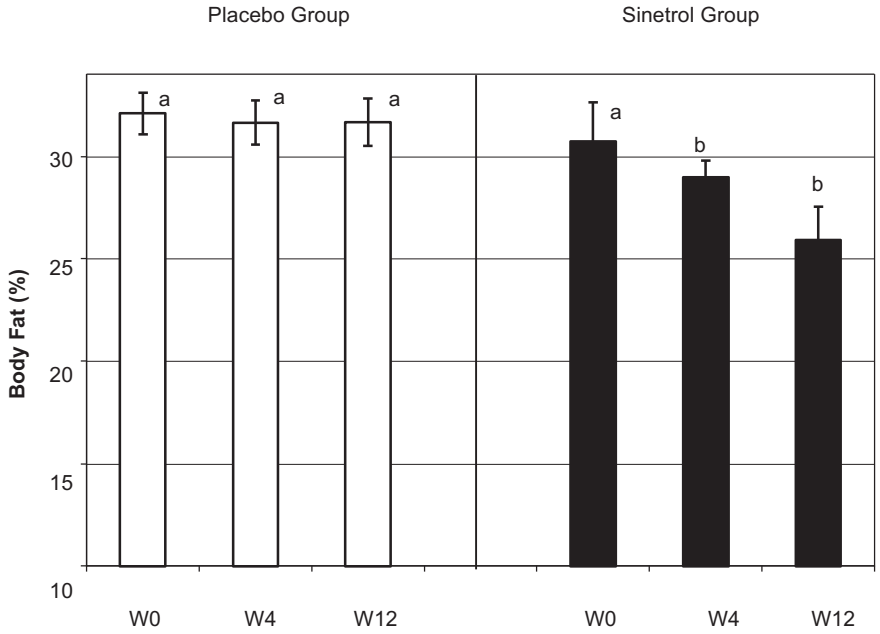

Fig. 4. Effects of supplementation with *placebo* and *SINETROL* on *body fat loss* (%) in 20 healthy volunteers before (0 weeks), during (4 weeks) and after 12 weeks of treatment. The tested products were administrated as 4 pieces hard capsules containing 350 mg each (1.4 g/day) Two capsules were administrated 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 extractstandardised at 60% of polyphenols and 15% maltodextrin. Values are expressed as mean7SE. Bars with different index letters aresignificantly 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, nonsignificant 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 ($p<0.05$) 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 = SINETROL > naringin > caffeine > guarana.

SINETROL is a potent inhibitor of PDE product (97% of inhibition; $p<0.001$). 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) ($p<0.001$).

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

Tested products	Concentration	PDE assay	PDE inhibition	
	(%)	Cpm	(%)	Mean (%)
Control	–	2153	−6	0 ± 5a
		2017	3	
		2016	3	
DMSO control	1	2178	−7	−5 ± 9a
		2282	−14	
		1986	5	
Caffeine	0.01	1176	56	56 ± 5b
		1242	51	
		1093	61	
Cyanidin-3 glucoside	0.01	388	105	99 ± 8c
		619	91	
		449	101	
Naringin	0.01	606	91	87 ± 6c
		782	80	
		637	89	
Guarana 12% caffeine	0.01	1989	5	7 ± 3a
		1905	10	
		1943	7	
SINETROL	0.01	625	90	97 ± 1c
		344	108	
		595	92	

Values are mean ± SE, $n = 3$, for each tested product.
Means within rows followed by the same superscript are not significantly different ($p<0.05$); Cpm = 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.

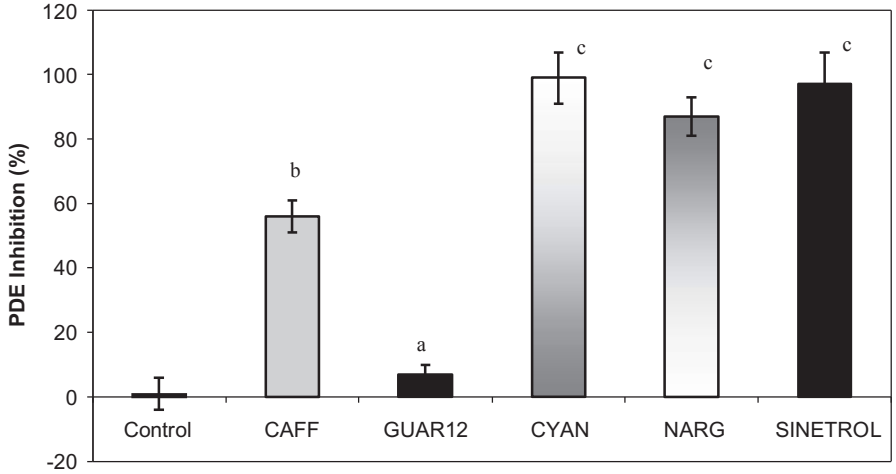

Fig. 5. Effects of various lipolytic products in *in vitro* PDE inhibition assay. PDE (50 mg/ml) were incubated during 10 min at 37 1C in the presence or not of tested products and $3^0,5^0$ cAMP (0.5 mCi/ml). The PDE assay was performed using SPA scintillation beads as described in Materials and methods. 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 compo- sition may help to decrease body weight and body fat.

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6 – SECOND CLINICAL STUDY

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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 (WO, 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 ($+329.73 \pm 14.68$%) was significantly greater than that for placebo ($+33.16 \pm 4.6$%) ($p < 0.0001$). 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 (μmol/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 (μmol/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 (μmol/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 (μmol/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 $22.87 \pm 7.30\%$ with Sinetrol-XPur, whereas they increased by $61.79 \pm 14.44\%$ with the placebo, and the difference between the two groups was highly significant ($p < 0.0001$). Fibrinogen levels decreased by $19.91 \pm 2.04\%$ 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 $14.03 \pm 1.18\%$ in the Sinetrol-XPur group compared with a slight increase in the placebo group ($2.76 \pm 1.61\%$) 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 ($17.38 \pm 4.08\%$ vs $2.19 \pm 3.66\%$, $p < 0.01$). GSH levels increased by $4.63 \pm 11.62\%$ in the Sinetrol-XPur group, whereas they decreased by $2.36 \pm 1.13\%$ 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 $9.95 \pm 1.87\%$ in the Sinetrol-XPur group, whereas it increased by $5.40 \pm 1.90\%$ in the placebo groups with a significant difference between the two groups ($p < 0.0001$). Concurrently, HbA1c rose slightly by $7.15 \pm 12.56\%$ in the Sinetrol-XPur group and by a higher level ($24.35 \pm 2.46\%$) 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 (μmol/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 (μmol/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 (μmol/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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7 - THIRD CLINICAL STUDY

Scientific clinical report

1 SINETROL XPUR – CLINICAL TRIAL

Clinical Study Report

Title	Double-blind, randomized, placebo-controlled study to evaluate the benefit of a polyphenolic-rich fruit and seed extract in managing body fat loss and in improving body composition in overweight and obese subjects
Investigational product	Sinetrol® Xpur
Indication	Body fat loss
Description	A 16-week randomized, double-blind, parallel design clinical trial comparing the benefit of Sinetrol® Xpur to placebo in managing body fat loss and in improving body composition
Sponsor	Fytexia SAS
Protocol number	CFE-46/14
Study period	March 2015 to September 2016
Principal investigator	Pedro E. Alcaraz Ramón, PhD
Investigators	Linda H. Chung, PhD Juana M. Morillas Ruiz, PhD Jacobo A. Rubio Arias, PhD Elena Marín Cascales, MSc Alejandro Martínez-Rodriguez, PhD
Compliance	This clinical investigation was performed per the principles established in the Declaration of Helsinki and in accordance with Good Clinical Practices defined in the ICH Harmonized Tripartite Guideline
Report date	APRIL-2018

2 SYNOPSIS

Study sponsor	FYTEXIA SAS ZAE via Europa 3, rue d'Athènes 34350 Vendres France
Name of study product	Sinetrol® Xpur
Characterization of the study product	Sinetrol® Xpur is a natural water-soluble fruit and seed extract
Title of the study	Double-blind, randomized, placebo-controlled study to evaluate the benefit of polyphenolic-rich fruit and seed extract in managing body fat loss and in improving body composition in overweight and obese subjects
Principal investigator	Pedro E. Alcaraz Ramón, PhD
Investigators	Linda H. Chung, PhD Juana M. Morillas Ruiz, PhD Jacobo A. Rubio Arias, PhD Elena Marín Cascales, MSc Alejandro Martínez-Rodriguez, PhD
Study center	UCAM's Research Center for High Performance Sport Avenida Jerónimos, 135 30107 Guadalupe, Murcia SPAIN
Study period	March 2015 to September 2016
Objectives	Evaluate the benefit of Sinetrol® Xpur in managing body fat loss and in improving body composition in overweight and obese subjects
Methodology	The clinical investigation was a 16-week, double-blind, randomized, placebo-controlled, parallel group study
Number of subjects	77 (VCAS)
Diagnosis and main criteria for inclusion	Overweight and obesity
Test product, dose, mode of administration and batch number	Sinetrol® Xpur (450 mg per dose of fruit and seed extract rich in polyphenols and caffeine), 2 capsules a day, per Sinetrol® Xpur oral, batch number XPUR141013 and XPUR150527
Duration of supplementation	16 weeks + 4-week follow-up
Reference product, dose,	Placebo (450 mg maltodextrin), 2 capsules a day, per oral, batch number MLT140120 and MLT150506

mode of administration and batch number	
Benefit criteria	• Body fat mass loss (DXA technology) after 16 weeks of supplementation as compared to placebo group • Changes in anthropometry and body composition (DXA technology) after 16 weeks of supplementation as compared to placebo group • Changes in resting energy expenditure (Respiratory Exchange Ratio) after 16 weeks of supplementation as compared to placebo group • Changes in metabolic parameters after 16 weeks of supplementation as compared to placebo group
Follow-up criteria	• Change in calorie intake (recommended vs reported) • Change of level of physical activity (pedometer)
Safety criteria	• Renal function parameters (urea, creatinine, sodium, potassium) • Liver function parameters (alanine transaminase, aspartate amino transferase, gamma-GT) • Heart rate at rest • Adverse events
Statistical methods	All variables included in the data collection are described by its statistical parameters (mean, standard deviation). An exploratory analysis of sample normality has been performed with the Kolmogorov-Smirnov test and both Liliefors and Shapiro-Wilk tests significance correction. The differences between groups and the effect of time have been evaluated with parametric procedures (*t*-test) to determine the intra- and inter-group differences. The level of significance is set at $p \leq 0.05$.

