CLINICALLY
PROVEN

sinetrol®

Adipose beiging benefits

SCIENCE PACK

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1 CITRUS POLYPHENOLS FOR ENHANCED LIPOLYSIS

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.

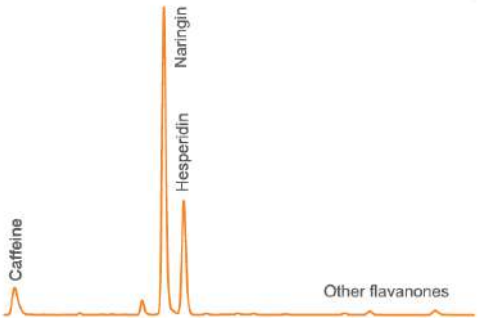
Sinetrol® is a largely citrus fruit-based ingredient made from the juice, peels, and seeds of fruit prepared by physical treatment (crushing, cold-pressure, extraction, centrifugation, filtration, and spray-drying) of specific varieties of sweet and blood oranges (*Citrus sinensis L.*),.), grapefruit (*Citrus paradisi Macfad.*), and guarana (*Paullinia cupana Kunth*).

Grapefruit

Orange

Guarana

Figure: Sinetrol® botanical composition



Example of Sinetrol® HPLC profile
Wavelength 280nm

The bioactives of Sinetrol® belong to the subfamily of flavanones and are extracted from citrus species, both grapefruit and orange. Naringin and hesperidin are the main phenolic biomarkers. Synergistically associated with a guarana seed extract providing a small quantity of caffeine, the bioavailability of the polyphenols from citrus are improved.

Citrus polyphenols enhance the physiological energetic pathway of lipolysis, destoring fat accumulated as triglycerides (TAGs) within white adipose cells. Bioactives in Sinetrol® also promote uncoupling of energy production within adipose tissue, which dissipates the released fat as heat. This effect of Sinetrol® is key to avoid further restorage of fat within adipose tissue.

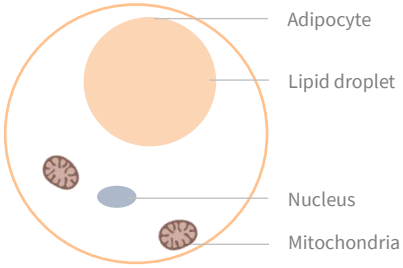
The beneficial effects of Sinetrol® are explained by these two complementary mechanisms of action, driven by the ability of Sinetrol® to promote adipose cell phenotype change from white to beige, i.e. from a storing category of adipose cells, to an energy metabolism-driven adipose cell category.

2 ADIPOSE TISSUE: PHENOTYPES & FUNCTIONS

Obesity is characterized primarily by an excess of white adipose tissue (WAT) and an enlargement in adipocyte size that results from increased triacylglycerols (TAGs) storage.

Sinetrol® is playing a role at the adipocyte level, helping to reach an equilibrium between energy intake and energy expenditure through a mechanism of action involving the beiging of adipocytes. Indeed, there are 3 types of fat-storing cells.

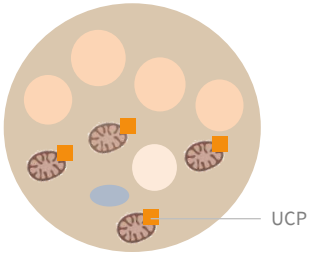
WHITE ADIPOSE TISSUE
(WAT)



The most common fat cell, used to store fat – mainly found in subcutaneous trunk areas

- Large lipid droplet
- Low mitochondrial density

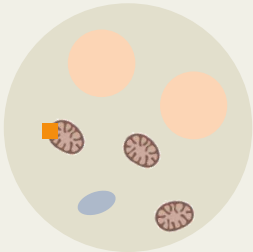
BROWN ADIPOSE TISSUE
(BAT)



Destores excess of fat released from WAT through an increase in resting energy expenditure, i.e. a higher rate of caloric burning

- Small lipid droplets
- High mitochondria density: uncoupling proteins (UCPs) positive

BEIGE ADIPOSE TISSUE
(BRITE) brown-in-white



Transitory phenotype cell within white tissue maturing to achieve fat-burning function thanks to the expression of UCPs

- Predominant lipid vacuole
- Medium mitochondrial density: UCPs positive

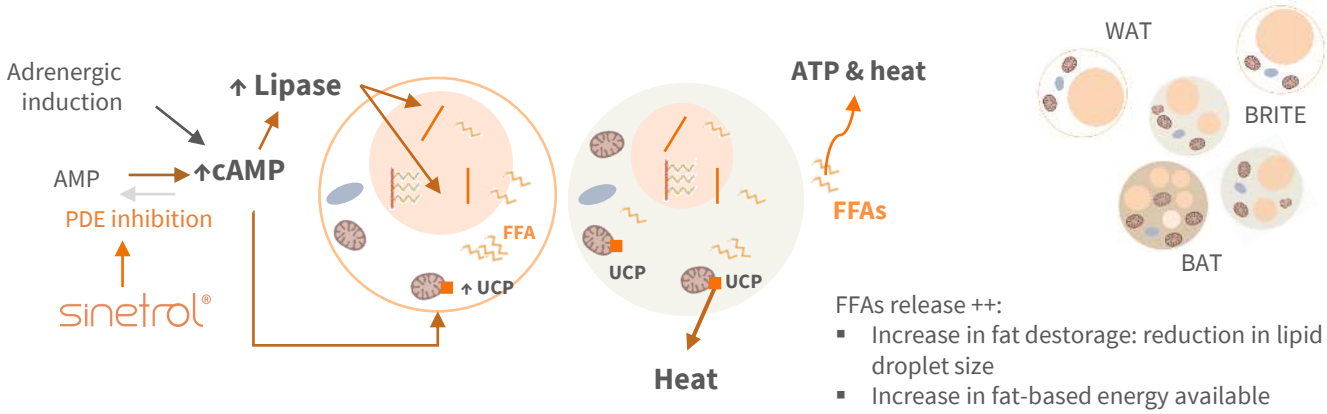
Uncoupling protein is a mitochondrial membranous protein devoted to adaptive thermogenesis, a specialized function performed by brown adipocytes.

Figure: Fat-storing cell phenotypes

3 LIPOLYSIS AMPLIFICATION WITH SINETROL: MECHANISM OF ACTION

Lipolysis is the physiological catabolic process in charge of energy homeostasis in the organism. Occurring in the adipocytes, lipolysis leads to the breakdown of TAGs into free fatty acids (FFAs) and glycerol.

Within physiological conditions, cAMP is secreted under adrenergic induction; phosphodiesterase (PDE) maintains a normal concentration of cAMP, which regulates the lipolytic activity. Upon release from fat cell, free fatty acids are converted into ATP (sustaining the energetic metabolism) and heat. Non-used FFAs are restored in white adipocytes.



FFAs release ++:

- Increase in fat destorage: reduction in lipid droplet size
- Increase in fat-based energy available
- Higher mitochondrial density in the adipocyte for an increased rate of calories burned

Figure: Lipolysis amplification

1 **Higher rate of lipolysis:** destoring of free fatty acids (FFAs)

Through the activity of Sinetrol® on phosphodiesterase inhibition, the rate of cyclic adenosine monophosphate (cAMP) produced by the physiological induction of lipolysis is maintained to a sufficient level that it enhances the basal lipolysis yield. Higher cAMP levels lead to an increase in FFAs release.

2 **Higher mitochondria density and higher expression of UCPs:** Intensified FFAs conversion as heat

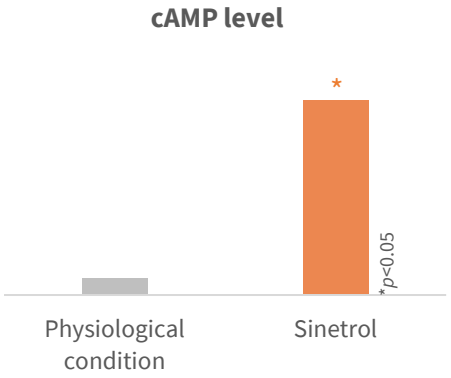
Thermogenesis is a specific metabolic pathway in beige adipose cells that uncouples the energy production pathway, dissipating released FFAs as heat, and, accordingly, avoiding further restorage of fat in adipose tissue. Through the activity of Sinetrol® on phosphodiesterase inhibition, the rate of cAMP is maintained to a sufficient level that it promotes UCP expression and mitochondrial biogenesis by way of adipose beiging.

▶ **Enhanced lipolytic rate** and **beiging benefits of adipocytes**

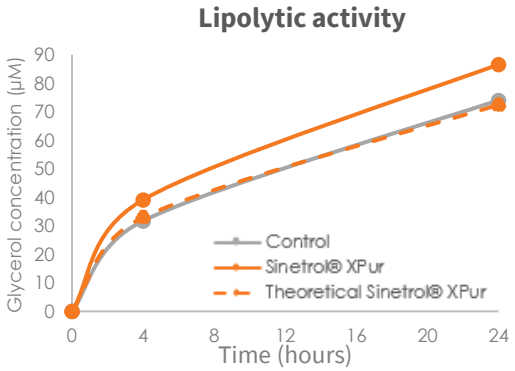
LIPOLYSIS AMPLIFICATION WITH SINETROL: MECHANISM OF ACTION

As highlighted during both ***ex vivo* adipose cell assay and a human clinical investigation**, Sinetrol®, by its action on phosphodiesterase inhibition, promotes the ratio of cAMP, activating both lipolysis and adipose beiging.

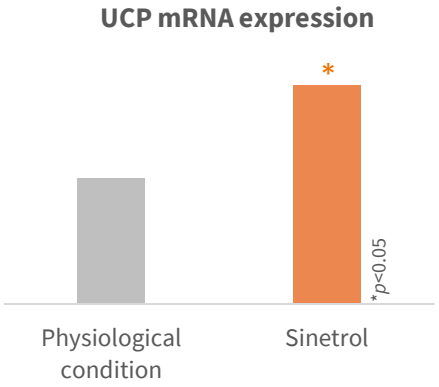
As a physiological consequence, it enhances the release of FFAs, which are further burned during thermogenesis, thereby increasing resting energy expenditure from beige adipose cells.



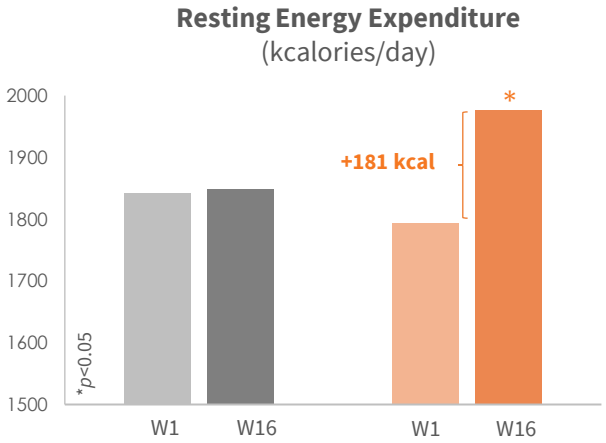
The increase of cAMP is the result of the inhibition of PDE by Sinetrol



Sinetrol increases fat destoring through enhanced rate of lipolysis



Sinetrol inhibits PDE which induces an increase in cAMP leading to an increased level of UCPs



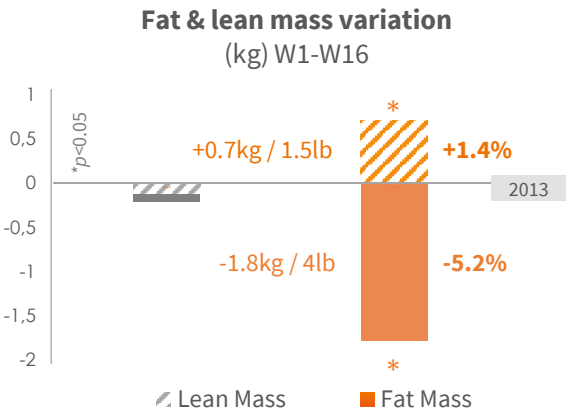
The demonstration of Sinetrol®'s mechanism of action helps explain its physiological effects on a sustained body fat mass reduction, providing prolonged health benefits for individuals at risk of overweight and obesity.

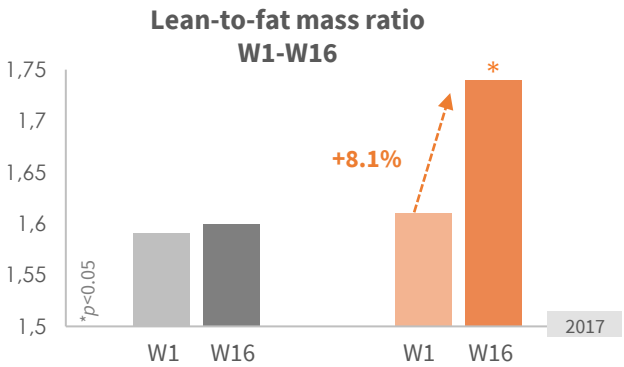
4 CLINICAL TRIALS – RESULTS OVERVIEW

Clinical study - 2013	Clinical study - 2017
France, Paris National Association of Medical Prevention	**Murcia, Spain** Research center, University of Murcia
Sinetrol 900 mg/day	**Sinetrol** 900 mg/day
12 weeks	**16 weeks + 4 weeks**
95 subjects (statistically powered) BMI: 26-30 Age: 22-45	**77 subjects** (statistically powered) BMI: 25-40 Age: 29-52
Normo-caloric diet	**Normo-caloric diet** *(Harris and Benedict)*
Physical activity < 30min/week	**Physical activity** As usual, recorded
VISCAN by Tanita	**Dual X-Ray Absorption** **GOLD STANDARD**
	
▪ Abdominal trunk fat analyzer ▪ Anthropometric measures ▪ Blood samples	▪ Body composition: fat & fat-free mass ▪ Resting Energy expenditure ▪ Blood Samples

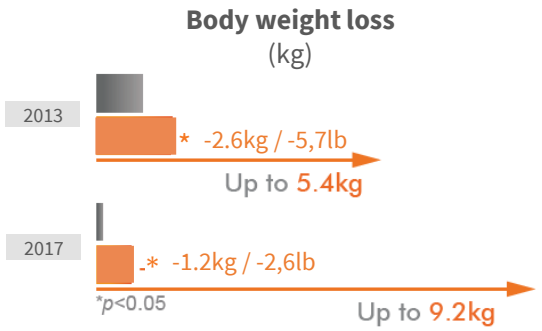
+ New independent clinical study published in 2020 on Asian population

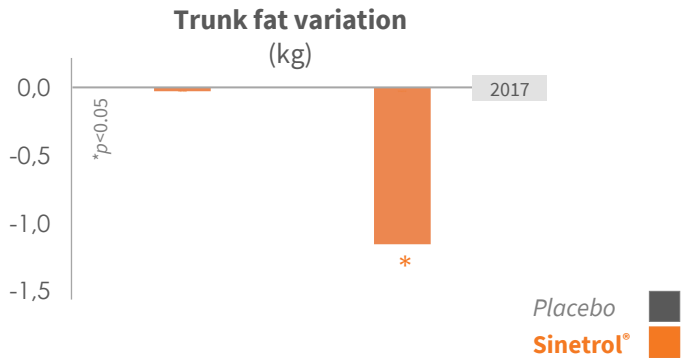
WEIGHT LOSS & BODY COMPOSITION IMPROVEMENT





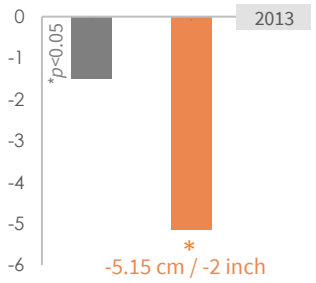
- **Significant weight loss** compared to the placebo group: subjects not only lost weight, but **their body fat mass decreased while their lean mass increased leading to a healthier body composition.**
- **65% of the total fat loss was from the trunk area: the Index of Central Obesity decreased significantly**.



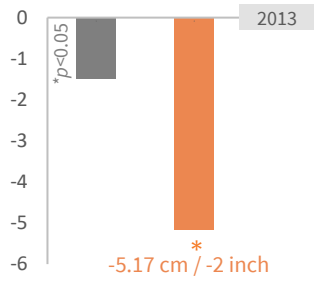


ANTHROPOMETRIC MEASURES

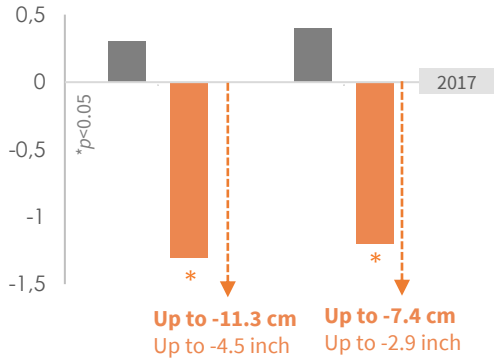
Waist size reduction
(cm) W1-W12



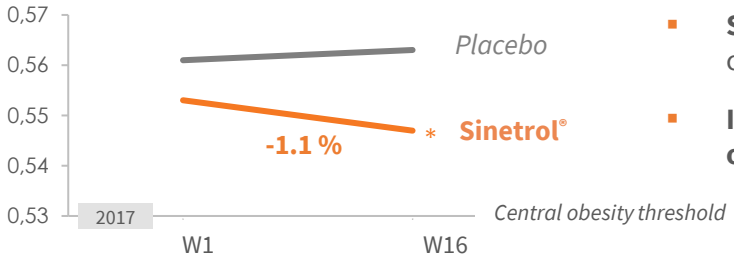
Hip size reduction
(cm) W1-W12



Waist & Hip size reduction
(cm) W1-W16



Index of Central Obesity variation (ICO)
(points) W1-W16



- **Significant decrease of ICO:** ratio of waist circumference to height§
- **ICO is a better indicator of central obesity** than waist size

LINGERING BENEFITS – PREVENTION OF THE REBOUND EFFECT

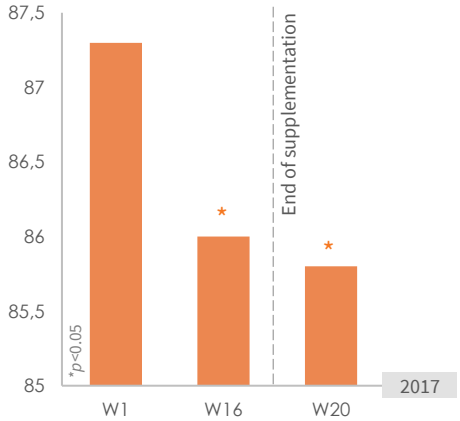
- **Sinetrol** 900 mg/day
- **16 weeks**
- **77 subjects**

FOLLOW-UP

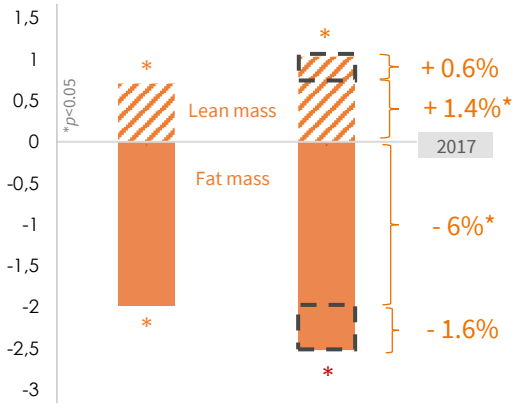
- **4 weeks after the end of the supplementation**
- **47 subjects**
  - 31 in Sinetrol group : 72%
  - 16 in the *placebo* group : 47%

One month after the end of the supplementation, not only did subjects of the Sinetrol group did not regain the weight lost but **they continued to improve their body composition: body fat mass kept decreasing.**

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



Body fat & lean mass variations
(kg) W1-W16 & follow-up W1-W20



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SCIENTIFIC SUPPORT & PUBLICATIONS

TRANSLATED FROM THE KOREAN PUBLICATION

The Effects of Sinetrol-XPur on leptin-deficient obese mice and activation of cAMP-dependent UCP-2

Jae Myeong Yoo[1], Minhee Lee[1], Han Ol Kwon[1], Sei Gyu Choi[2], Mun Hyoung Bae[2], Ok-Kyung Kim[3]

[1]Graduate School of East-West Science, Kyung Hee University, Gyeonggi 17104, Korea
[2]RPCorpCo. Ltd, Seoul 06170, Korea
[3]Division of Food and Nutritional Science, Chonnam National University, Gwangju 61186,
Korea

†Corresponding author. E-mail: 20woskxm@chonnam.ac.kr

Tel: +82-62-530-1318; Fax: +82-62-530-1339

The Effects of Sinetrol-XPur on leptin-deficient obese mice and activation of cAMP-dependent UCP-2

Abstract

The present study investigated the anti-obese effect of Sinetrol-XPur (polyphenolic citrus spp. and Paullinia cupana Kunth dry extract) and defined the action mode for cAMP-depedent UCP-2 activation. Leptin-deficient obese mice were treated with two different doses, 100 mg/kg body weight (B.W.) and 300 mg/kg B.W. in each AIN93G supplement, for 7 weeks. The treatment of obese mice with both low and high dose Sinetrol-XPur significantly reduced the body weight gain compare to control obese mice. White adipose tissue weight of mice was reduced in high dose supplemented groups 30.96%. Serum TC and TG were reduced with high dose of Sinetrol-XPur by 20.02% and 30.96% respectively. Serum level of HDL was significantly increased by treatment of both dose, resulting that the ratio of HDL to LDL was increased by 138.78% and 171.49%. respectively. In genetic expression of biochemical factors related lipid metabolism, there were significant decrease in FAS and increase in UCP-2 by treatment of high dose Sinetrol-XPur, but there were no significant difference in LPL and HSL. To define the cellular mechanism, intracellular cAMP levels in 3T3-L1 adipocytes significantly increased in a dose-dependent manner over the range of 50-250 μg/mL. And PDE inhibitor (IBMX) clearly blocked the cAMP, suggesting that Sinetrol-Xpur promotes lipolysis of adipocyte through inhibition of cAMP-dependent PDE, resulting in induction of CREB and UCP-2. These results suggest that Sinetrol-XPur supplementation is a viable option for reducing body weight and fat by improving serum lipid profiles and genetic expression of lipid metabolic factors, especially activation of cAMP-dependent UCP2.

Key words : anti-obesity, Sinetrol-XPur, Citrus, ob/ob mice

Introduction

Obesity is an incurable chronic disease which can lead to abnormal or excessive body fat accumulation associated with various metabolic syndromes, including hardening of the blood vessel and a high risk of type 2 diabetes, cardiovascular diseases. Obesity is mostly caused by excessive food intake, insufficient exercise and genetic susceptibility. Some cases are caused by medications, endocrine disorders or mental disorder. Changes to diet and exercise intervention are effective strategies for prevention and management of obesity. Various physiological factors involve in adipogenesis such as triglyceride, high density lipoprotein(HDL), low density lipoprotein(LDL), fatty acid synthase(FAS), uncoupling protein-2(UCP-2), hormone-sensitive lipase(HSL). Especially UCP-1 and HSL are associated with cAMP concentration in cells under control of phosphodiesterase(PDE) and protein kinase A(PKA) activation. Although a number of effective medications for weight loss are currently available, there are still limitations, including serious adverse effects and long-term safety. Non-pharmacological drugs can prevent obesity without side effects against weight gain.

Recent studies have been demonstrated that flavonoid plays an important role in polyphenol activation on adipogenesis including anthocyanin (malvidin, cyanidin; red orange, bluebarries and wine), flavanone (naringin, hesperidin, narirutin, etc.; orange, grapefruit), flavonols (quercetin, kaempherol; onions, broccoli). in addition, positive effects of flavonoid reported prevention of inflammation, cardiovascular disease, and ischemia and regulated food-intake via increased lipolytic activity with inhibition of cAMP-PDE. Sinetrol-XPur is a mixture natural plants of red orange(*Citrus sinensis* L. Osbeck), sweet orange(*Citrus sinensis* L. Osbeck), *Citrus paradisi* Macfad, and *Paullinia* cupana Kunth and its main compounds include flavonoid such as antocyanins and flavanone. A study suggested that Sinetrol-XPur enhanced

anti-obesity effects through inhibition of PDE. In the present study, we aimed to investigate the anti-obesity effects of Sinetrol-XPur in 3T3-L1 cells and obesity model mice.

Materials and methods

Obesity model mice. Sinetrol-XPur is provided by Fytexia. C57BL/6J ob/ob male mice(n=18), C57BL/6J male mice (n=6) were treated with Sinetrol-XPur 100 mg/kg B.W.(Sinetrol low), Sinetrol-XPur 300 mg/kg B.W.(Sinetrol high) for 7 weeks. Mice were maintained on a mixture of Sinetrol-XPur and AIN93G (Feedlab Co., Ltd., Hanam, Korea) and tap water (15 ml/day). All mice were housed individually in clear plastic cages under controlled temperature (23 ± 2°C), humidity (55 ± 5%), and illumination (12-hour light/dark cycle). The animal research protocol was approved by the Animal Care and Use Committee of the Kyounghee University and all experiments were conducted in accordance with the institutional guidelines established by the Committee (KHUASP(SE)-15-019).

Blood and tissue sampling. The animals were euthanized immediately before blood and tissue collection. Blood samples were collected from the left ventricle of anesthetized rats into heparinized syringes. Plasma was separated by centrifugation at 1200g for 15 min at 4°C and stored at –20°C until assayed for triglyceride(TG), total cholesterol(TC), HDL-cholesterol, LDL/VLDL using enzyme assay kit (Biovision Inc. Mountain view, CA, USA). The fat tissues were removed and washed with PBS. Weight of each fat tissues (white adipocytes: abdominal fat, visceral fat, epididymal fat, brown fat) were measured.

Quantitative reverse-transcription PCR (qRT-PCR). Total RNA was extracted from cultured cells using TRIzol reagent (Invitrogen, Carlsbad, USA), following the manufacturer′s instructions (Beckman Coulter, Brea, USA). The extracted RNA was subsequently reverse

transcribed using a RevertAid™ H Minus First Strand cDNA Synthesis Kit (Fermentas Inc., Hanover, USA), with oligo$(dT)_{15–18}$ as a random primer. All real-time RT-PCR measurements were performed using the ABI Prism 7000 Sequence Detection System (Applied Biosystems, Foster City, USA). All PCR amplifications (40 cycles) were performed in a total volume of 25 μl containing 150 ng of cDNA using an SYBR Green I qPCR kit (TaKaRa, Shiga, Japan), according to the manufacturer′s recommendations. By normalizing to Gapdh, the relative quantification of gene expression was performed using the comparative threshold (Ct) method, as described by the manufacturer (Applied Biosystems). The values were expressed as the fold change over the control. Relative gene expression was displayed as $2^{-\Delta Ct}$ ($\Delta Ct = Ct_{target\ gene} - Ct_{Gapdh}$). The fold change was calculated as $2^{-\Delta\Delta Ct}$ ($\Delta\Delta Ct = \Delta Ct_{control} - Ct_{treatment}$).