Summary-conclusions	<u>Benefit criteria</u> • Primary outcome achieved the 5% level of significance with a mean body fat loss (%BW) of 2 points (p=0.0003) within the Sinetrol® Xpur group after 16 weeks of supplementation. • All anthopometric parameters (BW, BMI, total lean mass, total fat mass, lean-to-fat mass ratio, trunk fat mass, ICO, waist and hip circumferences) were significantly improved within the Sinetrol® Xpur group after 16 weeks of supplementation. • Resting energy expenditure significantly improved within the Sinetrol® Xpur group after 16 weeks of supplementation. • Fibrinogen and free fatty acids concentration significantly improved within the Sinetrol® Xpur group after 16 weeks of supplementation. Levels of leptin and adiponectin stayed stable in both groups after 16 weeks of supplementation. <u>Follow-up criteria</u> • The mean difference between recommended intake and reported intake was less than 10% after 16 weeks in both groups. • The level of physical activity (pedometer) did not significantly changed in both groups throughout the course of the study. <u>Safety criteria</u> • Safety parameters here were no clinically significant changes in any of the laboratory parameters observed. • There were no clinically significant changes in heart rate. • No serious or related adverse events associated to the supplementation were reported. <u>Overall conclusion</u> It has been shown that supplementation with Sinetrol® Xpur within the 16-week period led to a statistically significantly total body fat mass loss while the placebo group did not experience any significant variation. Moreover, all related anthropometric parameters significantly improved with the Sinetrol® Xpur supplementation, as well as resting energy expenditure. In addition, it could be proven that Sinetrol® Xpur has an excellent safety profile and that no adverse events have been recorded.

3 TABLES OF CONTENTS

4 ABBREVIATIONS

BMI	Body Mass Index
BW	Body Weight
CVD	Cardiovascular Disease
DXA	Dual energy X-ray Absorptiometry
FFAs	Free Fatty Acids
FM	Fat Mass
ICO	Index of Central Obesity
LM	Lean Mass
NCD	Non Communicable Disease
REE	Resting Energy Expenditure
TFM	Trunk Fat Mass
VCAS	Valid Case Analysis Set
SMM	Skeletal Muscle Mass

5 ETHICS

The study was reviewed and approved by the Comité de Ética de la UCAM before study initiation.

This clinical investigation was performed according to the protocol, to the principles established in the current revised version of the Declaration of Helsinki (Seoul, 2008) and in accordance with the recommendations of Good Clinical Practice (1996) and guidelines from Good Epidemiological Practice (http://.ieatemp.com/goodEpiPractice.aspx). The Declaration of Helsinki can be obtained from the website of the World Medical Association in .wma.net/es/30publications/10policies/b3/17c_es.pdf.

Before enrolment, the investigator has informed each subject about the objective, the intended effect, possible impacts and risks as well as the exact chronological and procedural investigation process. The subject was also informed about the fact that he/she may revoke his/her written consent at any time, and thereby terminate participation in the study.

The participant declared his/her agreement to all the conditions of the investigation, by signing the informed consent form in the presence of the investigator, who countersigned the form including the date and location.

6 RATIONALE

Excessive body weight is currently the most common chronic health problem worldwide and one of the greatest public health challenges of the twenty-first century. A major cause of overweight and obesity is known to be the accumulation of excessive body fat due to such causes as an imbalance between calories consumption and energy expenditure, especially within population with sedentary behaviours. In addition to causing various physical disabilities and psychological problems, overweight and obesity, especially when excess of fat is accumulated within the abdominal area, drastically increase a person's risk of developing a number of non-communicable diseases (NCDs); including metabolic syndrome (MS), cardiovascular diseases (CVDs) and type 2 diabetes mellitus (T2DM) (Aballay *et al*. 2013; Balkau *et al*. 2007; Cardoso-Saldana *et al*. 2010; Janus *et al*. 2007; Kaysen *et al*. 2009; Wadden & Phelan, 2002), which dramatically affect average life expectancy, making overweight and obesity the fifth leading risk factor for global death (WHO 2013). Nevertheless, overweight, obesity and their consequences are preventable.

During fat accumulation throughout the progression of overweight or obesity, it has been 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 modified diets during weight loss intervention programs in overweight populations with excessive abdominal fat (Janssen & 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 (Cases *et al*. 2015).

Therefore, 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), and it is critical that such body weight

loss programs participate to preserve a balanced body composition (lean/fat ratio). Hence, a precise measurement of the percentage body fat associated to lean mass ratio is considered the reference method for defining overweight or obesity. Accordingly, Dual energy X-ray Absorptiometry (DXA), appears as the highest sensitive approach able to determine the entire body composition (both body fat & body lean mass), is now considered as the gold standard methodology (Shawk *et al*. 2007); but still anthropometric indices such as BMI, waist and hip circumferences and index of central obesity (ICO) are the most commonly used indicators for assessing abdominal overweight or obesity (Singh *et al*. 1998; Parikh *et al*. 2006; Mushtaa *et al*. 2011).

Among the most dietary patterns studied for their health effects, it appears that adherence to a Mediterranean diet is correlated to a lower risk for NCDs. It has been assumed that some bioactive constituents of Mediterranean foods, namely polyphenols, are responsible for the observed health-promoting effects ascribed to this dietary style (Ros *et al*. 2014). The biological effects of polyphenols have been largely attributed to their antioxidant properties; however, recent data suggest that polyphenols can exert modulatory action in cells by interacting with the cell signaling machinery. Thus, several polyphenols can affect metabolic pathways involved in either appetite, adipogenesis and energy homeostasis (Maydani & Hasan 2010). Hence, these bioactives might be useful in the management of metabolic disorders generally associated to overweight and obesity.

The product to be investigated, Sinetrol® Xpur, is a proprietary combination of extracts from grapefruit and orange, providing Mediterranean polyphenols, and guarana providing natural caffeine.
In a 12-week double-blind, placebo-controlled trial (Dallas *et al* 2014) with 95 overweight subjects (BMI=26.0-29.9), supplementation with Sinetrol® Xpur associated with a normo-caloric diet and 30 min/week of physical activity, induced a significant body weight loss (-2.6kg) associated with a body fat decrease (-3.6%) and reduction of both waist and hip circumferences.

Moreover, in a double-blind, placebo-controlled pilot trial (Cases *et al*. 2015) involving 25 overweight men, 12 weeks of supplementation with Sinetrol® Xpur induced a significant decrease of body fat (-2.6%) while metabolic markers of muscle catabolism stayed stable after 12 weeks, indicating preservation of SMM during fat loss.

Based upon these results, the aim of the present study was (1) to confirm the benefits of a supplementation with Sinetrol® Xpur in decreasing body fat mass within a population including both overweight and obese subjects and (2) to confirm and evaluate the preservation of SMM during fat loss in integrating a reliable measure of body composition (fat mass and lean mass) using the DXA Technology.

7 STUDY OBJECTIVE

Main objective of this 16-week double-blind, randomized, placebo-controlled study, is improvement of total body weight, mainly as body fat loss – At least 2 points difference body fat percentage between D0 and D112 within the Sinetrol® Xpur-supplemented group.

- **Primary outcome:** Total body fat percentage loss versus body weight (BW) by DXA measurement
- **Secondary outcomes measured:** Body weight loss, body lean mass gain, lean-to-fat mass ratio improvement, excess body fat mass improvement *versus* theoretical fat mass, trunk fat mass reduction, index of central obesity improvement, waist circumference & hip size improvement, Resting Exchange Ratio (REE) improvement, metabolic parameters improvement, change in calorie intake.

8 INVESTIGATIONAL PLAN

8.1 Overall study design and plan: description

The clinical investigation was a 16-week, double-blind, randomized, placebo-controlled study.

All subjects were instructed to ingest two capsules, one at breakfast time and one at lunch time. Thus, the daily dose was 2 capsules.

In addition, subjects were coached by a dietician to follow and maintain a nutritionally balanced and normal-calorie diet based on individual diet plans. Based on gender, body weight, age and height, the individual Resting Energy Expenditure (REE) was calculated from the revised equation of Harris-Benedict (Roza & Shizgal, 1984) and adjusted to their individual level of physical activity assessed with an oral interview.