Table 1. Primer sequences used in real-time PCR quantification of mRNA

Gene	Primer sequences
FAS	F 5’-GAAGTGTCTGGACTGTGTCATTTTTAC-3’
	R 5’-TTAATTGTGGGATCAGGAGAGCAT-3’
LPL	F 5‘-CAAGATTCACTTTTCTGGGACTGA-3’
	R 5‘-GCCACTGTGCCGTACAGAGA-3’
HSL	F 5‘-CACTAGTCCCTCCCCCAGTTT-3’
	R 5’-AGCTGGCACAGCAGGTCTGT-3’
UCP-2	F 5’-GCCCCTTCACCTCTTTAGCA-3’
	R 5’-CCAAGCACTGGGAAGGTCTAA-3’
GAPDH	F 5’-CATGGCCTTCCGTGTTCCTA-3’
	R 5’-GCGGCACGRCAGATCCA-3’

Reagents and cell culture materials. Mouse 3T3-L1 preadipocyte cells were maintained in High-DMEM supplemented with 10% FBS, penicillin (100 U/mL) and streptomycin (100 μg/mL). The 3T3-L1 differentiation were induced by 0.5 mM 3-isobutyl-1-methylxanthine, 1

mm dexamethasone and 1ug.ml insulin (MDI). After 3days of differentiation, the cells were incubated with 1ug/ml insulin for 5~7 days. The medium was changed every 3 days. All cultured cells were incubated in a humidified atmosphere at 37° C and at 5% CO_2.

cAMP assay. Cells were incubated in 6-well plates for 24 hours and serum-starved for 18 hours. Cells were treated with 0 and 1 IU of TSH for 30 minutes. For the cAMP assay, cells were treated with 0.1N hydrochloric acid to stop endogenous phosphodiesterase activity. After cell lysis, samples were centrifuged at 600g at room temperature and supernatant was collected. cAMP was measured using the cAMP direct immunoassay kit (Millpore) following the manufacturer's instructions. Each experiment was repeated three times.

Statistical analysis. All the experiments were repeated at least three times and the results were presented as the means ± standard deviation (SD), as indicated. Statistical analysis was done using PASW Statistics, version 17.0 (SPSS Inc., Chicago, IL, USA). Statistically significant differences between groups were tested using a one-way ANOVA (Duncan's multiple range test) P values <0.05 were considered significant.

Results

Effects of Sinetrol-XPur on total body, liver and adipocyte tissue weight

To investigate anti-obesity effect of Sinetrol-XPur, we determined changes in total body, liver and adipocyte tissue weight. Body weight significantly was increased genetical factor in ob/ob mice groups (Obese; 20.23±2.38 g, Sinetrol low; 16.75±2.07 g, Sinetrol high; 14.92±1.88 g),

compared to control group (ob/ob mice 12.67±1.29 g). However, 100 and 300 mg/kg B.W of Sinetrol-XPur-treated groups significantly reduced weight gain 17.18% and 26.23%, respectively. Interestingly, no differences were found between Sinetrol high-treated group and control group. In addition, high and low Sinetrol-XPur-treated groups were decreased liver weight and significant reduced liver weight was observed in high Sinetrol-XPur-treated group. Similar results in white and brown fat tissue weight presented that Sinetrol-XPur prevented adipocyte accumulation in high and low treated groups and Sinetrol high-treated group significantly decreased white and brown fat tissue weight. These results suggested that Sinetrol inhibited adipocyte accumulation and weight gain in ob/ob mice.

Table 2. Effect of Sinetrol-XPur on body weight gain and tissue weight in obese mice

	Lean			Obese			Sinetrol low			Sinetrol high		
Weight gain (g)	12.67	±	1.29[c]	20.23	±	2.38[a]	**16.75**	**±**	**2.07[b]**	**14.92**	**±**	**1.88[b]**
Tissue weight (g)												
Liver	1.09	±	0.06[c]	3.53	±	0.86[a]	2.97	±	0.68[ab]	**2.44**	**±**	**0.21[b]**
White adipose tissue	3.55	±	0.99[c]	13.75	±	1.24[a]	11.23	±	2.55[a]	**8.10**	**±**	**2.24[b]**
Brown adipose tissue	0.25	±	0.02[b]	0.31	±	0.06[a]	0.36	±	0.06[a]	0.31	±	0.12[a]

All data are presented as mean±standard deviation. Statistical analyses were performed by Duncan's multiple range tests after one-way ANOVA and using SPSS software. Differences were considered statistically significant at $P<0.05$.

Sinetrol low ; Obese + Sinetrol 100 mg/kg B.W., Sinetrol high ; Obese + Sinetrol 300 mg/kg B.W.

Effects of Sinetrol-XPur on lipid metabolism.

To investigate the effects of Sinetrol-XPur on lipid metabolism, TC, TG, HDL-cholesterol, LDL/VLDL- cholesterol were measured. Concentrations of TC, TG, HDL-cholesterol, LDL/VLDL- cholesterol in Obese, Sinetrol low and Sinetrol high groups were significantly

increased, compared to control group. However, Sinetrol low and Sinetrol high-treated groups decreased concentrations of TC (14.77% in Sinetrol low and 20.02% in Sinetrol high) and TG (15.70% in Sinetrol low and 30.96% in Sinetrol high). However, no significant different HDL-cholesterol concentrations were found between obese and Sinetrol treated groups. The level of LDL/VLDL- cholesterol in Sinetrol low and Sinetrol high reduced 20.36% and 32.9%, respectively and significant decreased LDL/VLDL- cholesterol was observed in Sinetrol high-treated group. In addition, HDL/LDL ratio in Sinetrol low and Sinetrol high significantly increased 138.78%, 171.49%, compared to obese group. These results suggested that Sinetrol improved serum level of TC, TG, HDL-cholesterol, LDL/VLDL- cholesterol in ob/ob mice.

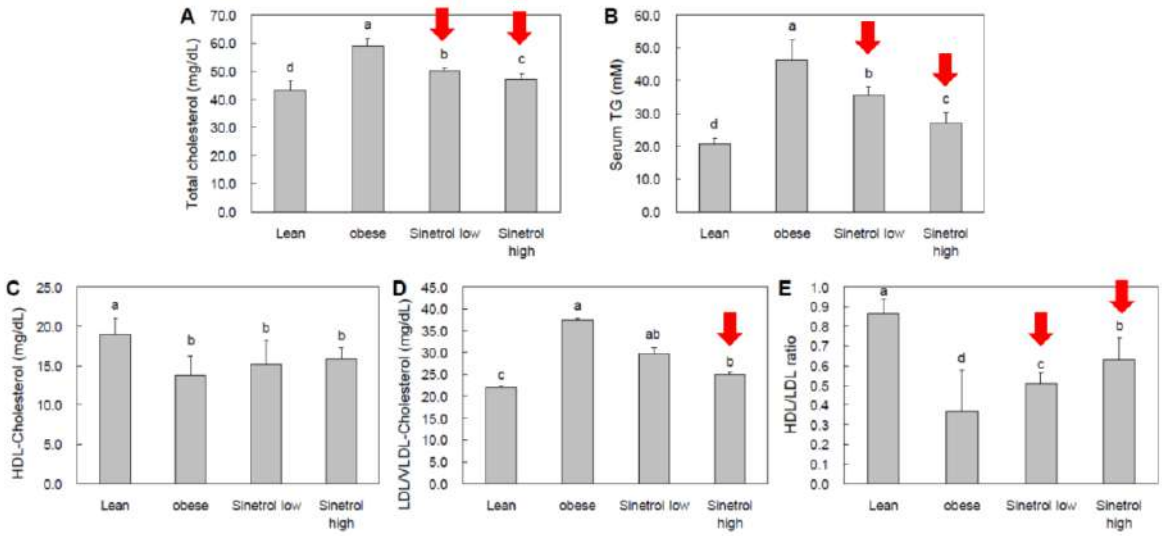

Fig. 1. Effect of Sinetrol-XPur on lipid profiles in obese mice.

All data are presented as mean±standard deviation. Statistical analyses were performed by Duncan's multiple range tests after one-way ANOVA using SPSS software. Differences were considered statistically significant at $P<0.05$. Sinetrol low ; Obese + Sinetrol 100 mg/kg B.W., Sinetrol high ; Obese + Sinetrol 300 mg/kg B.W. (A) Total cholestrol, (B) Serum triglyceride, (C) HDL-cholesterol, (D) LDL/VLDL-cholesterol, (E) HDL/LDL ratio

Effects of Sinetrol-XPur on adipogenetic mRNA expression in fat tissue

To investigate the anti-obesity effect of Sinetrol-XPur on mRNA expressions in fat tissue, FAS, lipoprotein lipase(LPL), HSL, and UCP-2 were accessed by qRT-PCR. The mRNA expressions of FAS, lipoprotein lipase(LPL) and HSL in ob/ob mice was higher than control group, while mRNA expression of UCP-2 in obesity groups was lower than control group. However, Sinetrol low and Sinetrol high-treated ob/ob mice groups reduced mRNA expression of FAS and HSL,

compared to non-treated ob/ob mice group but LPL mRNA level did not differ between control and treated groups. Significant decreased mRNA expression of FAS (36.69%) was observed between Sinetrol high-treated and control groups. The mRNA expressions of UCP-2 tended to be a decreased in both Sinetrol low and Sinetrol high-treated ob/ob mice groups and significant higher UCP-2 expression was found in Sinetrol high-treated group, compared to control group. These results suggested that Sinetrol inhibited adipogenesis through the down-regulation of FAS and up-regulation of UCP-2 expressions in ob/ob mice.

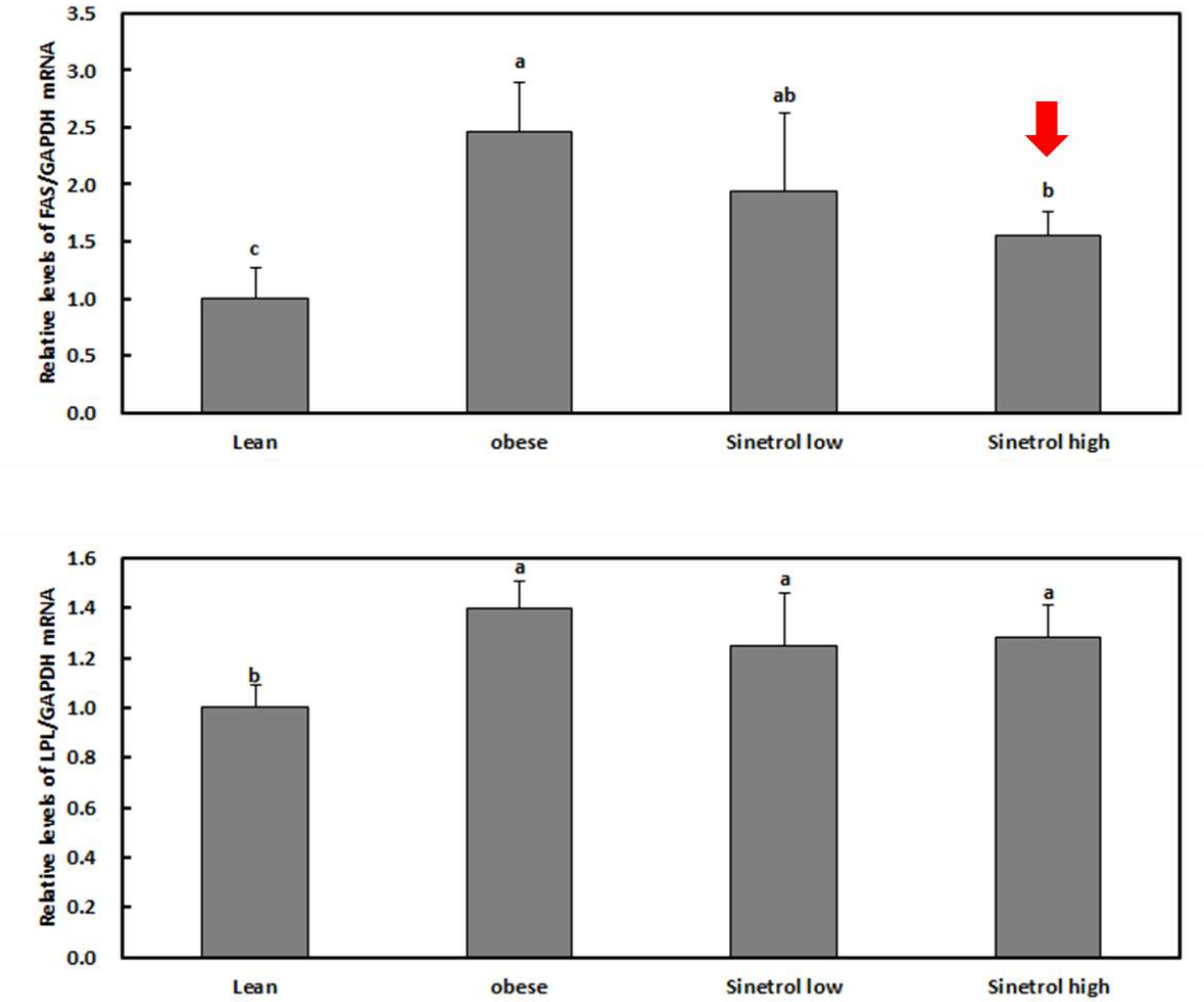

Fig.2. Effect of Sinetrol-XPur on expression of fatty acid synthase (FAS) and lipoprotein lipase (LPL) in obese mice.

All data are presented as mean±standard deviation. Statistical analyses were performed by Duncan's multiple range tests after one-way ANOVA using SPSS software. Differences were considered statistically significant at $P<0.05$.

Sinetrol low ; Obese + Sinetrol 100 mg/kg B.W., Sinetrol high ; Obese + Sinetrol 300 mg/kg B.W.