The REE was calculated as follows:

Men	REE = 88.362 + (13.397 x weight(kg)) + (4.799 x height(cm)) - (5.677 x age(years))
Women	REE = 447.593 + (9.247 x weight(kg)) + (3.098 x height(cm)) - (4.330 x age(years))

Then, REE is adjusted according the level of physical activity.

Little to no exercise	Daily kcalories needed = REE x 1.2
Light exercise (1-3 days per week)	Daily kcalories needed = REE x 1.375
Moderate exercise (3-5 days per week)	Daily kcalories needed = REE x 1.55
Heavy exercise (6-7 days per week)	Daily kcalories needed = REE x 1.725
Very heavy exercise (Twice per day)	Daily kcalories needed = REE x 1.9

Participants were encouraged to maintain their usual level of physical activity and to follow the individual diet throughout the 16-week intervention-period. At the beginning and at the end of the studied period, volunteers performed a 24-hour diet recall interview (2 interviews during the week and 1 during the weekend) to check compliance to instructions.

Volunteers have been submitted to 6 visits during the study.

Pre-inclusion visit (W_0):

- Oral and written information about the nature, purpose, possible risks and benefits of the study provided to the subjects by the investigator
- Written consent of the subject to participate, he/she understands the requirements of the clinical investigation and is willing to comply
- Verification that the inclusion criteria are met and that there are no violations of the exclusion criteria
- Assessment of anthropometrics
- Blood sampling for the assessment of safety parameters

Baseline visit (W1):

- Assessment of anthropometrics and body composition (DXA)
- Assessment of REE with indirect calorimetry
- Interview, determination of calorie intake and nutritional coaching
- Blood sampling for metabolic analyses
- Issue of first pill box and instructions for correct use
- Issue of pedometer and diary and instructions for correct use

Follow-up visits (W4, W8 and W12):

- Return of subject's diary
- Return of any unused investigational product for compliance control
- Questioning and documentation of possible occurrence of adverse events
- Issue of next pill dispenser for 4 weeks

Final visit (W16):

- Assessment of anthropometrics and body composition (DXA)
- Assessment of REE with indirect calorimetry
- Interview and determination of calorie intake
- Blood sampling for metabolic analyses and safety parameters

- Questioning and documentation of possible occurrence of adverse events
- Return of any unused investigational product for compliance control

8.2 Selection of study population

8.2.1 Inclusion criteria

- Age 25 to 55 years olds
- Overweight and obese subjects (25 kg/m^2 ≤ BMI ≤ 42.5 kg/m^2)

8.2.2 Exclusion criteria

- Having a metabolic and/or chronical disease for which subjects are treated (diabetes, dyslipidaemia, thyroiditis, inflammatory disease, immunological disease, infectious disease, asthma, anxiety and depression…)
- Having a food allergy to the ingredients of the product (grapefruit, orange, caffeine and/or guarana)
- Have been involved in the prior 6 months in a chronic treatment program, a weight loss program, having a history of eating disorders, have been subjected to weight reduction surgery
- Having started or quit smoking, having a high alcohol consumption
- Being pregnant, breastfeeding or wanting to have a baby
- Menopausal women (no period since at least 12 months)

8.2.3 Removal of subjects from therapy

Subjects were free to discontinue their participation in the study at any time, without prejudice to further intervention. Further, investigators could withdraw individual subjects at their discretion if judged necessary.
Specific reasons for discontinuing a subject from the study were:

- Intolerance to the investigational product
- Required additional therapy due to other complaints, which could influence the results of the investigation
- Serious adverse events

- Clinically significant illness or intake of concomitant medication according to exclusion criteria, which could influence the results of the investigation
- Insufficient compliance by the subject
- Withdrawal of informed consent

8.3 Investigational product / Supplementation

8.3.1 Supplementation administered

For the 16 weeks following randomization, subjects received either Sinetrol® Xpur or placebo and ingested one capsule with breakfast and one with lunch, daily.

8.3.2 Identity of the investigational product

Sinetrol® Xpur is a proprietary combination of fruit extracts. It is standardized to contain at least 20% of polyphenols in the form of flavanones extracted from grapefruit (*Citrus paradisi* Macfad) and orange (*Citrus sinensis* L.). The product also contains a source of caffeine delivered from a guarana extract (*Paullinia cupana* Kunth).
The dry extract was packaged in red cellulose capsules (450 mg per capsule).
Identical-looking capsules are filled with 450 mg of maltodextrin each and used as placebo.

8.3.3 Methods of assigning subjects to supplementation groups

The randomization number was generated using a simple block randomization of 1:1 with an additional stratification for sex (40% minimum and 60% maximum each sex) with separate randomization list.
The label of the issued investigational product contained the randomization number.
Randomization occurred at visit 1 (W1) when inclusion criteria are met, subjects complied with the protocol and no violation of exclusion criteria had occurred. A 3-letter code is then attributed to each subject.

8.3.4 Blinding

As this clinical investigation was performed double blind, the investigator received sealed envelopes containing allocation information to Sinetrol® Xpur or placebo. The emergency envelopes should be opened by the investigator in emergency cases in which the investigator suspected a causal relation with the investigational product requiring unblinding.
For data and biological analysis, the scientists involved only accessed to the random number labelled on samples. They did not have any information concerning the sex and the arm.

8.4 Methods for assessment of benefit and safety variables

8.4.1 Safety analysis

After sampling, venous blood samples were transported on the same day in cooler boxes to a central laboratory (Complejo Hospitalario Universitario de Cartagena - Spain) for analysis of safety parameters including:

- Serology: Human Immunodeficiency Virus 1 & 2 (HIV 1&2), Hepatits B Virus (HBV), Hepatitis C Virus (HCV)
- Hormones: Human Chorionic Gonadotropin (hCG)
- Kidney function: urea, creatinine, sodium (Na), potassium (K), glomerular filtration
- Liver function: alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), gamma-glutamyltransferase (gamma-GT)
- Heart rate: A polar heart rate band (Polar Electro Inc., NY, USA) was strapped over the volunteer’s chest to measure heart rate while at rest.

8.4.2 Benefit variables

8.4.2.1 Body composition

Anthropometry:

Body weight (kg) was measured in subjects wearing light clothes and no shoes using calibrated weighing scales (Tanita Corporation, IL, USA).

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 with a non-stretchable tape.

The ICO was calculated as the waist-to-height ratio.

Theoretical fat mass was calculated according to the equations of Deurenberg *et al.* (Deurenberg *et al.*, 1991):

Body fat (%): (1.20 x BMI) + (0.23 x age) – (10.8 x sex) -5.4; with sex=1 for men and sex=0 for women.

Dual-Energy X-ray Absorptiometry:

Body composition was assessed using DXA-scan of the whole body (XR-46; Norland Corp., Fort Atkinson, WI, USA). Discrimination of whole-body fat mass (FM), trunk fat mass (TFM) and lean mass (LM) was assessed with a computerized analysis of DEXA-scan (Software Illuminatus DXA 4.4.0, Visual MED, Inc. and Norland CooperSurgical Company).

8.4.2.2 Resting energy expenditure (REE)

REE was measured while the volunteer was at rest. Volunteer wore a mask to measure gas exchange using indirect calorimetry (MetaLyzer Cortex 3B, Leipzig, Germany).

8.4.2.3 Physical activity

The subjects were provided with a pedometer in order to assess the daily number of steps by detecting the motion of the subject's hip.

8.4.2.3 Diet

At the beginning and at the end of the studied period, volunteers performed a 24-hour diet recall interview (2 interviews during the week and 1 during the weekend) to check compliance to individual recommended intake according to the revised equation of Harris-Benedict (Roza & Shizgal, 1984).

8.4.2.5 Metabolic outcomes

For metabolic analysis, 20mL of blood was collected at baseline (W_1) and at the end of the study (W_{16}), from the basilica vein using a vacutainer system and 4 tubes Terumo Venoject (Terumo, Leuven, Belgium) with EDTA, heparin or dry tube. Some samples were centrifuged at 3000 r.p.m for 10 minutes at 4°C. Immediately after centrifugation, plasma has been extracted and proportionally divided in aliquots of 0.5 to 1mL (Eppendorf tubes). Samples has been frozen at -80°C for further analysis. A total of 40 blood aliquots per volunteer (20 aliquots at baseline and 20 aliquots at the end of the study) are stored in a Serum Bank:

SERUM BANK	
Heparinized blood	8 x 750 µL plasma
	2 x 500 µL red blood cells
EDTA blood	6 x 500 µL plasma
Dry blood	4 x 2 mL serum

First blood analysis performed included plasma concentration of fibrinogen, FFAs, leptin and adiponectin; additional metabolic outcomes will possibly be analyzed in order to go more in deep into the mechanism of action of the supplement.
FFAs quantification was assessed with a colorimetric method on a Pentra 400 Chemistry Analyzer (Horiba ABX). Fibrinogen, leptin and adiponectin quantification were assessed with Multiplex assay on a Luminex 100 LS (Luminex, Inc., Austin, TX) using commercial kits (Millipore, Billerica, MA).