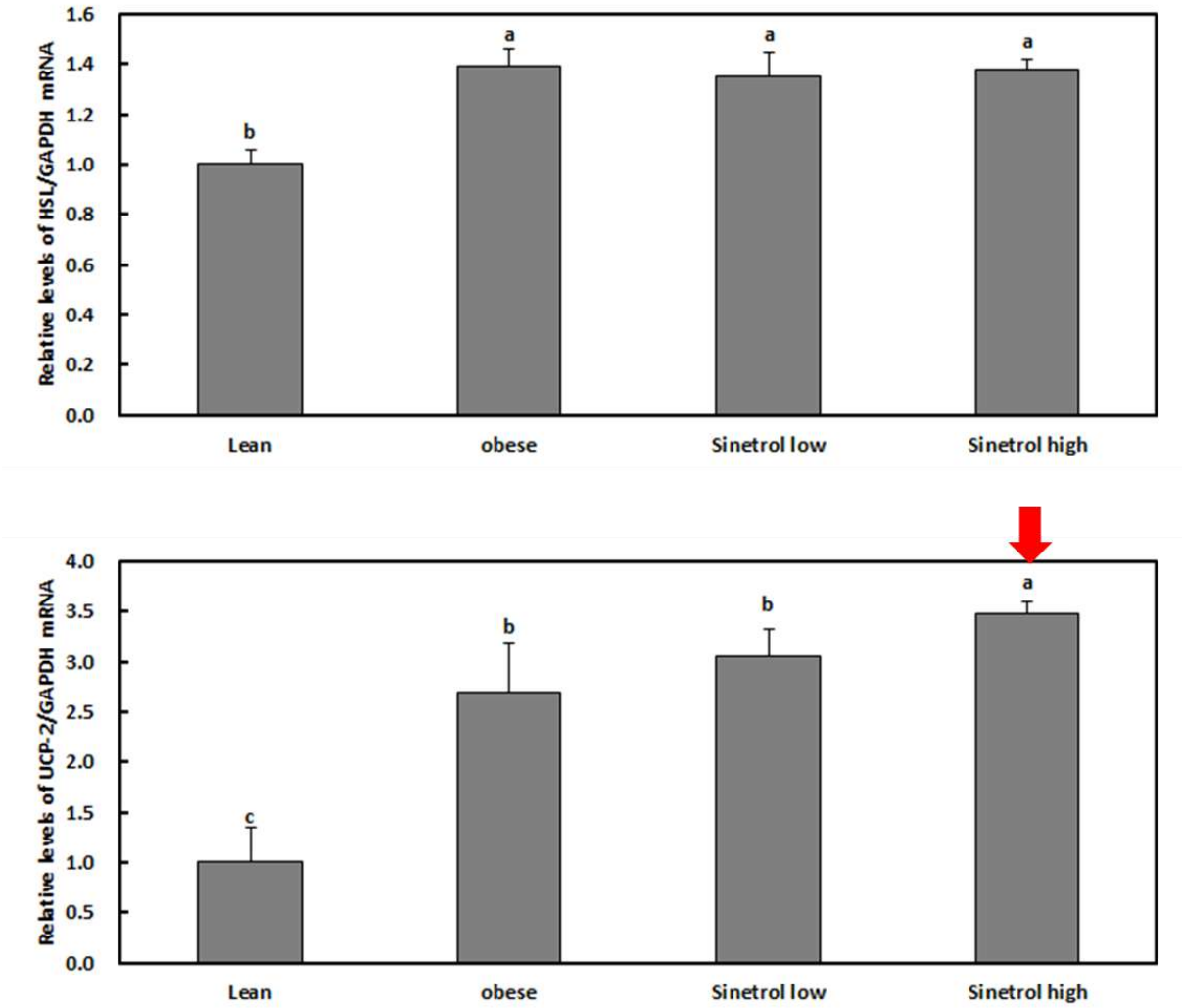

Fig.3. Effect of Sinetrol-XPur on expression of hormone-sensitive lipase (HSL) and UCP-2 in obese mice.

All data are presented as mean±standard deviation. Statistical analyses were performed by Duncan's multiple range tests after one-way ANOVA using SPSS software. Differences were considered statistically significant at $P<0.05$.

Sinetrol low ; Obese + Sinetrol 100 mg/kg B.W., Sinetrol high ; Obese + Sinetrol 300 mg/kg B.W.

Effects of Sinetrol-XPur on cAMP and mRNA expression of UCP-2

To confirm the effects of Sinetrol-XPur on adipogenesis, we examined the levels of cAMP and mRNA expression of UCP-2 in 3T3-L1 cells. Differentiation of 3T3-L1 pre-adipocyte cells were induced by 0.5 mM 3-isobutyl-1-methylxanthine, 1 mm dexamethasone and 1ug.ml insulin (MDI) for 3 days and incubated with 1ug/ml insulin for 5days. After treatment of

Sinetrol-XPur, cAMP levels were dose-dependently increased and mRNA expression UCP-2 was increased by treatment of Sinetrol-XPur. To further investigate whether Sinetrol-XPur increased cAMP via PDE, we used PDE inhibitor (IBMX) and cAMP inhibitor (cyclosporinA/FK506). Co-treated with IBMX + Sinetrol and cyclosporinA/FK506+ Sinetrol increased cAMP level and mRNA expression UCP-2, compared to single treated group. These results suggested that Sinetrol promoted inhibition of fat accumulation through activation of cAMP-dependent UCP2 rather than HSL level in 3T3-L1 cells.

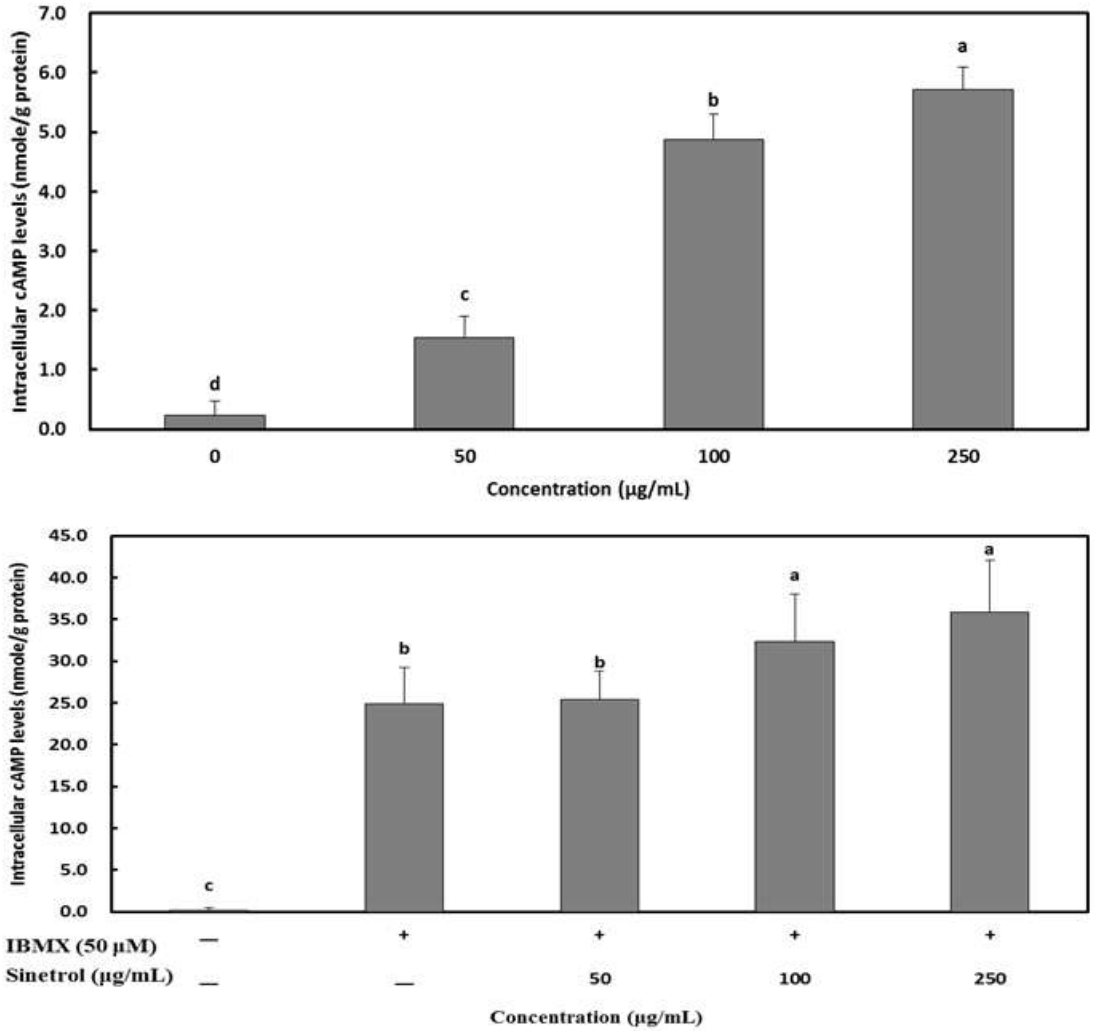

Fig.4. Effect of Sinetrol-XPur on intracellular cAMP levels in 3T3-L1 cells.

All data are presented as mean±standard deviation. Statistical analyses were performed by Duncan's multiple range tests after one-way ANOVA using SPSS software. Differences were considered statistically significant at $P<0.05$.

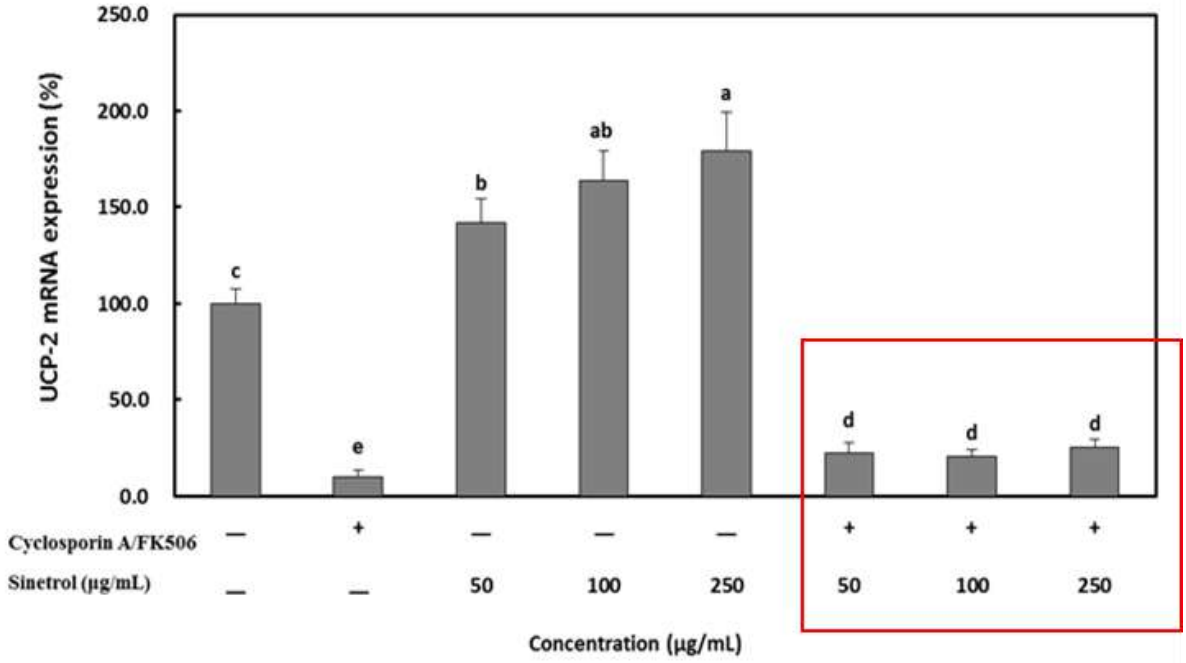

Fig.5. Effect of Sinetrol-XPur on expression of UCP-2 gene in 3T3-L1 cells.

All data are presented as mean±standard deviation. Statistical analyses were performed by Duncan's multiple range tests after one-way ANOVA using SPSS software. Differences were considered statistically significant at $P<0.05$.

Summary

This study investigated the anti-obesity effects of Sinetrol in ob/ob mice and in 3T3-L1 adipocytes. Our data showed that Sinetrol inhibited weight gain with decreased liver and white adipose tissue weight, indicating inhibited adipocyte accumulation and weight gain in ob/ob mice. Serum levels of lipid metabolism factors such as TC, TG, HDL-cholesterol, LDL/VLDL-cholesterol were improved by treatment of Sinetrol in ob/ob mice through activation of cAMP-dependent UCP2, resulting in reduction of fat accumulation and lipogenesis. Finally, our observations of anti-obesity effects of Sinetrol in ob/ob mice and in 3T3-L1 adipocytes that Sinetrol may be a potential therapeutic agent for the treatment and prevention of obesity.

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Efficacy and Safety of Sinetrol-XPur on Weight and Body Fat Reduction in Overweight or Obese Adults: A 12-Week, Randomized, Double-Blind, Parallel, Placebo-Controlled Trial

Seon-Joo Park,[1] Anshul Sharma,[1] Mun Hyoung Bae,[2] Ha Chang Sung,[2] Nam Ki Kim,[2] Eunju Sung,[3] and Hae-Jeung Lee[1,4]

[1]*Department of Food and Nutrition, College of BioNano Technology, Gachon University, Seongnam, Gyeonggi-do, Korea.*
[2]*Rpbio Co. Ltd. Korea, Seoul, Korea.*
[3]*Department of Family Medicine, Kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine, Seoul, Korea.*
[4]*Institute for Aging and Clinical Nutrition Research, Gachon University, Gyeonggi-do, Korea.*

ABSTRACT This study investigated the effect of Sinetrol-XPur on weight and body fat reduction in overweight or obese Korean participants. Among 100 overweight or obese participants enrolled in a 12-week randomized, double-blinded, controlled study, 86 participants completed the trial. Participants took either two Sinetrol-XPur tablets (450 mg per tablet) or two placebo tablets once a day. Bodyweight, body fat percentage, body mass index (BMI), body fat mass, waist circumference, and various safety parameters were measured. After the 12-week intervention, a significant reduction was observed in the body fat mass ($P = .030$) by dual-energy X-ray absorptiometry (DEXA), body weight ($P = .002$), and BMI ($P = .002$) compared to the placebo. Body fat percentage ($P = .007$) by DEXA showed a significant reduction in the Sinetrol-XPur group, but no difference compared to the control group. Abdominal metabolic risks by computed tomography and blood biochemistry analysis were significantly decreased in the Sinetrol-XPur group, but there were no differences between the Sinetrol-XPur and placebo groups. Safety profiles were not different between the two groups. These results suggested that Sinetrol-XPur significantly reduced body weight, body fat mass, and BMI in obese Korean subjects, which confirms the antiobesity effect of Sinetrol-XPur in the Korean population.