8.5 Data quality assurance

During the clinical investigation, a monitor had regular contacts with the investigational site, including visits to verify that al data in the CRFs are completed and recorded in a timely manner and are consistent with the source data, that signed and dated informed consent forms have been obtained from each subject at the time of enrollment, that the study is being performed according the study protocol.

8.6 Statistical plan

8.6.1 Determination of sample size

The sample size calculation is based upon the results of a previous study with Sinetrol® Xpur (Dallas *et al.*, 2014). After 12 weeks of investigation, the study population (*n*=95) showed a significant reduction in body fat of (3.6 ± 1.6) points difference (%) in the Sinetrol® Xpur group compared to (1.0 ± 0.7) points difference (%) in the placebo group; it corresponds to a 2.6 ± 0.1 difference between groups.
Based on this result, current study objective was to improve body composition in order to reach difference of total body fat variation (% BW) by -2.0 points minimum within the Sinetrol® Xpur group after 16 weeks of supplementation as it was set for the primary outcome in this previous clinical study.
Since in the general population, the total body fat is significantly different between men and women, the use of a variance weighted by gender is required.
Given a significance level of 5%, a power of 80%, a weighted variance of $2.82^2 = 7.95$; a sample size of 32 subjects per group is required. Assuming a drop-out and failure rate of 40%, inclusion of 107 subjects was recommended.

8.6.2 Statistical analysis

Data has been analysed using statistical package SPSS v20.0 for MAC. A descriptive analysis of the variables has been performed to provide the mean, standard deviation, maximum and minimum ranges. Subsequently, an exploratory analysis of sample normality has been performed with the Kolmogorov-Smirnov test and both

Liliefors and Shapiro-Wilk tests significance correction. A study of variables homoscedasticity and heteroscedasticity has been performed.
The differences between groups and the effect of time have been evaluated with a student t-test General Linear Model (pairwise comparison) in order to determine the intra- and inter-group differences. The level of significance is set at $p \leq 0.05$.
Potential confounding factors have been identified during a secondary statistical analysis and net variations have been analyzed using linear regression analysis.

9 STUDY SUBJECTS

9.1 Disposition of subjects

During the length of time between March 2015 and September 2016, 107 subjects were enrolled.
58 subjects (58 of 107; 54%) were assigned to the Sinetrol® Xpur arm and 49 subjects (49 of 107; 46%) to the placebo arm.

9.1.1 Activity level at the beginning of the study (VCAS)

At the beginning of the study, all subjects declared to practice either no physical activity or less than 1 time per week.

9.1.2 Recommended and reported calories intake (VCAS)

There are no significant differences in the VCAS population, at the beginning of the study, between the placebo and the Sinetrol® Xpur regarding the recommended calories intake (p=0.768).

Recommended intake (kcal)	*N*	Mean	SD	Min	Median	Max
TOTAL	77	2097	308	1593	2051	2860
Sinetrol® Xpur	43	2107	333	1593	2026	2860
Placebo	34	2086	276	1655	2053	2579
p-value		0.768				

There are no significant differences in the VCAS population, at the beginning of the study, between the placebo and the Sinetrol® Xpur regarding the reported calories intake (p=0.078).

Reported intake (kcal)	*N*	Mean	SD	Min	Median	Max
TOTAL	77	1881	507	341	1825	3316
Sinetrol® Xpur	43	1971	521	341	1952	3316
Placebo	34	1759	470	1019	1688	3107
p-value		0.078				

9.2 Dropouts and protocol deviations

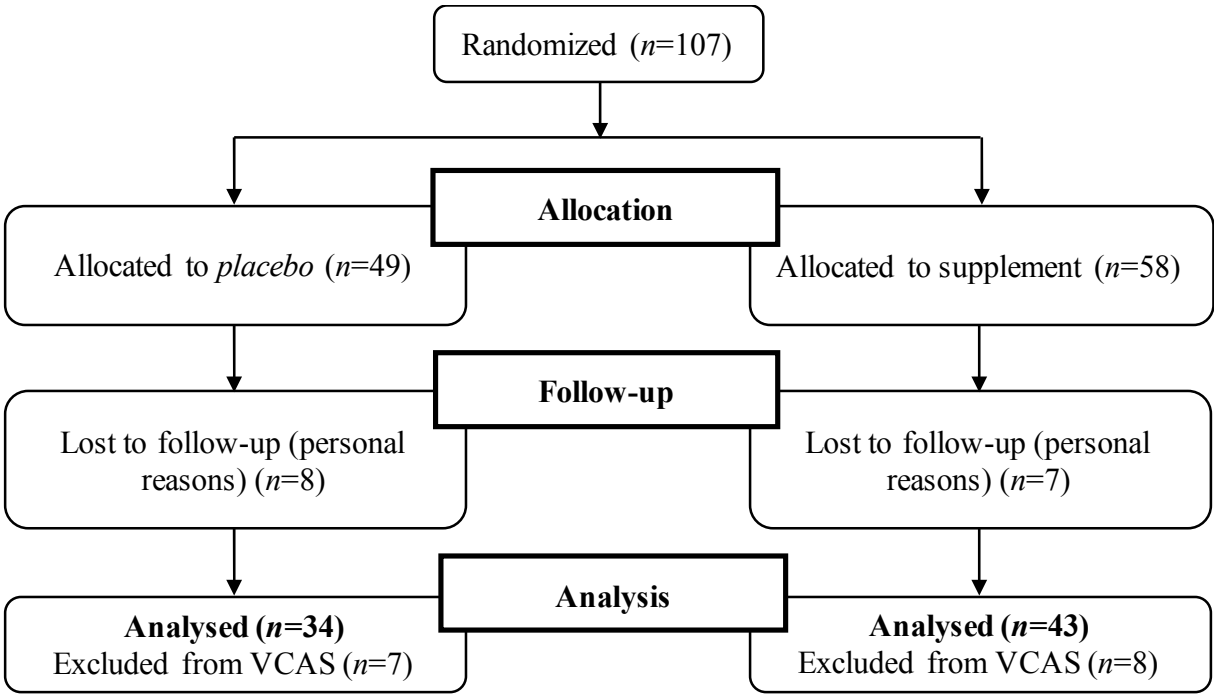
9.2.1 Dropouts

The dropout rate was 14.0%: 15 volunteers, 7 within the Sinetrol® Xpur Xpur-supplemented group (3 females and 4 males) and 8 within the placebo-supplemented group (5 females and 3 males) dropped out for personal reasons and were excluded from VCAS population.

9.2.2 Protocol deviations

15 subjects were excluded from VCAS population, 8 within the Sinetrol® Xpur-supplemented group (3 females and 5 males) and 7 within the placebo-supplemented group (3 females and 4 males) because of protocol deviations such as inconsistency of the DXA measurement or non-compliance to the protocol. These volunteers were excluded from VCAS population.

9.2.3 VCAS population: CONSORT Flow Chart



10 BENEFIT EVALUTION

10.1 Data sets analyzed

According to dropouts and protocol deviations, a total of 30 subjects were excluded from the VCAS.

	TOTAL		Sinetrol® Xpur group		Placebo group	
	Number	Percentage	Number	Percentage	Number	Percentage
Study population	107	100%	58	54%	49	46%
VCAS	77	72%	43	56%	34	44%

10.2 Baseline characteristics

10.2.1 Age

There is no statistical difference in the VCAS population regarding the age distribution between Sinetrol® Xpur and the placebo group (p = 0.197).

Age (years)	*N*	Mean	SD	Min	Median	Max
TOTAL	77	41.2	5.5	29	41	52
Sinetrol® Xpur	43	42.0	5.1	31	41	50
Placebo	34	40.3	5.9	29	40	52
p-value		0.197				

10.2.2 Gender

There is no statistical difference in the VCAS population regarding the gender distribution between Sinetrol® Xpur and the placebo group (p = 0.685).

Gender	*N*	Male		Female	
		Number	Percentage	Number	Percentage
TOTAL	77	36	47%	41	53%
Sinetrol® Xpur	43	21	49%	22	51%
Placebo	34	15	44%	19	56%
p-value	0.685				

10.2.3 Height

There is no statistical difference in the VCAS population regarding the height between Sinetrol® Xpur and the placebo group (*p* =0.307).

Height (m)	*N*	Mean	SD	Min	Median	Max
TOTAL	77	169.3	9.3	150.6	167	192.0
Sinetrol® Xpur	43	170.2	10.4	150.6	169	192.0
Placebo	34	168.0	7.6	156.6	166	187.3
p-value		0.307				

10.2.4 Body weight at inclusion

There is no statistical difference in the VCAS population regarding the body weight between Sinetrol® Xpur and the placebo group (*p* = 0.930).

Body weight (kg)	*N*	Mean	SD	Min	Median	Max
TOTAL	77	89.2	13.2	63.9	90.5	122.8
Sinetrol® Xpur	43	89.0	14.1	63.9	90.8	122.8
Placebo	34	89.3	12.2	67.7	88.7	115.6
p-value		0.930				

10.2.5 BMI at inclusion

There is no statistical difference in the VCAS population regarding the BMI between Sinetrol® Xpur and the placebo group (*p* = 0.423).

BMI (kg/m^2)	*N*	Mean	SD	Min	Median	Max
TOTAL	77	30.0	3.7	24.6	29.3	39.9
Sinetrol® Xpur	43	29.7	4.0	24.6	28.4	39.9
Placebo	34	30.3	3.3	24.6	30.1	38.4
p-value		0.423				

10.2.6 Theoretical fat mass at inclusion

There is no statistical difference in the VCAS population regarding the theoretical fat mass between Sinetrol® Xpur and the placebo group (*p* = 0.282).

Theoretical FM (g)	*N*	Mean	SD	Min	Median	Max
TOTAL	77	32633	10033	17104	30333	64218
Sinetrol® Xpur	43	32042	9604	17104	30333	60873
Placebo	34	33382	10650	21574	29838	64218
p-value		0.282				

10.3 Benefit results

10.3.1 Primary outcome: Total body fat percentage loss (%BW)

At baseline (W_1), there is no statistical difference in the VCAS population regarding the total body fat mass (%BW) between Sinetrol® Xpur and the placebo group (p = 0.759).
At the end of the study (W_{16}), there is no statistical difference in the VCAS population regarding the total body fat mass (%BW) between Sinetrol® Xpur and the placebo group (p = 0.119).

Regarding intragroup significance, there is no statistical difference for the total body fat mass (%BW) between W_1 and W_{16} in the placebo group (p = 0.427) while there is a statistical difference between W_1 and W_{16} in the Sinetrol® Xpur group (p = 0.0003).

Objective was to reach a – 2.0 points total body fat mass (%BW) variation between W_1 and W_{16} in the Sinetrol® Xpur group. It was here obtained a - 2.0 points variation; delta between both groups is statistically significant (p = 0.016).

Total body fat	W_1 (%BW)	W_{16} (%BW)	*p*-value (intragroup)	Delta W_1-W_{16} (%BW)	Delta W_1-W_{16} (%)
Sinetrol® Xpur	38.7±8.4	36.7±8.5	0.0003*	- 2.0±3.5	- 5.2
Placebo	39.4±9.3	39.2±9.7	0.427	- 0.1±3.9	- 0.5
p-value (intergroup)	0.759	0.119		0.016*	

The secondary statistical analysis of confounding factors indicated that the variation of daily steps might confound the association between intake of the supplement and the net variation of total body fat (% BW) between Sinetrol® Xpur and placebo groups.

Statistical regression analysis indicated a significant variation of total body fat mass net variation in both non-adjusted model (p = 0.016) and daily steps-adjusted model (p = 0.011).

Net variation of total body fat mass (% BW)			
Non-adjusted model		Daily steps-adjusted model	
-1.86±3.78	0.016*	-1.99±3.77	0.011*

10.3.2 Secondary outcome: Body weight variation

At baseline (W_1), there is no statistical difference in the VCAS population regarding the body weight between Sinetrol® Xpur and the placebo group (p = 0.930).
At the end of the study (W_{16}), there is no statistical difference in the VCAS population regarding the body weight between Sinetrol® Xpur and the placebo group (p = 0.355).
Regarding intragroup significance, there is no statistical difference for body weight between W_1 and W_{16} in the placebo group (p = 0.338) while there is a statistical difference between W_1 and W_{16} in the Sinetrol® Xpur group (p = 0.015) with a mean body weight loss of 1.1 kg (-1.2%).

Body weight (kg)	W_1 (kg)	W_{16} (kg)	p-value (intragroup)	Delta W_1-W_{16} (kg)	Delta W_1-W_{16} (%)
Sinetrol® Xpur	89.0±14.1	87.9±13.8	0.015*	- 1.1±3.2	- 1.2
Placebo	89.3±12.2	89.1±12.5	0.338	- 0.2±3.1	- 0.2
p-value (intergroup)	0.930	0.355		0.117	

The secondary statistical analysis of confounding factors indicated that the variation of daily steps might confound the association between intake of the supplement and the net variation of body weight between Sinetrol® Xpur and placebo groups.
Statistical regression analysis indicated a non significant variation of body weight net variation in non-adjusted model (p = 0.117) and a significant variation in daily steps-adjusted model (p = 0.041).

Net variation of body weight (kg)			
Non-adjusted model		Daily steps-adjusted model	
-0.86±3.15	0.117	-1.99±3.77	0.041*

10.3.3 Secondary outcome: BMI variation

At baseline (W_1), there is no statistical difference in the VCAS population regarding the BMI between Sinetrol® Xpur and the placebo group (p = 0.358).
At the end of the study (W_{16}), there is no statistical difference in the VCAS population regarding the BMI between Sinetrol® Xpur and the placebo group (p = 0.112).

Regarding intragroup significance, there is no statistical difference for BMI between W_1 and W_{16} in the placebo group (p = 0.311) while there is a statistical difference between W_1 and W_{16} in the Sinetrol® Xpur group (p = 0.023) with a mean BMI loss of -0.4 kg/m^2 (-1.0%).

BMI (kg/m^2)	W_1 (kg/m^2)	W_{16} (kg/m^2)	p-value (intragroup)	Delta W_1-W_{16} (kg/m^2)	Delta W_1-W_{16} (%)
Sinetrol® Xpur	30.7±4.4	30.4±4.3	0.023*	- 0.4±1.2	- 1.0
Placebo	31.6±4.0	31.5±3.9	0.311	- 0.1±1.1	- 0.3
p-value (intergroup)	0.358	0.112		0.154	

10.3.4 Secondary outcome: Total body lean mass variation

At baseline (W_1), there is no statistical difference in the VCAS population regarding the total body lean mass between Sinetrol® Xpur and the placebo group (p = 0.785).
At the end of the study (W_{16}), there is no statistical difference in the VCAS population regarding the total body lean mass between Sinetrol® Xpur and the placebo group (p = 0.278).

Regarding intragroup significance, there is no statistical difference for total body lean mass between W_1 and W_{16} in the placebo group (p = 0.362) while there is a

statistical difference between W_1 and W_{16} in the Sinetrol® Xpur group (p = 0.006) with a mean total body lean mass increase of 0.7 kg (+1.4%).

Total body lean mass (g)	W_1 (g)	W_{16} (g)	p-value (intragroup)	Delta W_1-W_{16} (g)	Delta W_1-W_{16} (%)
Sinetrol® Xpur	51810±12529	52518±12401	0.006*	+ 708±1746	+ 1.4
Placebo	51077±10438	50944±10429	0.362	- 132±2168	- 0.3
p-value (intergroup)	0.785	0.278		0.032*	

10.3.5 Secondary outcome: Total body fat mass variation

At baseline (W_1), there is no statistical difference in the VCAS population regarding the total body fat mass between Sinetrol® Xpur and the placebo group (p = 0.629).
At the end of the study (W_{16}), there is no statistical difference in the VCAS population regarding the total body fat mass between Sinetrol® Xpur and the placebo group (p = 0.104).

Regarding intragroup significance, there is no statistical difference for total body fat mass between W_1 and W_{16} in the placebo group (p = 0.444) while there is a statistical difference between W_1 and W_{16} in the Sinetrol® Xpur group (p = 0.0002) with a mean total body fat reduction of 1.8 kg (-5.2%).

Total body fat mass (g)	W_1 (g)	W_{16} (g)	p-value (intragroup)	Delta W_1-W_{16} (g)	Delta W_1-W_{16} (%)
Sinetrol® Xpur	34201±8284	32411±8199	0.0002*	- 1789±2996	- 5.2
Placebo	35236±10412	35152±10761	0.444	- 83±3413	- 0.2
p-value (intergroup)	0.629	0.104		0.011*	

The secondary statistical analysis of confounding factors indicated that the variation of daily steps might confound the association between intake of the supplement and the net variation of total body fat mass (g) between Sinetrol® Xpur and placebo groups.
Statistical regression analysis indicated a significant variation of total body fat mass (g) net variation in both non-adjusted model (p = 0.011) and daily steps-adjusted model (p = 0.008).

Net variation of total body fat mass (g)			
Non-adjusted model		Daily steps-adjusted model	
-1706±3278	0.011*	-1830±3250	0.008*

10.3.6 Secondary outcome: Beneficial variation of body composition

Beneficial variation of body composition refers to the variation between delta Fat mass minus delta Lean mass.

While there is no change regarding beneficial variation of body composition in the placebo group (+0.049 kg), the Sinetrol® Xpur group experienced a – 2.5kg of beneficial variation after 16 weeks of supplementation; the delta between both groups is statistically significant (*p*=0.005).

Delta fat mass – delta lean mass (g)	Delta W_1-W_{16} (g)
Sinetrol® Xpur	- 2498±3737
Placebo	+ 49±4821
p-value (intergroup)	0.005*

10.3.7 Secondary outcome: Lean-to-fat mass ratio

At baseline (W_1), there is no statistical difference in the VCAS population regarding the lean-to-fat mass ratio between Sinetrol® Xpur and the placebo group (p = 0.881).

At the end of the study (W_{16}), there is no statistical difference in the VCAS population regarding the lean-to-fat mass ratio between Sinetrol® Xpur and the placebo group (p = 0.254).

Regarding intragroup significance, there is no statistical difference for lean-to-fat mass ratio between W_1 and W_{16} in the placebo group (p = 0.234) while there is a statistical difference between W_1 and W_{16} in the Sinetrol® Xpur group (p = 0.00004) with a mean lean-to-fat mass ratio increase of 0.13 (+8.1%).