KEYWORDS: • ***body fat mass*** • ***body mass index*** • ***bodyweight*** • ***obesity*** • ***Sinetrol-XPur***

INTRODUCTION

Obesity is a multifactorial and persistent international issue.[1] Obesity refers to excess fat buildup in the body, which is influenced by genetic, behavioral, and environmental factors.[2] The westernized diet and sedentary lifestyles are responsible for obesity. As per the recent data, body mass index (BMI) equal to or >30 has led to 61% of global deaths.[1] In addition, obesity has many negative repercussions on the human body leading to hypertension, cardiovascular diseases, fatty liver, and type 2 diabetes mellitus.[3–6] Obesity as a risk factor poses a physical, economic, and psychological global burden.[7–9] According to a recent report, by 2025, the obesity rate for women and men are expected to rise 21% and 18%, respectively.[10] The obesity prevalence has also been increasing in South Korea for persons aged 19 years or older. A report by the Ministry of Health and Welfare published that the obesity prevalence in the Korean population has been risen by 42.3% for men and 26.4% for women by 2016 (www.index.go.kr). Furthermore, the number of obese adolescents and children has also been continually growing.[11] The conventional approaches to prevent or treat obesity and associated complications include a balanced diet, increased energy expenditure, and lifestyle modification. However, in recent years, the trend has been shifted toward natural health functional foods as an alternative way to treat obesity. Accordingly, a recent review has summarized the plausible roles of specific bioactive compounds, from green tea, green coffee, berries, pomegranate, ginger, nuts, and seeds, for obesity prevention, weight management, and obesity-related complications.[12]

Recently, a polyphenolic dietary compound, Sinetrol, has turned out to be an active topic for improving obesity and obesity-associated complications.[13–15] Sinetrol is a mixture of flavonoids, including anthocyanins and flavanones from citrus-based foods. Sinetrol-XPur is extracted by physical

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Address correspondence to: Hae-Jeung Lee, PhD, Department of Food and Nutrition, College of BioNano Technology, Gachon University, 1342 Seongnamdaero, Sujeong-gu, Seongnam-si, Gyeonggi-do 13120, Korea, E-mail: skysea@gachon.ac.kr; skysea1010@gmail.com *or Eunju Sung, PhD, Department of Family Medicine, Kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine, Seoul 03181, Korea, E-mail:* eju.sung@samsung.com

methods in a concentrated foam using particular varieties of orange, grapefruit, and guarana.[13,16]

The potential mechanisms of Sinetrol-XPur against obesity may be based on antioxidation,[14] antiinflammation,[14] and lowering blood triglyceride (TG), and cholesterol contents.[16] One study attributed the effect of Sinetrol-XPur on lipid profiles to the activation of cAMP-dependent uncoupling protein-2 (*UCP-2*) in leptin-deficient obese mice.[16] Another study showed the correlation of the lipolytic effect and the increased gene expressions of adipose triglyceride lipase (*ATGL*), A-kinase anchoring protein 1 (*AKAP1*), and perilipin-1 (*PLIN*) and reduced expression of phosphodiesterase 3B (*PGE3B*).[17]

Although few human studies have been published on the effects of Sinetrol-XPur, suggesting that this flavonoid compound has a potential antiobesity effect, Dallas *et al.* showed that Sinetrol-XPur reduced BMI after 12 weeks of supplementation, although it was a small intervention with 20 overweight subjects.[13] In a continued work, Dallas *et al.* reported beneficial effects in body fat composition, including the improvement in glycemic, inflammatory, and oxidative status in 12-week, in a double-blinded, parallel, clinical trial with 95 healthy overweight French.[14] Along the same line, Sinetrol-XPur supplementation led to a significant reduction in abdominal fat, waist circumference, and improved skeletal muscle mass in 25 overweight male French subjects.[15]

It is well established that genetic factors are among the risk factors for obesity.[18] There may be ethnic differences that are unknown.[19,20] The types of obesity vary in western and Asian countries. Koreans mostly have apple-type obesity, but many westerners have the pear-shaped type of obesity.[21,22] In addition, different lifestyle and dietary environment could have an impact on obesity.[18,23]

To the best of our information, no clinical trial has been conducted on the efficacy and safety of Sinetrol-XPur in overweight or obese Korean subjects. Therefore, this study aimed to assess the Sinetrol-XPur's effect against obesity *via* randomized, double-blind, placebo-controlled clinical trial.

MATERIALS AND METHODS

Study subjects, inclusion, and exclusion criteria

The study subjects were recruited from Kangbuk Samsung Hospital, Seoul, Korea. A total of 100 healthy obese and overweight male and female subjects were enrolled in the study. The subjects were >19 years and <60 years and had BMIs of >25.0 kg/m^2 or <30.0 kg/m^2. All agreed to participate, and those who were not diagnosed with any disease were included in this study. Exclusion criteria included the following: having any cerebrovascular disease (cerebral infarction, cerebral hemorrhage, and so on) within the last 6 months; have a heart illness (angina, myocardial infarction, heart failure, and arrhythmia requiring treatment); any history of cerebrovascular disease and heart disease, but clinically stable; consumed drugs affecting body weight within the last month (inhibitors and appetite suppressants); have consumed functional food/supplement for obesity improvement; central nervous system disorder, sign of depression, treated with drugs, or rehabilitation; taking psychiatric drugs, beta-blockers, diuretics, contraceptives, steroids, and female hormones; uncontrolled hypertension >160/100 mmHg, fasting blood sugar (126 mg/dL or more), random blood sugar (200 mg/dL or more); diabetic taking autologous or oral hypoglycemic agents or insulin; person with <0.1 μU/mL of TSH or >10 μU/mL; creatinine level more than twice; AST or ALT is three times higher; gastrointestinal disorders, alcohol use, medical history; unable to exercise due to musculoskeletal disorders, joined any commercial obesity program within the last 3 months; participated or planned to participate in other clinical trials within the last month, pregnant or plan to become pregnant or nursing mothers; lost >5% of their weight in the last 3 months; and a person deemed inappropriate by the investigator for other reasons were excluded from the trial.

Test material

Sinetrol-Xpur is mainly a citrus fruit extract of grapefruit (*Citrus paradisi*), orange (*Citrus sinensis* Osbeck), and guarana (*Paullinia cupana*). In particular, Sinetrol-Xpur is a polyphenolic-rich ingredient, of which naringin and hesperidin are the main markers of grapefruit and orange, respectively. And the guarana extract provides a small amount of caffeine.[13–15] The participants were instructed to take two tablets once a day before a meal for 12 weeks; a test tablet contained 450 mg of Sinetrol-XPur. The placebo has an identical quantity of maltodextrin. The placebo tablet was produced with the same look, taste, and energy content. The Rpbio Company, Korea, prepared the test and placebo tablets.

Study design

This 12-week randomized, double-blind, placebo-controlled parallel trial on overweight and obese Korean was conducted from June 2018 to February 2019. The Ethics Board Committee of Kangbuk Samsung Hospital, Seoul, Korea approved the study protocol (IRB No. 2018-04-003).

The prescreening was conducted by a telephone conversation and subjects who met the eligibility criteria were scheduled for a baseline visit. Participants were satisfied with the inclusion and exclusion criteria. Written informed consent was obtained from all participants before enrollment. Assessments were conducted every 6 weeks, that is, week 6 and week 12 after randomization (first visit for screening: −2 weeks, second visit: 0 weeks, third visit: 6 weeks, fourth visit: 12 weeks). At the baseline visit (second visit), subjects were randomly assigned to receive either Sinetrol-XPur supplements or placebo. Randomization lists were computer-generated by a statistician. Subjects, as well as investigators, were blind to the intervention assignment until the end of the study.

The sample size was calculated with a significance between treatment and placebo groups at $P = .05$ and a power of 80% using the result of body fat mass and percentage reported by Nosaka *et al.*[24] The required number of subjects

was determined using a power calculation according to published guidelines for human dietary intervention studies.[25,26]

The Sinetrol-XPur and placebo tablets were provided by the investigators every 6 weeks, and compliance was assessed at every follow-up. Compliance was monitored through a trained researcher by calculating remnant tablet strips from the participants at the third and fourth visits. Before and after the intervention, both groups were evaluated for various parameters, namely anthropometric, biochemical assessments, vital signs, energy intake, and exercise. The participants were also examined for any adverse effects during the intervention.

Efficacy measurement

On each visit, the anthropometric parameters, including height, weight, hip circumference, waist circumference, diastolic blood pressure (DBP), and systolic blood pressure (SBP) were measured and recorded by the trained workforce. As for the primary outcome, the dual-energy X-ray absorptiometry (DEXA; Lunar Prodigy Advance, GE, USA) was used to assess the total body fat percentage and body fat mass (kg) before and after the 12-week intervention. As for trunk fat, computed tomography (CT; Light Speed VCT XTE, GE, Japan) was used to assess visceral fat, subcutaneous fat, total abdominal fat, and visceral subcutaneous ratio. Bioelectrical impedance analysis (BIA, Inbody 720; Biospace, Korea) was conducted to measure body fat mass, body fat percentage, and visceral fat area.

The blood samples were collected after 12 h overnight fasting. Blood samples were assessed for lipid profile (total cholesterol [TC], TG, high-density lipoprotein [HDL] cholesterol, and low-density lipoprotein [LDL] cholesterol), fasting glucose, and high-sensitivity C-reactive protein (hs-CRP).

Safety measurement

For all the subjects before and after 12 weeks of intervention, safety assessments included the following: blood parameters such as white blood cells, red blood cells, hemoglobin, hematocrit, platelets, lymphocytes, aspartate aminotransferase, alanine aminotransferase, total bilirubin, gamma-glutamyl transpeptidase, creatinine, blood urea nitrogen, uric acid, and pulse.

Diet and exercise

All participants were provided education on diet and physical activity during the intervention period. It was recommended to reduce the energy intake to 500 kcal/day less than usual. In addition, they were trained to perform exercises corresponding to 300 kcal or more every day. Participants were instructed to carefully consume high fat and high carbohydrates and alcohol drink, and refrain from high-calorie snacks or midnight snacks. The participants were instructed to submit their dietary records and exercise logs on the next visit. The dietary intake data were estimated using the CAN PRO 4.0 program (The Korean Nutrition Society, Seoul, Korea).

Statistics

Statistical data were analyzed using SAS 9.4 (SAS Institute, Cary, North Carolina, USA). Significances of efficacy assessment and safety assessment were performed in the per-protocol groups ($n = 86$) and intention-to-treat group

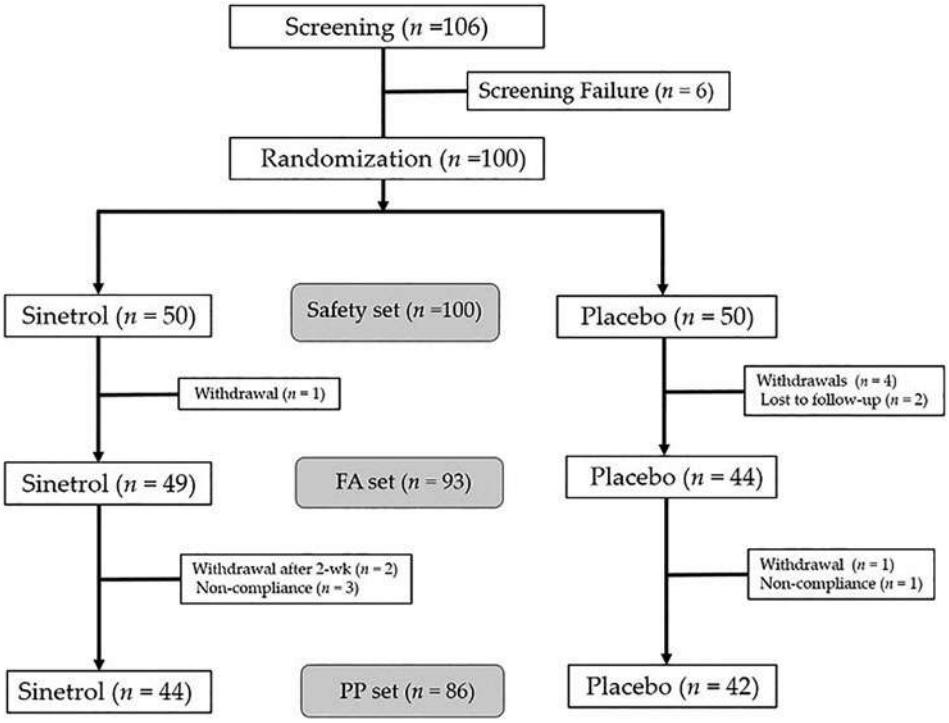

FIG. 1. Flow diagram of study design. FA, full analysis; PP, per-protocol.

Table 1. Demographic Characteristics of Participants

	Test group (n=*44*)	*Control* (n=*42*)	*Total* (n=*86*)	P
Sex, *n* (%)				
Male	19 (43.18)	26 (61.90)	45 (52.33)	0.082[a]
Female	25 (56.82)	16 (38.10)	41 (47.67)	
Age (three)				
Mean ± SD	41.86 ± 8.39	40.83 ± 7.80	41.36 ± 8.08	0.557[b]
Min, max	27.00, 62.00	25.00, 57.00	25.00, 62.00	
Obesity family history, *n* (%)				
No	38 (86.36)	35 (83.33)	73 (84.88)	0.694[a]
Yes	6 (13.64)	7 (16.67)	13 (15.12)	
Exercise and physical activity (regular exercise at least three times a week), *n* (%)				
No	25 (56.82)	25 (59.52)	50 (58.14)	0.799[a]
Yes	19 (43.18)	17 (40.48)	36 (41.86)	
Among those who perform regular exercise at least three times a week				
When to start, *n* (%)				
>1 month	9 (47.37)	9 (52.94)	18 (50.00)	1.000[c]
1–3 months	5 (26.32)	4 (23.53)	9 (25.00)	
4 months to 1 year	1 (5.26)	1 (5.88)	2 (5.56)	
Over 1 year	4 (21.05)	3 (17.65)	7 (19.44)	

[a]*P*-value by chi-square test.
[b]*P*-value by two sample *t*-test.
[c]*P*-value by Fisher's exact test.