LM/FM	W_1	W_{16}	*p*-value (intragroup)	Delta W_1-W_{16}	Delta W_1-W_{16} (%)
Sinetrol® Xpur	1.61±0.6	1.74±0.7	0.00004*	+ 0.13±0.19	+ 8.1
Placebo	1.59±0.6	1.63±0.8	0.234	+ 0.04±0.31	- 2.5
p-value (intergroup)	0.881	0.254		0.064	

10.3.8 Secondary outcome: Percentage excess FM vs theoretical FM

At baseline (W_1), there is no statistical difference in the VCAS population regarding the percentage excess FM vs theoretical FM between Sinetrol® Xpur and the placebo group (p = 0.465).
At the end of the study (W_{16}), there is no statistical difference in the VCAS population regarding the percentage excess FM vs theoretical FM between Sinetrol® Xpur and the placebo group (p = 0.230).

Regarding intragroup significance, there is no statistical difference for percentage excess FM vs theoretical FM between W_1 and W_{16} in the placebo group (p = 0.416) while there is a statistical difference between W_1 and W_{16} in the Sinetrol® Xpur group (p = 0.0002) with a mean percentage excess FM vs theoretical FM decrease of 5.7 points (-63.3% of the excess).

Percentage excess FM *vs* theoretical FM	W_1 (%)	W_{16} (%)	p-value (intragroup)	Delta W_1-W_{16}	Delta W_1-W_{16} (%)
Sinetrol® Xpur	9.0±15.4	3.3±16.9	0.0002*	-5.7±9.7	- 63.3
Placebo	6.6±13.1	6.2±16.4	0.416	-0.4±11.4	- 6.1
p-value (intergroup)	0.465	0.230		0.015*	

10.3.9 Secondary outcome: Body trunk fat mass variation

At baseline (W_1), there is no statistical difference in the VCAS population regarding the body trunk fat mass between Sinetrol® Xpur and the placebo group (p = 0.792).
At the end of the study (W_{16}), there is a no statistical difference in the VCAS population regarding the body trunk fat mass between Sinetrol® Xpur and the placebo group (p = 0.118).

Regarding intragroup significance, there is no statistical difference for body trunk fat mass between W_1 and W_{16} in the placebo group (p = 0.461) while there is a statistical difference between W_1 and W_{16} in the Sinetrol® Xpur group (p = 0.00001) with a mean body trunk fat mass reduction of -1154 g (-6.5%).

Body trunk fat mass	W_1 (g)	W_{16} (g)	*p*-value (intragroup)	Delta W_1-W_{16} (g)	Delta W_1-W_{16} (%)
Sinetrol® Xpur	17837±4638	16683±4577	0.00001*	- 1154±1609	- 6.5
Placebo	18141±5443	18114±5933	0.461	-27±1622	- 0.1
p-value (intergroup)	0.792	0.118		0.002*	

10.3.10 Secondary outcome: ICO (Index of Central Obesity) variation

At baseline (W_1), there is no statistical difference in the VCAS population regarding the ICO between Sinetrol® Xpur and the placebo group (p = 0.577).
At the end of the study (W_{16}), there is a no statistical difference in the VCAS population regarding the ICO between Sinetrol® Xpur and the placebo group (p = 0.117).

Regarding intragroup significance, there is no statistical difference for ICO between W_1 and W_{16} in the placebo group (p = 0.234) while there is a statistical difference between W_1 and W_{16} in the Sinetrol® Xpur group (p = 0.023) with a mean ICO reduction of 0.006 points (-1.1%).

ICO (points)	W_1 (points)	W_{16} (points)	*p*-value (intragroup)	Delta W_1-W_{16} (points)	Delta W_1-W_{16} (%)
Sinetrol® Xpur	0.553±0.06	0.547±0.06	0.023*	- 0.006±0.02	- 1.1
Placebo	0.561±0.05	0.563±0.05	0.234	+ 0.002±0.01	+ 0.4
p-value (intergroup)	0.577	0.117		0.025*	

10.3.11 Secondary outcome: Waist circumference variation

At baseline (W_1), there is no statistical difference in the VCAS population regarding the waist circumference between Sinetrol® Xpur and the placebo group (p = 0.980).
At the end of the study (W_{16}), there is a no statistical difference in the VCAS population regarding the waist circumference between Sinetrol® Xpur and the placebo group (p = 0.266).

Regarding intragroup significance, there is no statistical difference for waist circumference between W_1 and W_{16} in the placebo group (p = 0.235) while there is a

statistical difference between W_1 and W_{16} in the Sinetrol® Xpur group (p = 0.018) with a mean waist circumference reduction of 1.1cm (-1.3%).

Waist circumference (cm)	W_1 (cm)	W_{16} (cm)	p-value (intragroup)	Delta W_1-W_{16} (cm)	Delta W_1-W_{16} (%)
Sinetrol® Xpur	94.2±11.3	93.0±10.8	0.018*	- 1.1±3.4	- 1.3
Placebo	94.2±9.9	94.5±9.6	0.235	+ 0.3±2.2	+ 0.3
p-value (intergroup)	0.980	0.266		0.020*	

10.3.12 Secondary outcome: Hip circumference variation

At baseline (W_1), there is no statistical difference in the VCAS population regarding the hip circumference between Sinetrol® Xpur and the placebo group (p = 0.384).
At the end of the study (W_{16}), there is a no statistical difference in the VCAS population regarding the hip circumference between Sinetrol® Xpur and the placebo group (p = 0.080).

Regarding intragroup significance, there is no statistical difference for hip circumference between W_1 and W_{16} in the placebo group (p = 0.159) while there is a statistical difference between W_1 and W_{16} in the Sinetrol® Xpur group (p = 0.002) with a mean hip circumference reduction of 1.2cm (-1.2%).

Hip circumference (cm)	W_1 (cm)	W_{16} (cm)	p-value (intragroup)	Delta W_1-W_{16} (cm)	Delta W_1-W_{16} (%)
Sinetrol® Xpur	109.0±7.2	107.7±6.7	0.002*	- 1.2±2.6	- 1.2
Placebo	110.5±8.2	110.1±8.0	0.159	- 0.4±2.2	- 0.4
p-value (intergroup)	0.384	0.080		0.070	

The secondary statistical analysis of confounding factors indicated that the variation of daily steps might confound the association between intake of the supplement and the net variation of hip circumference between Sinetrol® Xpur and placebo groups.
Statistical regression analysis indicated a non significant variation of hip circumference variation in non-adjusted model (p = 0.070) and a significant variation in daily steps-adjusted model (p = 0.033).

Net variation of hip circumference (cm)			
Non-adjusted model		Daily steps-adjusted model	
-0.84±2.47	0.070	-1.05±2.48	0.033*

10.3.13 Secondary outcome: Resting Energy Expenditure (REE)

At baseline (W_1), there is no statistical difference in the VCAS population regarding the REE between Sinetrol® Xpur and the placebo group (p = 0.695).
At the end of the study (W_{16}), there is a no statistical difference in the VCAS population regarding the REE between Sinetrol® Xpur and the placebo group (p = 0.174).

Regarding intragroup significance, there is no statistical difference for REE between W_1 and W_{16} in the placebo group (p = 0.459) while there is a statistical difference between W_1 and W_{16} in the Sinetrol® Xpur group (p = 0.012) with a mean REE increase of 181 kcal/d (+10.1%).

REE (kcal/d)	W_1 (kcal/d)	W_{16} (kcal/d)	p-value (intragroup)	Delta W_1-W_{16} (kcal/d)	Delta W_1-W_{16} (%)
Sinetrol® Xpur	1794±476	1976±495	0.012*	+ 181±425	+ 10.1
Placebo	1841±355	1849±473	0.459	+ 8±376	+ 0.4
p-value (intergroup)	0.695	0.174		0.063	

10.3.14 Secondary outcome: Metabolic parameters

10.3.14.1 Fibrinogen

At baseline (W_1), there is no statistical difference in the VCAS population regarding the fibrinogen concentration between Sinetrol® Xpur and the placebo group (p = 0.073).
At the end of the study (W_{16}), there is a no statistical difference in the VCAS population regarding the fibrinogen concentration between Sinetrol® Xpur and the placebo group (p = 0.169).

Regarding intragroup significance, there is no statistical difference for fibrinogen concentration between W_1 and W_{16} in the placebo group (p = 0.067) while there is a statistical difference between W_1 and W_{16} in the Sinetrol® Xpur group (p = 0.001) with a mean fibrinogen concentration reduction of 21 mg/dL (-5.7%).

Fibrinogen (mg/dL)	W_1 (mg/dL)	W_{16} (mg/dL)	p-value (intragroup)	Delta W_1-W_{16} (mg/dL)	Delta W_1-W_{16} (%)
Sinetrol® Xpur	387±58	365±68	0.001*	- 21±42	- 5.7
Placebo	360±63	381±66	0.067	+ 21±73	+ 5.8
p-value (intergroup)	0.073	0.169		0.001*	

10.3.14.2 Free Fatty Acids (FFAs)

At baseline (W_1), there is no statistical difference in the VCAS population regarding the FFAs concentration between Sinetrol® Xpur and the placebo group (p = 0.715).
At the end of the study (W_{16}), there is a no statistical difference in the VCAS population regarding the FFAs concentration between Sinetrol® Xpur and the placebo group (p = 0.075).

Regarding intragroup significance, there is no statistical difference for FFAs concentration between W_1 and W_{16} in the placebo group (p = 0.146) while there is a statistical difference between W_1 and W_{16} in the Sinetrol® Xpur group (p = 0.009) with a mean FFAs concentration increase of 0.16 mmol/L (+15.1%).

FFAs (mmol/L)	W_1 (mmol/L)	W_{16} (mmol/L)	p-value (intragroup)	Delta W_1-W_{16} (mmol/L)	Delta W_1-W_{16} (%)
Sinetrol® Xpur	1.06±0.40	1.22±0.35	0.009*	+ 0.16±0.41	+ 15.1
Placebo	1.03±0.44	1.10±0.36	0.146	+ 0.07±0.34	+ 6.8
p-value (intergroup)	0.715	0.075		0.176	

10.3.14.3 Leptin

At baseline (W_1), there is no statistical difference in the VCAS population regarding the leptin concentration between Sinetrol® Xpur and the placebo group (p = 0.997).
At the end of the study (W_{16}), there is a no statistical difference in the VCAS population regarding the leptin concentration between Sinetrol® Xpur and the placebo group (p = 0.254).

Regarding intragroup significance, there is no statistical difference for leptin concentration between W_1 and W_{16} in the placebo group (p = 0.080); there is no statistical difference between W_1 and W_{16} in the Sinetrol® Xpur group (p = 0.261).

Leptin (ng/mL)	W_1 (ng/mL)	W_{16} (ng/mL)	p-value (intragroup)	Delta W_1-W_{16} (ng/mL)	Delta W_1-W_{16} (%)
Sinetrol® Xpur	9.06±6.60	8.68±7.31	0.261	- 0.38±3.83	- 4.2
Placebo	9.05±7.47	9.87±7.36	0.080	+ 0.82±2.98	+ 9.1
p-value (intergroup)	0.997	0.254		0.084	

10.3.14.3 Adiponectin

At baseline (W_1), there is no statistical difference in the VCAS population regarding the adiponectin concentration between Sinetrol® Xpur and the placebo group (p = 0.999).

At the end of the study (W_{16}), there is a no statistical difference in the VCAS population regarding the adiponectin concentration between Sinetrol® Xpur and the placebo group (p = 0.397).

Regarding intragroup significance, there is no statistical difference for adiponectin concentration between W_1 and W_{16} in the placebo group (p = 0.161); there is no statistical difference between W_1 and W_{16} in the Sinetrol® Xpur group (p = 0.415).

Adiponectin (µg/mL)	W_1 (µg/mL)	W_{16} (µg/mL)	p-value (intragroup)	Delta W_1-W_{16} (µg/mL)	Delta W_1-W_{16} (%)
Sinetrol® Xpur	20.19±14.50	20.45±17.33	0.415	- 0.26±7.79	+ 1.3
Placebo	20.20±14.11	19.25±13.06	0.161	- 0.95±4.88	- 4.7
p-value (intergroup)	0.999	0.397		0.238	

10.4 Follow-up of protocol requirements

10.4.1 Recommended and reported dietary intake

At baseline (W_1), there is no statistical difference in the VCAS population regarding the recommended intake between Sinetrol® Xpur and the placebo group (p = 0.768).

At the end of the study (W_{16}), there is a no statistical difference in the VCAS population regarding the recommended intake between Sinetrol® Xpur and the placebo group (p = 0.906).
Regarding intragroup significance, there is no statistical difference for recommended intake between W_1 and W_{16} in the placebo group (p = 0.383) while there is a statistical difference between W_1 and W_{16} in the Sinetrol® Xpur group (p = 0.012) with a mean recommended intake reduction of 15 kcal/d.

Recommended intake (kcal)	W_1 (kcal)	W_{16} (kcal)	p-value (intragroup)
Sinetrol® Xpur	2107±333	2092±329	0.012*
Placebo	2086±276	2084±281	0.383
p-value (intergroup)	0.768	0.906	

At baseline (W_1), there is no statistical difference in the VCAS population regarding the reported intake between Sinetrol® Xpur and the placebo group (p = 0.078).
At the end of the study (W_{16}), there is a no statistical difference in the VCAS population regarding the reported intake between Sinetrol® Xpur and the placebo group (p = 0.440).
Regarding intragroup significance, there is a statistical difference for reported intake between W_1 and W_{16} in the placebo group (p = 0.015) while there is no statistical difference between W_1 and W_{16} in the Sinetrol® Xpur group (p = 0.194).

Reported intake (kcal)	W_1 (kcal)	W_{16} (kcal)	p-value (intragroup)
Sinetrol® Xpur	1971±521	1904±508	0.194
Placebo	1759±470	1885±522	0.015*
p-value (intergroup)	0.078	0.440	

When compared the recommended intake and the reported intake at baseline (W_1), the Sinetrol® Xpur group intake is 6.5% lower than the recommendation while the placebo group is 15.7% lower; however, at the end of the study (W_{16}), the reported intake within the placebo group is only 9.5% lower than the recommendation, which is less than a 10% difference and thus acceptable. The Sinetrol® Xpur group intake, at the end of the study, is 9.0% lower than the recommended intake which is still an acceptable difference.

10.4.2 Daily steps variation (pedometer)

At baseline (W_1), there is no statistical difference in the VCAS population regarding the mean daily steps between Sinetrol® Xpur and the placebo group (p = 0.449).
At the end of the study (W_{16}), there is no statistical difference in the VCAS population regarding the mean daily steps between Sinetrol® Xpur and the placebo group (p = 0.481).

Regarding intragroup significance, there is no statistical difference for mean daily steps between W_1 and W_{16} in the placebo group (p = 0.130); there is no statistical difference between W_1 and W_{16} in the Sinetrol® Xpur group (p = 0.399).
Taken together, the level of physical activity, assessed with recording of daily steps, is stable throughout the course of the study with no significant differences both between and within groups.

Daily steps (steps)	W_1 (steps/day)	W_{16} (steps/day)	p-value (intragroup)	Delta W_1-W_{16} (steps/day)	Delta W_1-W_{16} (%)
Sinetrol® Xpur	7236±2289	7310±2682	0.399	+ 74±1859	+ 1.0
Placebo	6826±2267	7280±2612	0.130	+ 455±2202	+ 6.7
p-value (intergroup)	0.449	0.481		0.213	

11 FOLLOW-UP OF BODY COMPOSITION VARIABLES

Four weeks after the end of the supplementation period (W_{16} + 4 weeks), participants, on a voluntary-basis, came back to the Research Center in order to perform a DXA-scan and to evaluate body composition four weeks after completion of the supplementation.
The dataset analyzed included 61% of partipants.

	TOTAL		Sinetrol® Xpur group		Placebo group	
	Number	Percentage	Number	Percentage	Number	Percentage
VCAS	77	100%	43	56%	34	44%
Follow-up	47	61%	31	72%	16	47%

Four weeks after the supplementation was stopped, body composition of volunteers from the Sinetrol® Xpur group continued to improve with additional reduction of body weight, BMI, total fat mass and trunk fat mass. Simultaneously, total lean mass and lean-to-fat mass ratio continued to increase.

	W_1	W_{16} + 4 weeks	*p*-value (intragroup)	Delta W_1-W_{20}	Delta W_1-W_{20} (%)
Body weight (kg)					
Sinetrol® Xpur	87.3±13.9	85.8±13.0	0.009*	- 1.5±3.3	- 1.7
Placebo	86.6±11.4	86.9±11.9	0.356	+ 0.3±2.9	+ 0.3
p-value (intergroup)	0.869	0.390		0.040*	
BMI (kg/m^2)					
Sinetrol® Xpur	30.0±3.8	29.5±3.5	0.018*	- 0.5±1.3	- 1.7
Placebo	30.0±3.4	30.1±3.7	0.312	+ 0.1±0.9	+ 0.3
p-value (intergroup)	0.953	0.269		0.046*	
Total fat mass (g)					
Sinetrol® Xpur	33327±8322	30801±7661	0.0001*	- 2526±3440	- 7.6
Placebo	30876±7769	30691±8863	0.428	- 184±3974	- 0.6
p-value (intergroup)	0.333	0.483		0.021*	
Total fat mass (%BW)					
Sinetrol® Xpur	38.5±8.7	35.7±8.7	0.0002*	- 2.8±3.9	- 7.3
Placebo	35.7±7.2	35.4±8.5	0.402	- 0.3±4.6	- 0.8
p-value (intergroup)	0.276	0.452		0.029*	
Trunk fat mass (g)					
Sinetrol® Xpur	17473±4876	16016±4521	0.0002*	- 1457±2002	- 8.3
Placebo	16064±442	16407±5084	0.281	+ 343±2312	- 2.1
p-value (intergroup)	0.338	0.394		0.004*	
Total lean mass (g)					
Sinetrol® Xpur	51002±12468	52034±12292	0.0008*	+ 1033±1651	+ 2.0
Placebo	52690±9595	53133±9406	0.247	+ 443±2520	+ 0.8
p-value (intergroup)	0.638	0.378		0.169	
LM/FM ratio					
Sinetrol® Xpur	1.64±0.60	1.82±0.690	0.00002*	+ 0.18±0.21	+ 11.0
Placebo	1.81±0.56	1.93±0.97	0.197	+ 0.13±0.57	+ 6.6
p-value (intergroup)	0.352	0.321		0.316	

12 SAFETY EVALUTION

12.1 Clinical laboratory values

12.1.2 Liver function parameters

There was no clinically significant difference within and between the Sinetrol® Xpur and the placebo groups for liver function parameters that all are within the healthy range at baseline (W_1) and at the end of the study (W_{16}).