($n = 100$), respectively. Differences in efficacy parameters before and after 12 weeks intervention were determined using the paired *t*-test. Between groups with changes before and after treatment, the two sample *t*-test or Wilcoxon rank-sum tests were used according to normal distribution or not. Finally, obesity could be associated with sex and baseline characteristics so that the final significant differences between Sinetrol-XPur and placebo groups were determined using an ANCOVA test adjusted for baseline values and sex. The difference of safety parameters within and between groups was determined by using the paired *t*-test and two-sample *t*-test, respectively. All continuous data are shown as mean ± standard deviation, and the *P*-value <.05 was considered as statistically significant.

RESULTS

The baseline characteristics and dietary assessment

One hundred six (106) subjects were screened for this intervention trial. One hundred subjects were randomly assigned to the Sinetrol-XPur ($n = 50$) group and placebo ($n = 50$) groups. Fourteen participants were excluded due to consent being withdrawn ($n = 4$), primary efficacy test was lost ($n = 1$), participants with <75% compliance ($n = 2$), subjects forgot to return remnant blister strips ($n = 2$), subjects missed visit window ($n = 3$), and subjects lost to follow-up ($n = 2$). At the end of the study, 86 subjects, Sinetrol-XPur ($n = 44$) and placebo ($n = 42$) were included in the study (Fig. 1). The baseline demographic characteristics were similar for both test and control groups. For energy intake and physical activity for each group during the intervention period, no significant differences were observed (Table 1). No adverse effects were reported for the participants of the full analysis set.

Efficacy assessment

After 12 weeks of intervention, the body weight was significantly decreased by 1.81% (1.35 ± 2.40 kg; $P = .000$) in the Sinetrol-XPur group compared with 0.25% reduction in the control group (0.20 ± 1.63 kg; $P = .431$) (Fig. 2A). The change observed was statistically significant ($P = .003$ by Wilcoxon rank sum test; $P = .002$ as determined by ANCOVA test) (Table 2). The BMI in the Sinetrol-XPur group was reduced by 2.32% (0.64 ± 0.85 kg/m^2; $P < .000$), compared with the control group 0.65% (0.18 ± 0.62 kg/m^2; $P = .065$). A statistically significant difference was observed between the two groups ($P = .002$ by Wilcoxon rank sum test; $P = .002$ by ANCOVA test) (Table 2 and Fig. 2B).

Following 12 weeks of Sinetrol-XPur intervention, the waist circumference (−1.97 ± 3.81 cm; $P = .001$ by paired *t*-test), the visceral fat area (−14.60 ± 24.72 cm^2; $P = .000$ by paired *t*-test), and total abdominal fat area (−22.64 ± 51.10 cm^2; $P = .005$ by paired *t*-test) as measured by CT were significantly changed. However, the changes between the groups were not statistically significant. No significant differences were observed for the subcutaneous fat area and visceral/subcutaneous fat ratio between the groups (Table 2).

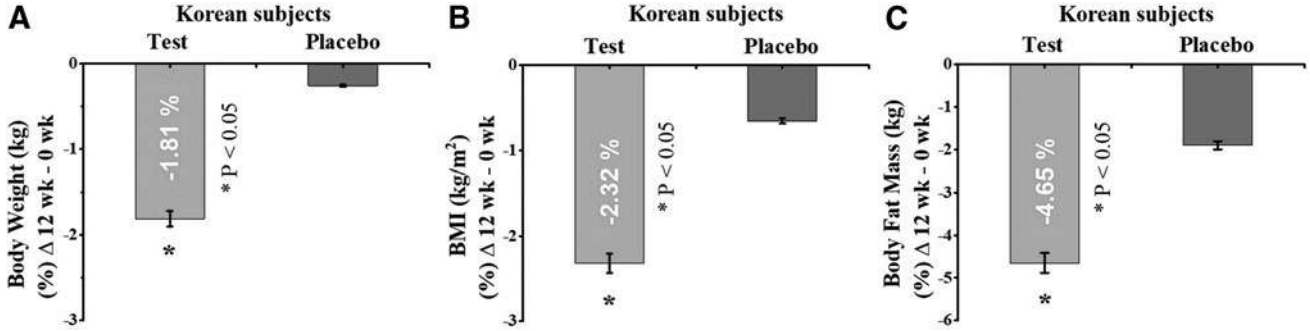

FIG. 2. Effect of Sinetrol-XPur supplementation on **(A)** body weight, **(B)** BMI, and **(C)** DEXA measured body fat mass between treatment and placebo groups at week 12. The body weight ($P = .002$), BMI ($P = .002$), and body fat mass ($P = .030$) decreased significantly in the treatment group compared to control as determined using ANCOVA. BMI, body mass index; DEXA, dual-energy X-ray absorptiometry.

Table 2. Efficacy Assessment in Anthropometrics, Blood Chemistry, and Blood Pressure Before and After 12-Week Treatment

	Test group (n=*44*)				*Placebo* (n=*42*)					
	0 Week	*12 Week*	*Change (12 weeks–0 weeks)*	P[a]	*0 Week*	*12 Week*	*Change (12 weeks—0 weeks)*	P[a]	P[b]	P[c]
Body weight (kg)	74.49±8.61	73.14±8.17	−1.35±2.40	0.000	77.80±10.13	77.60±10.08	−0.20±1.63	0.431	0.003	0.002
BMI (kg/m^2)	27.18±1.44	26.55±1.80	−0.64±0.85	<0.000	27.44±1.53	27.26±1.60	−0.18±0.62	0.065	0.002	0.002
WC (cm)	93.48±5.15	91.51±4.76	−1.97±3.81	0.001	94.73±6.38	93.58±6.26	−1.14±3.62	0.047	0.105	0.157
Waist-to-hip ratio	0.91±0.05	0.90±0.04	−0.01±0.04	0.092	0.91±0.05	0.90±0.04	−0.01±0.03	0.349	0.168	0.643
DEXA measurement										
Body fat (%)	36.30±6.81	35.43±7.17	−0.87±2.07	0.007	35.37±6.79	34.95±6.90	−0.42±1.76	0.126	0.656	0.124
Body fat (kg)	25.8±4.51	24.6±4.85	−1.17±2.11	0.000	26.3±5.29	25.8±5.31	−0.51±1.63	0.046	0.162	0.030
CT measurement										
Visceral fat area (cm^2)	143.47±47.96	128.87±43.63	−14.60±24.72	0.000	144.87±48.97	135.02±42.22	−9.85±25.53	0.016	0.383	0.344
Subcutaneous fat area (cm^2)	233.61±59.29	225.57±63.97	−8.04±37.83	0.165	242.92±73.01	237.19±70.41	−5.73±23.41	0.120	0.941	0.387
Total abdominal fat area (cm^2)	377.08±73.17	354.44±74.72	−22.64±51.10	0.005	387.79±90.61	372.22±81.53	−15.58±37.19	0.009	0.497	0.197
Visceral/subcutaneous fat ratio	0.66±0.31	0.62±0.29	−0.04±0.17	0.134	0.65±0.28	0.62±0.28	−0.02±0.14	0.262	0.548	0.875
BIA analysis										
Body fat mass (kg)	23.90±4.18	23.34±4.84	−0.56±2.09	0.081	23.75±4.87	23.69±4.85	−0.05±1.81	0.845	0.132	0.083
Body fat percentage (%)	32.48±6.48	32.33±7.12	−0.15±2.26	0.671	30.89±6.82	31.00±6.81	0.11±1.87	0.699	0.083	0.275
Visceral fat cross-sectional area (cm^2)	108.57±27.91	107.78±31.10	−0.79±14.30	0.716	106.77±30.31	107.18±30.59	0.41±9.85	0.787	0.234	0.291
Blood chemistry										
Total cholesterol (mg/dL)	199.09±34.22	194.82±36.36	−4.27±22.83	0.221	194.74±34.48	196.93±36.23	2.19±22.08	0.523	0.185	0.171
Triglyceride (mg/dL)	142.95±75.51	130.32±115.14	−12.64±114.70	0.468	131.83±72.74	130.21±73.33	−1.62±47.75	0.827	0.089	0.810
LDL-C (mg/dL)	135.18±33.60	131.02±39.44	−4.16±21.59	0.208	134.33±34.71	136.10±35.89	1.76±21.31	0.594	0.204	0.190
HDL-C (mg/dL)	61.34±15.13	60.43±15.63	−0.91±8.61	0.487	58.07±12.40	60.14±13.44	2.07±6.04	0.031	0.065	0.061
Fasting blood sugar (mg/dL)	98.86±9.49	96.61±7.79	−2.25±8.27	0.078	96.83±7.33	97.29±8.47	0.45±8.00	0.716	0.127	0.353
hs-CRP (mg/L)	0.19±0.53	0.10±0.08	−0.10±0.54	0.231	0.10±0.12	0.10±0.09	−0.00±0.10	0.841	0.212	0.807
Systolic blood pressure (mmHg)	126.41±9.93	126.77±9.70	0.36±8.91	0.787	131.38±11.68	133.57±11.61	2.19±7.23	0.056	0.300	0.065
Diastolic blood pressure (mmHg)	79.27±8.66	79.43±8.55	0.16±6.92	0.879	81.40±9.07	81.24±7.72	−0.17±7.18	0.881	0.830	0.828

Values are presented as mean±SD.

[a]Compared within groups: *P*-value by paired *t*-test.

[b]Compared between groups: *P*-value by Wilcoxon rank sum test.

[c]Compared between groups: *P*-value by ANCOVA adjusted baseline values and sex.

BIA, bioelectrical impedance analysis; BMI, body mass index; CT, computed tomography; DEXA, dual-energy X-ray absorptiometry; HDL, high-density lipoprotein; hs-CRP, high-sensitivity C-reactive protein; LDL-C, low-density lipoprotein cholesterol; WC, waist circumference.

Table 3. Safety Assessment in Blood Biochemistry Before and After 12-Week Treatment

	Test group (n = *44*)				*Placebo* (n = *42*)				
	0 Week	*12 Week*	*Change (12 weeks–0 weeks)*	P[a]	*0 Week*	*12 Week*	*Change (12 weeks–0 weeks)*	P[a]	P[b]
WBC ($10^3/\mu$L)	6.73 ± 1.59	6.69 ± 1.74	0.04 ± 1.38	0.845	6.30 ± 1.33	6.34 ± 1.4	0.05 ± 1.01	0.759	0.974
RBC ($10^6/\mu$L)	4.83 ± 0.43	4.74 ± 0.45	−0.09 ± 0.21	0.005	4.89 ± 0.47	4.86 ± 0.46	−0.07 ± 0.16	0.005	0.671
Hb (g/dL)	14.41 ± 1.49	14.38 ± 1.42	−0.07 ± 0.63	0.418	14.66 ± 1.33	14.77 ± 1.29	0.01 ± 0.47	0.899	0.477
Hct (%)	42.73 ± 3.68	42.50 ± 3.76	−0.34 ± 1.91	0.222	43.47 ± 3.56	43.46 ± 3.36	−0.24 ± 1.38	0.239	0.787
Platelet ($10^3/\mu$L)	270.58 ± 53.02	268.49 ± 50.55	1.04 ± 23.84	0.761	271.76 ± 48.00	271.49 ± 53.41	2.40 ± 28.35	0.572	0.801
Lymphocyte (%)	33.28 ± 6.04	34.34 ± 7.22	1.07 ± 6.22	0.232	36.64 ± 7.96	35.86 ± 7.18	−0.18 ± 5.64	0.831	0.310
AST (IU/L)	22.62 ± 9.26	20.43 ± 6.64	−2.02 ± 6.62	0.037	22.72 ± 7.65	21.67 ± 6.04	−1.04 ± 7.49	0.354	0.504
ALT (IU/L)	27.68 ± 17.82	23.18 ± 14.24	−3.57 ± 12.85	0.057	25.90 ± 15.57	22.02 ± 8.68	−3.24 ± 10.40	0.042	0.893
Total bilirubin (mg/dL)	0.82 ± 0.37	0.80 ± 0.41	−0.02 ± 0.26	0.604	0.76 ± 0.28	0.82 ± 0.29	0.05 ± 0.22	0.168	0.191
ALP (IU/L)	59.86 ± 13.86	60.96 ± 12.29	0.71 ± 7.09	0.483	66.74 ± 15.14	67.76 ± 15.50	1.00 ± 8.28	0.422	0.857
Creatinine (mg/dL)	0.81 ± 0.19	0.80 ± 0.18	−0.01 ± 0.09	0.491	0.85 ± 0.16	0.87 ± 0.18	0.01 ± 0.08	0.483	0.328
BUN (mg/dL)	11.81 ± 3.25	11.64 ± 2.70	−0.05 ± 2.72	0.904	12.69 ± 3.39	12.66 ± 3.72	−0.08 ± 2.81	0.849	0.953
Uric acid (mg/dL)	5.33 ± 1.39	5.27 ± 1.41	−0.05 ± 0.62	0.599	5.69 ± 1.42	5.64 ± 1.55	−0.05 ± 0.61	0.558	0.959
γ-GTP (IU/L)	36.50 ± 41.52	28.69 ± 27.83	−5.92 ± 18.26	0.027	38.72 ± 48.20	33.84 ± 35.62	−5.91 ± 23.20	0.094	0.998
Vital sign									
Pulse (times/min)	73.52 ± 9.86	73.31 ± 7.67	−0.29 ± 7.95	0.802	72.92 ± 8.59	73.53 ± 6.43	0.20 ± 8.80	0.879	0.779

Values are presented as mean ± SD.

[a]Compared within groups: *P*-value by paired *t*-test.

[b]Compared between groups: *P*-value by two sample *t*-test.

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; γ-GTP, gamma-glutamic transpeptidase; Hb, hemoglobin; Hct, hematocrit; RBC, red blood cell; WBC, white blood cell.

Compared to 1.9% reduction in placebo (0.51 ± 1.63; $P = .046$), the body fat mass was decreased by 4.65% (1.17 ± 2.11; $P = .000$) in the Sinetrol-XPur group as measured by DEXA. The ANCOVA test showed a statistically significant ($P = .030$) difference between groups (Table 2 and Fig. 2C). Compared to placebo, change in body fat percentage (−0.87 ± 2.07; $P = .007$ by paired *t*-test) was significant in the test group (Table 2); however, no significant differences were seen between the Sinetrol-XPur and placebo groups. After 12 weeks of intervention, no significant changes were observed in body fat mass, body fat percentage, and visceral fat cross-sectional area, measured by BIA (Table 2).

Changes in blood chemistry parameters during the 12-week Sinetrol-XPur intervention are shown in Table 2. No significant changes were observed between groups for lipid profile, fasting blood sugar, and hs-CRP (an inflammation marker). No significant change was observed for SBP and DBP (Table 2).

Safety

The safety evaluation was performed on blood parameters and vital signs. Compared to baseline, the hepatotoxicity markers, such as ALT, AST, gamma-glutamic transpeptidase (γ-GTP), showed a significant reduction in the intervention group. No significant differences were observed between the two groups for safety parameters (Table 3). At each visit, the subjects were evaluated for side effects or symptoms. No moderate or severe side effects were observed during the intervention study (Table 4). Total calorie intake and physical activity by exercise were not different between Sinetrol-XPur and placebo group, respectively (data not shown).

DISCUSSION

We investigated the effects of Sinetrol-XPur supplementation on body fat (%) and body fat content by DEXA, anthropometric parameters, and blood chemistry in overweight/obese Korean subjects.

Obesity and excess body fat are threatening the global population during the past decade. Many studies have shown that health/functional foods have emerged as a substitute to prevent or treat obesity.[27] The bioactivities of functional foods have been ascribed to the presence of several polyphenols that have their impact on the metabolic pathways.[28]

Several studies have revealed that the inhibitory action of flavonoids on cAMP-phosphodiesterase activity leads to

Table 4. Adverse Events Between Sinestrol and Placebo Group During 12-Week Intervention

	Test group (n = *50*)		*Placebo* (n = *50*)		*Total* (n = *100*)	
	N	*Incidence (%)*	N	*Incidence (%)*	N	*Incidence (%)*
Mild	12	100.00	11	100.00	23	100.00
Moderate	0	0.00	0	0.00	0	0.00
Severe	0	0.00	0	0.00	0	0.00

rising cellular cAMP levels.[13,29] Elevated cAMP levels stimulate lipolysis by activating cAMP-dependent protein kinase A (PKA).[30] The latter activates AMP response element-binding protein (*CREB*),[31,32] leading to the increased expression of *UCP-2*. *UCP-2* plays an important role in body weight regulation, energy stability, and thermoregulation in humans.[33] Hence, these flavonoids could plausibly be used in the management of obesity.

There has been a growing interest in the use of citrus derivatives to treat obesity. The animal studies based on blood orange juice[34] and grapefruit juice[35] have provided insight. Later, a clinical study by Fujioka *et al.* described the weight loss effect of grapefruit consumption in ninety-one obese adults. Compared to placebo, a 12-week supplementation of naringin (flavanone) rich raw grapefruit, grapefruit juice, and grapefruit capsules (500 mg) resulted in 1.6, 1.5, and 1.1 kg weight loss, respectively.[36] In contrast, another study showed that the intake of grapefruit or its juice had no significant effects on physiological variables except for a slight increase in HDL in obese subjects.[37] Still, the data on the potential health benefits of fruits containing polyphenols on obesity are inconclusive.

As a polyphenolic compound, Sinetrol-XPur showed a significant effect on fat reduction in human subjects mainly from the western countries.[13–15] However, no clinical study has been conducted on the Korean population. Ethnically, Koreans are between non-Korean western and African populations. Notably, the intake of flavonoid (Sinetrol) concentration in natural foam (fruits) is less compared to Sinetrol-XPur (prepared with much higher concentration).

In this study, Sinetrol-XPur supplementation resulted in a significant reduction of body weight in the Korean subjects. Our results are supported by clinical trial (12-weeks) findings by Dallas *et al.* and Cases *et al.*[14,15] We have shown that the body fat mass content was significantly reduced with Sinetrol-XPur supplementation compared with placebo, similar to the study findings of Dallas *et al.*, where Sinetrol-XPur resulted in a significant reduction of body fat compared with control.[14] In contrast, no significant difference was reported for body fat percentage in healthy overweight adults, as described in a previous study. The authors highlighted that the grapefruit had a specific effect on central obesity.[38] On BMI reduction, in contrast to our study, no statistically significant change was reported by Dallas *et al.*[14] As per the recent studies, BMI is an important tool to assess abdominal obesity.[39,40]

In this study, supplementation with Sinetrol-XPur was effective for reducing body fat (%) and waist circumference. However, the difference was not statistically significant. In contrast, body fat (%), waist circumference, and hip circumference were significantly changed after the supplementation of Sinetrol-XPur in a previous clinical study.[14] Similarly, Cases *et al.* showed that Sinetrol-XPur supplementation led to a significant change in abdominal fat, waist circumference, hip circumference, and the waist-to-hip ratio.[15]

Another study by Dow *et al.* reported that grapefruit consumption for 6 weeks significantly reduced waist circumference (a measure of central obesity) in healthy overweight adults, without any significant between-treatment difference.[38] Previous studies have shown significant reductions in body fat percentage[14] and waist circumference.[14,15] Although there were no significant differences in the above parameters between the Sinetrol-XPur and the placebo groups, the parameters still showed the reductions. Similar to previous report,[14] no significant changes in TC, TG, LDL-C, HDL-C, hs-CRP, SBP, and DBP were observed in this study after 12 weeks of Sinetrol-XPur supplementation. In contrast, a significant reduction was reported for hs-CRP, as reported by previous studies.[14,15] This contrariwise continent-dependent difference in the effect of Sinetrol-XPur may be the result of not only the race of Koreans but also other dietary environments.

The strengths of the present study include the design of the study, which is a randomized, double-blind, placebo-controlled parallel trial, and the large sample size. In addition, the study results have supported the findings of previous clinical trials. The participants were recruited from one clinical center of Korea; this could cause selection bias. Therefore, to verify this result, a longer intervention study is required in multiple clinical centers. In this study, a 12-week supplementation of Sinetrol-XPur displayed a reducing trend for body weight, body fat mass, and BMI, suggesting a plausible role of Sinetrol-XPur in obesity reduction without any adverse effects. This is the first clinical study assessing the effects of Sinetrol-XPur on overweight/obese Korean subjects.

In conclusion, in the present study, our outcomes indicated that 12 weeks of Sinetrol-XPur intake might have a positive effect on ameliorating body weight, body fat mass, and BMI in overweight and obese Korean individuals. No adverse effects were observed in the participants. Our data have supported the previously reported clinical studies from France. Taken together, we suggested that Sinetrol-XPur could act as a potential antiobesity compound. Further studies are warranted to investigate the long-term effects of Sinetrol-XPur.

AUTHOR DISCLOSURE STATEMENT

N.K.K., H.C.S., and M.H.B. are employees of Rpbio Co. Ltd. Republic of Korea. For all other authors, no competing financial interests exist.

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REFERENCES

1. Collaborators GO: Health effects of overweight and obesity in 195 countries over 25 years. *N Engl J Med* 2017;377:13–27.
2. Rodríguez-Pérez C, Segura-Carretero A, del Mar Contreras M: Phenolic compounds as natural and multifunctional anti-obesity agents: A review. *Crit Rev Food Sci Nutr* 2019;59:1212–1229.
3. Van Gaal LF, Mertens IL, Christophe E: Mechanisms linking obesity with cardiovascular disease. *Nature* 2006;444:875–880.

4. Hall JE, do Carmo JM, da Silva AA, Wang Z, Hall ME: Obesity-induced hypertension: Interaction of neurohumoral and renal mechanisms. *Circ Res* 2015;116:991–1006.
5. Kusminski CM, Bickel PE, Scherer PE: Targeting adipose tissue in the treatment of obesity-associated diabetes. *Nat Rev Drug Discov* 2016;15:639–660.
6. Longo M, Zatterale F, Naderi J, *et al.*: Adipose tissue dysfunction as determinant of obesity-associated metabolic complications. *Int J Mol Sci* 2019;20:2358.
7. Ji C, Guo X: The clinical potential of circulating microRNAs in obesity. *Nat Rev Endocrinol* 2019;15:731–743.
8. Ghaben AL, Scherer PE: Adipogenesis and metabolic health. *Nat Rev Mol Cell Biol* 2019;20:242–258.
9. Tompuri TT, Lakka TA, Hakulinen M, *et al.*: Assessment of body composition by dual-energy X-ray absorptiometry, bioimpedance analysis and anthropometrics in children: The Physical Activity and Nutrition in Children study. *Clinic Physiol Funct Imaging* 2015;35:21–33.
10. Risk N: Factor Collaboration (NCD-RisC). Trends in adult body-mass index in 200 countries from 1975 to 2014: A pooled analysis of 1698 population-based measurement studies with 19.2 million participants. *Lancet* 2016;387:1377–1396.
11. Jung J-H, Hur Y-I: The effect of Maté extract on body weight and fat reduction in obese women: A randomized placebo-controlled clinical trial. *Korean J Obes* 2016;25:197–206.
12. Konstantinidi M, Koutelidakis AE: Functional foods and bioactive compounds: A review of its possible role on weight management and obesity's metabolic consequences. *Medicines* 2019;6:94.
13. Dallas C, Gerbi A, Tenca G, Juchaux F, Bernard F-X: 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). *Phytomedicine* 2008;15:783–792.
14. Dallas C, Gerbi A, Elbez Y, Caillard P, Zamaria N, Cloarec M: 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. *Phytother Res* 2014;28:212–218.
15. Cases J, Romain C, Dallas C, Gerbi A, Rouanet JM: 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. *Int J Food Sci Nutr* 2015;66: 471–477.
16. Yoo JM, Lee M, Kwon HO, Choi SG, Bae MH, Kim O-K: Effects of Sinetrol-XPur on leptin-deficient obese mice and activation of cAMP-dependent UCP-2. *J Korean Soc Food Sci Nutr* 2016;45:484–491.
17. Lee M, Kwon HO, Choi SG, Bae MH, Kim O-K: The effects of Sinetrol-XPur on lipolysis of leptin-deficient obese mice. *J Korean Soc Food Sci Nutr* 2017;46:389–393.
18. Hebebrand J, Hinney A: Environmental and genetic risk factors in obesity. *Child Adolesc Psychiatr Clin N Am* 2009;18:83–94.
19. Stryjecki C, Alyass A, Meyre D: Ethnic and population differences in the genetic predisposition to human obesity. *Obesity Rev* 2018;19:62–80.
20. Ramachandran A, Chamukuttan S, Shetty SA, Arun N, Susairaj P: Obesity in Asia–Is it different from rest of the world. *Diabetes Metab Res Rev* 2012;28:47–51.
21. Han T-K, So W-Y: Effects of FABP2 Ala54Thr gene polymorphism on obesity and metabolic syndrome in middle-aged Korean women with abdominal obesity. *Cent Eur J Public Health* 2019;27:37–43.
22. Wahi G, Anand SS: Race/ethnicity, obesity, and related cardiometabolic risk factors: A life-course perspective. *Curr Cardiovasc Risk Rep* 2013;7:326–335.
23. Roda C, Charreire H, Feuillet T, *et al.*: Lifestyle correlates of overweight in adults: A hierarchical approach (the SPOTLIGHT project). *Int J Behav Nutr Phys Act* 2016;13:114.
24. Nosaka N, Maki H, Suzuki Y, *et al.*: Effects of margarine containing medium-chain triacylglycerols on body fat reduction in humans. *J Atheroscler Thromb* 2003;10:290–298.
25. Hickey GL, Grant SW, Dunning J, Siepe M: Statistical primer: Sample size and power calculations—Why, when and how? *Eur J Cardiothorac Surg* 2018;54:4–9.
26. Lucey A, Heneghan C, Kiely M: Guidance for the design and implementation of human dietary intervention studies for health claim submissions. *Nutr Bull* 2016;41:378–394.
27. Trigueros L, Peña S, Ugidos A, Sayas-Barberá E, Pérez-Álvarez J, Sendra E: Food ingredients as anti-obesity agents: A review. *Crit Rev Food Sci Nutr* 2013;53:929–942.
28. Wang S, Moustaid-Moussa N, Chen L, *et al.*: Novel insights of dietary polyphenols and obesity. *J Nutr Biochem* 2014;25:1–18.
29. Kuppusamy U, Das N: Effects of flavonoids on cyclic AMP phosphodiesterase and lipid mobilization in rat adipocytes. *Biochem Pharmacol* 1992;44:1307–1315.
30. Lumeng CN, Saltiel AR: Inflammatory links between obesity and metabolic disease. *J Clin Invest* 2011;121:2111–2117.
31. Sassone-Corsi P: Coupling gene expression to cAMP signalling: Role of CREB and CREM. *Int J Biochem Cell Biol* 1998;30: 27–38.
32. Mayr B, Montminy M: Transcriptional regulation by the phosphorylation-dependent factor CREB. *Nat Rev Mol Cell Biol* 2001;2:599–609.
33. Kim K-H, Park Y: Food components with anti-obesity effect. *Annu Rev Food Sci Technol* 2011;2:237–257.
34. Titta L, Trinei M, Stendardo M, *et al.*: Blood orange juice inhibits fat accumulation in mice. *Int J Obes* 2010;34:578–588.
35. Chudnovskiy R, Thompson A, Tharp K, Hellerstein M, Napoli JL, Stahl A: Consumption of clarified grapefruit juice ameliorates high-fat diet induced insulin resistance and weight gain in mice. *PLoS One* 2014;9:e108408.
36. Fujioka K, Greenway F, Sheard J, Ying Y: The effects of grapefruit on weight and insulin resistance: Relationship to the metabolic syndrome. *J Med Food* 2006;9:49–54.
37. Silver HJ, Dietrich MS, Niswender KD: Effects of grapefruit, grapefruit juice and water preloads on energy balance, weight loss, body composition, and cardiometabolic risk in free-living obese adults. *Nutr Metab* 2011;8:8.
38. Dow CA, Going SB, Chow H-HS, Patil BS, Thomson CA: The effects of daily consumption of grapefruit on body weight, lipids, and blood pressure in healthy, overweight adults. *Metabolism* 2012;61:1026–1035.
39. Bell JA, Carslake D, O'Keeffe LM, *et al.*: Associations of body mass and fat indexes with cardiometabolic traits. *J Am Coll Cardiol* 2018;72:3142–3154.
40. Ortega FB, Sui X, Lavie CJ, Blair SN: Body mass index, the most widely used but also widely criticized index: Would a criterion standard measure of total body fat be a better predictor of cardiovascular disease mortality? Paper presented at: *Mayo Clinic Proc* 2016;91:443–455.