Alanine transaminase (ALT)	W_1 (U/L)	W_{16} (U/L)	*p*-value (intragroup)	Delta W_1-W_{16} (U/L)	Delta W_1-W_{16} (%)
Reference values*	**7 to 55 U/L**				
Sinetrol® Xpur	26.0±12.9	22.6±11.7	0.013*	- 3.4±9.8	- 13.1
Placebo	21.5±8.7	20.9±8.2	0.330	- 0.6±7.2	- 2.8
p-value (intergroup)	0.107	0.253		0.097	
Aspartate aminotransferase (AST)	W_1 (U/L)	W_{16} (U/L)	*p*-value (intragroup)	Delta W_1-W_{16} (U/L)	Delta W_1-W_{16} (%)
Reference values*	**8 to 48 U/L**				
Sinetrol® Xpur	21.4±5.3	20.6±8.2	0.256	- 0.8±8.1	- 3.7
Placebo	20.1±5.5	19.5±4.2	0.271	- 0.6±5.5	- 3.0
p-value (intergroup)	0.324	0.252		0.461	
Gamma-Glutamyltransferase (GGT)	W_1 (U/L)	W_{16} (U/L)	*p*-value (intragroup)	Delta W_1-W_{16} (U/L)	Delta W_1-W_{16} (%)
Reference values*	**6 to 48 U/L**				
Sinetrol® Xpur	23.1±13.2	23.0±13.3	0.447	- 0.1±6.8	- 0.4
Placebo	19.5±12.0	20.2±12.1	0.212	+ 0.7±4.6	+ 3.6
p-value (intergroup)	0.247	0.189		0.282	

*www.mayoclinic.org

12.1.3 Renal function parameters

There was no clinically significant difference within and between the Sinetrol® Xpur and the placebo groups for renal function parameters that all are within the healthy range at baseline (W_1) and at the end of the study (W_{16}).

Urea	W_1 (mg/dL)	W_{16} (mg/dL)	*p*-value (intragroup)	Delta W_1-W_{16} (mg/dL)	Delta W_1-W_{16} (%)
Reference values*	**15 to 46 mg/dL**				
Sinetrol® Xpur	31.7±7.9	31.8±8.2	0.483	0.0±7.3	+ 0.3

Placebo	36.4±8.9	33.0±7.7	0.009*	- 3.4±7.1	- 9.3
p-value (intergroup)	0.025*	0.265		0.028*	
Creatinine	W_1 (mg/dL)	W_{16} (mg/dL)	*p*-value (intragroup)	Delta W_1-W_{16} (mg/dL)	Delta W_1-W_{16} (%)
Reference values*	**0.6 to 1.3 mg/dL**				
Sinetrol® Xpur	0.76±0.16	0.78±0.15	0.152	+ 0.02±0.10	+ 2.6
Placebo	0.81±0.18	0.75±0.16	0.011*	- 0.06±0.13	- 7.4
p-value (intergroup)	0.269	0.211		0.003*	
Sodium (Na)	W_1 (mmol/L)	W_{16} (mmol/L)	*p*-value (intragroup)	Delta W_1-W_{16} (mmol/L)	Delta W_1-W_{16} (%)
Reference values*	**135 to 145 mmol/L**				
Sinetrol® Xpur	140.8±3.4	140.6±2.3	0.391	- 0.1±3.3	- 0.1
Placebo	141.2±1.3	141.2±2.1	0.467	0.0±2.3	- 0.0
p-value (intergroup)	0.510	0.152		0.442	
Potassium (K)	W_1 (mmol/L)	W_{16} (mmol/L)	*p*-value (intragroup)	Delta W_1-W_{16} (mmol/L)	Delta W_1-W_{16} (%)
Reference values*	**3.6 to 5.2 mmol/L**				
Sinetrol® Xpur	4.3±0.5	4.4±0.4	0.187	+ 0.1±0.4	+ 2.3
Placebo	4.3±0.3	4.3±0.2	0.417	0.0±0.4	0.0
p-value (intergroup)	0.777	0.199		0.340	

*www.mayoclinic.org

12.2 Heart rate

There was no significant difference within and between the Sinetrol® Xpur and the placebo groups for resting heart rate at baseline (W_1) and at the end of the study (W_{16}).

Heart rate (b.p.m)	W_1 (b.p.m)	W_{16} (b.p.m)	*p*-value (intragroup)	Delta W_1-W_{16} (b.p.m)	Delta W_1-W_{16} (%)
Sinetrol® Xpur	70.8±9.1	70.6±8.8	0.449	- 0.2±9.6	- 0.3
Placebo	72.1±10.7	72.3±9.1	0.474	+ 0.1±7.6	+ 0.3
p-value (intergroup)	0.635	0.258		0.448	

12.3 Adverse events

Neither adverse events nor side effects were recorded throughout the course of the study.

13 OVERALL CONCLUSIONS

The intention of this investigation was to evaluate benefit -primarily as total body fat loss- of a 16-week supplementation with Sinetrol® Xpur in a randomized, double-blind, placebo-controlled study conducted in overweight and obese subjects.

The primary endpoint has been reached. The Sinetrol® Xpur-supplemented group significantly lost body fat mass (%BW) (-2.0 points) while the body fat mass (%BW) of the placebo group stayed stable after 16 weeks.

The secondary endpoints focused on the evaluation of the benefit of Sinetrol® Xpur on body composition (fat mass variation, trunk fat mass variation, lean mass variation, lean-to-fat mass ratio) and anthropometrics (waist & hip circumferences). All those secondary endpoints were significantly improved after 16 weeks of supplementation with Sinetrol® Xpur while the placebo group did not experience any positive variation. Taken together, these results confirmed the weight management benefits of Sinetrol® Xpur which is able to positively rebalance body composition in decreasing body fat mass while significantly increasing lean mass and hence, improving the lean-to-fat mass ratio. Linked-anthropometric parameters were all improved in the supplemented group while no positive shifts were seen within the placebo group.

In summary, it has been shown that supplementation with Sinetrol® Xpur within a 16-week period induces beneficial changes in body composition in positively rebalancing the total lean and fat mass. In addition, the supplementation did not induce any adverse nor side effects.

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8 – ADDITIONAL PUBLICATION

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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.

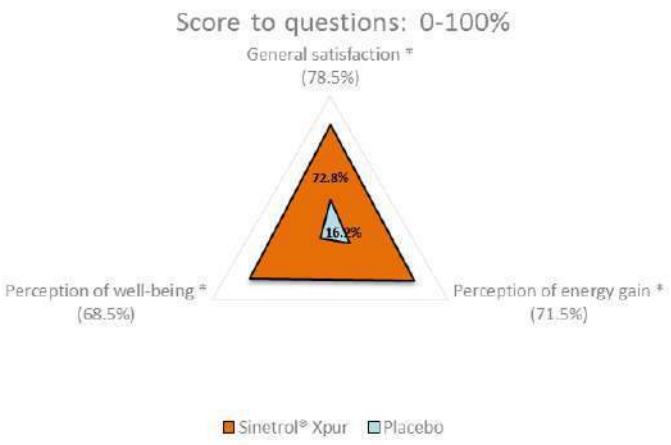

Figure 1. Perception of Sinetrol® Xpur efficacy after 12 weeks of supplementation. Values are means ± SD, *n*=13 (*placebo*) or *n*=12 (Sinetrol® XPur). *p<0.001 indicate a difference between *placebo* and and Sinetrol® Xpur.

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 partial inductor of SMM reduction during weight loss. Finally, volunteers supplemented with

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 results show a genuine benefit of the product in managing *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

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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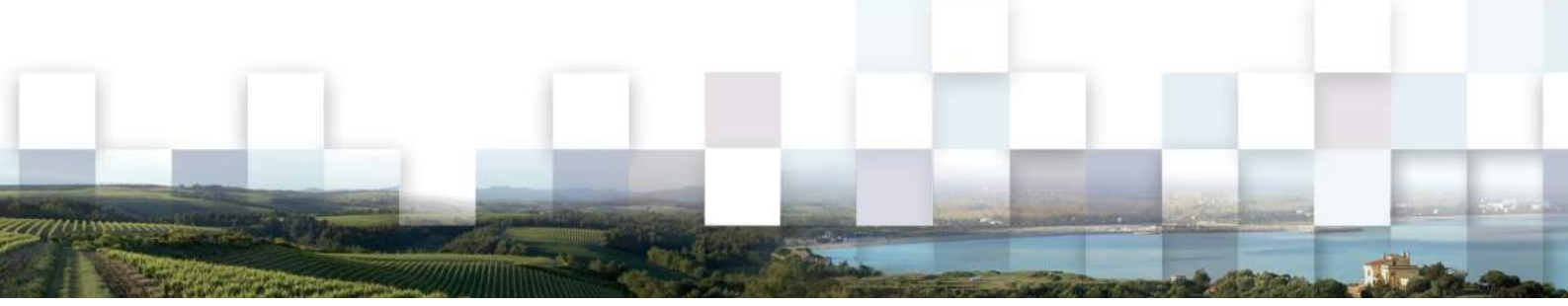

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