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SCIENTIFIC SUPPORT & PUBLICATIONS

1 TITLE PAGE

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

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

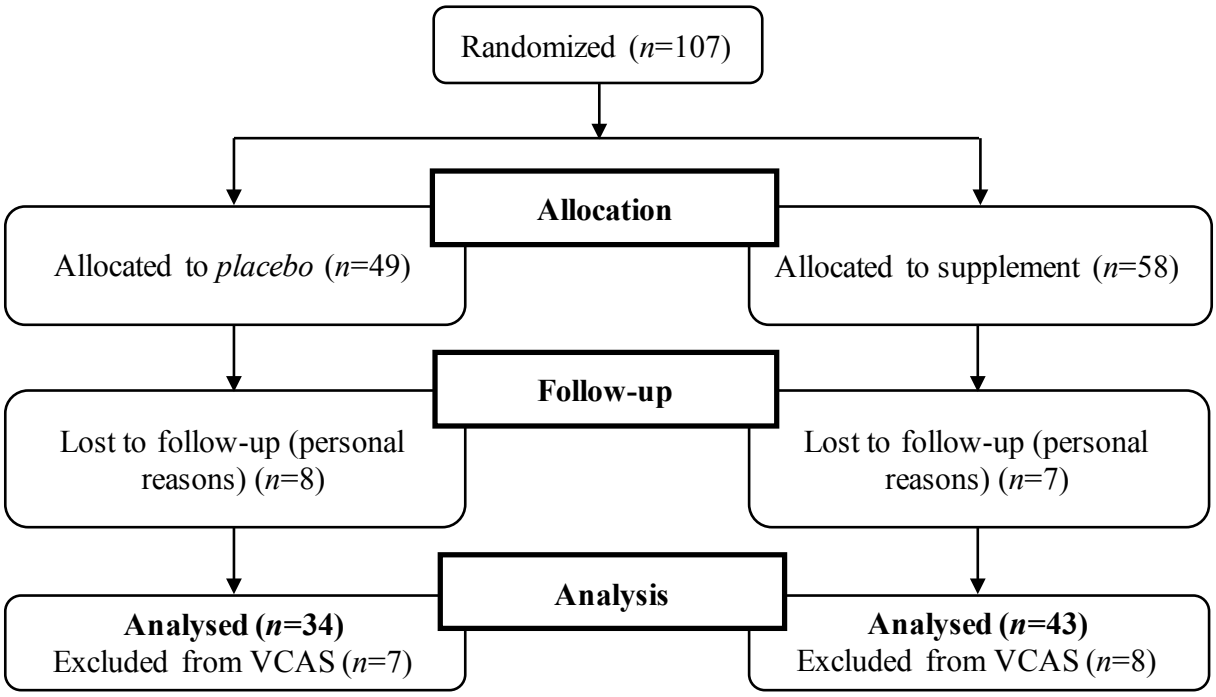
9.2.1 Dropouts

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	-1.27±3.18	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.

14 REFERENCES

Aballay LR, Eynard AR, Diaz MP, Navarro A, Munoz SE. Overweight and obesity: a review of their relationship to metabolic syndrome, cardiovascular disease, and cancer in South America. *Nutr. Rev.* 2013; 71:168–179.

Balkau B, Valensi P, Eschwege E, Slama G. A review of the metabolic syndrome. *Diabetes Metab.* 2007b; 33:405–413.

Cardoso-Saldana GC, Yamamoto-Kimura L, Medina-Urrutia A, Posadas- Sanchez R, Caracas-Portilla NA, Posadas-Romero C. Obesity or overweight and metabolic syndrome in Mexico City teenagers. *Arch. Cardiol. Mex.* 2010; 80:12–18.

Cases J, Romain C, Dallas C, Gerbi A, Rouanet JM. 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. *Int. J. Food Sci. Nutr.* 2015; 66(4):471-477.

Dallas C, Gerbi A, Elbez , Caillard P, Zamaria N, Cloarec M. 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. *Phytother. Res.* 2014; 28(2):212-218.

Deurenberg P, Weststrate JA, Seidell JC. Body mass index as a measure of body fatness: age- and sex-specific prediction formulas. *Br. J. Nutr.* 1991; 65(2):105-114.

Janssen I, Ross R. Effects of sex on the change in visceral, subcutaneous adipose tissue and skeletal muscle in response to weight loss. *Int. J. Obes. Relat. Metab. Disord.* 1999; 23:1035–1046.

Janus ED, Laatikainen T, Dunbar JA, Kilkkinen A, Bunker SJ, Philpot B, Tideman PA, et al. Overweight, obesity and metabolic syndrome in rural southeastern Australia. *Med. J. Aust.* 2007; 187:147–152.

Kaysen GA, Kotanko P, Zhu F, Sarkar SR, Heymsfield SB, Kuhlmann MK, Dwyer T, et al. Relationship between adiposity and cardiovascular risk factors in prevalent hemodialysis patients. *J. Ren. Nutr.* 2009; 19:357–364.

Kim TN, Park MS, Ryu JY, Choi HY, Hong HC, Yoo HJ, Kang HJ, et al. Impact of visceral fat on skeletal muscle mass and vice versa in a prospective cohort study: the Korean Sarcopenic Obesity Study (KSOS). *PLoS One* 2014; 9:e115407.

Meydani M, Hasan ST. Dietary polyphenols and obesity. *Nutrients* 2010; 2(7):737-751.

Mushtaq MU, Gull S, Abdullah HM, Shahid U, Shad MA, Akram J. Waist circumference, waist-hip ratio and waist-height ratio percentiles and central obesity among Pakistani children aged five to twelve years. *BMC Pediatr*. 2011; 11:105.

Parikh RM, Joshi SR, Menon PS, Shah NS. Index of central obesity - A novel parameter. *Med. Hypotheses* 2006; 68(6):1272-5.

Ros E, Martinez-Gonzalez MA, Estruch R, Salas-Salvdo J, Fito M, Martinez JA, Corella D. Mediterranean diet and cardiovascular health: teachings of the PREDIMED study. *Adv. Nutr*. 2014; 5(3):330S-336S.

Ross R, Rissanen J, Pedwell H, Clifford J, Shragge P. Influence of diet and exercise on skeletal muscle and visceral adipose tissue in men. *J. Appl. Physiol*. 1996; 81:2445–2455.

Roza AM, Shizgal HM. The Harris Benedict equation reevaluated: resting energy requirements and the body cell mass. *Am. J. Clin. Nutr*. 1984; 40(1):168-182.

Shaw KA, Srikanth VK, Fryer JL, Blizzard L, Dwyer T, Venn AJ. Dual energy X-ray absorptiometry body composition and aging in a population-based older cohort. *Int. J. Obes.* 2007; 31:279–284.

Shen X, Tang Q, Wu J, Feng Y, Huang J, Cai W. Effect of vitamin E supplementation on oxidative stress in a rat model of diet-induced obesity. *Int. J. Vitam. Nutr. Res.* 2009; 79:255–263.

Singh RB, Ghosh S, Beegom R, Mehta AS, De AK, Haque M, Dube GK, et al. Prevalence and determinants of central obesity and age- specific waist: hip ratio of people in five cities: the Indian Women's Health Study. *J. Cardiovasc. Risk*. 1998; 5:73–77.

Wadden TA, Phelan S. Assessment of quality of life in obese individuals. *Obes. Res.* 2002; 10:50S–57S.

World Health Organization. 2013. Available at: http://www.euro.who.int/__data/assets/pdf_file/0019/249022/WHO-European-Ministerial-

Conference-on-the-Prevention-and-Control-of-Noncommunicable-Diseases-in-the-Context-of-Health-2020.pdf?ua1/41. Accessed on 19 January 2015.

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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

Constantin Dallas,[1]* Alain Gerbi,[2] Yves Elbez,[3] Philippe Caillard,[4] Nicolas Zamaria[5] and Maurice Cloarec[6]

[1]Fytexia, 3 rue d'Athènes-VIA EUROPA 34350 Vendres, France
[2]RDVC Produits Santé, 102 Avenue René Coty, 76600 Le Havre, France
[3]Centre Médical, 5 place de la Bastille, 750043 Paris, France
[4]Centre d'Exploration Vasculaire, 1 rue saint Antoine, 75004 Paris, France
[5]Centre d'Exploration Biologique, 49 avenue de Versailles 75016 Paris, France
[6]Association National de Prévention des Maladies, 8 rue duguay Trouin, 75006 Paris, France

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).

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,

* Correspondence to: Constantin Dallas, Fytexia, 3 rue d'Athènes-VIA EUROPA, 34350 Vendres, France.
E-mail: cdallas@fytexia.com

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

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

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

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

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

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

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

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

	Placebo			Sinetrol-XPur		
	Baseline	W12	% change	Baseline	W12	% change
Kidney function						
Na (mmol/L)	134 ± 1	133 ± 1	−0.45 ± 0.82	136 ± 1	134 ± 1[a]	−1.53 ± 0.55, NS
K (mmol/L)	4.4 ± 0.1	3.9 ± 0.1[a]	−10.86 ± 1.48	4.5 ± 0.1	4 ± 0.1[a]	−9.43 ± 1.55, NS
Urea (mmol/L)	6.3 ± 0.2	7 ± 0.2[a]	18.87 ± 5.27	6.5 ± 0.3	7.5 ± 0.87[a]	28.93 ± 7.06, NS
Creatinine (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 ($\approx 23\%$) and fibrinogen ($\approx 20\%$). 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 ($\approx 17\%$ and $\approx 5\%$, 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.

REFERENCES

Balkau B, Deanfield JE, Despre S, *et al.* 2007. International Day for the Evaluation of Abdominal Obesity (IDEA): a study of waist circumference, cardiovascular disease, and diabetes mellitus in 168,000 primary care patients in 63 countries. *Circulation* **116**: 1942–1951.

Chen D, Daniel KG, Kuhn DJ, *et al.* 2004. Green tea and tea polyphenols in cancer prevention. *Front Biosci* **9**: 2618–2631.

Crujeiras AB, Parra MD, Rodríguez MC, Martínez de Morentin BE, Martínez JA. 2006. A role for fruit content in energy-restricted diets in improving antioxidant status in obese women during weight loss. *Nutrition* **22**: 593–599.

Dai SX, Zou Y, Feng YL, Liu HB, Zheng XB. 2012. Baicalin down-regulates the expression of macrophage migration inhibitory factor (MIF) effectively for rats with ulcerative colitis. *Phytother Res***26**(4): 498–504.

Dallas C, Gerbi A, Tenca G, Juchaux F, Bernard FX. 2008. 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). *Phytomedicine* **15**: 783–792.

Dallas C, Laureano O. 1994a. Effects of pH, sulphur dioxide, alcohol content, temperature and storage time on colour composition of a young Portuguese red table wine. *J Sci Food Agric* **65**: 477–485.

Dallas C, Laureano O. 1994b. Effect of SO_2 on the extraction of individual anthocyanins and colored matter of three Portuguese grape varieties during winemaking. *Vitis* **33**: 41–47.

De Ferranti S, Mozaffarian D. 2008. The perfect storm: obesity, adipocyte dysfunction, and metabolic consequences. *Clin Chem* **54**: 945–955.

Décordé K, Teissèdre PL, Sutra T, Ventura E, Cristol JP, Rouanet JM. 2009. Chardonnay grape seed procyanidin extract supplementation prevents high-fat diet-induced obesity in hamsters by improving adipokine imbalance and oxidative stress markers. *Mol Nutr Food Res* **53**: 659–666.

Dembinska-Kiec A, Mykkänen O, Kiec-Wilk B, Mykkänen H. 2008. Antioxidant phytochemicals against type 2 diabetes. *Br J Nutr* **99** (ESuppl 1): ES109–ES117.

Fain JN, 2010. Release of inflammatory mediators by human adipose tissue is enhanced in obesity and primarily by the nonfat cells: a review. *Mediators Inflamm* **2010**: 513948.

Festa A, D'Agostino R Jr, Williams K, *et al.* 2001. The relation of body fat mass and distribution to markers of chronic inflammation. *Int J Obes Relat Metab Disord* **25**: 1407–1415.

Frankel EN, Kanner J, German JB, Parks E, Kinsella JE. 1993. Inhibition of oxidation of human low-density lipoprotein by phenolic substances in red wine. *Lancet* **341**: 454–457.

Fujioka K, Greenway F, Sheard J, Ying Y. 2006. The effects of grapefruit on weight and insulin resistance: relationship to the metabolic syndrome. *J Med Food* **9**: 49–54.

Furukawa S, Fujita T, Shimabukuro M, *et al.* 2004. Increased oxidative stress in obesity and its impact on metabolic syndrome. *J Clin Invest* **114**: 1752–1761.

Goldwasser J, Cohen PY, Yang E, Balaguer P, Yarmush ML, Nahmias Y. 2010. Transcriptional regulation of human and rat hepatic lipid metabolism by the grapefruit flavonoid naringenin: role of PPARalpha, PPARgamma and LXRalpha. *PLoS One* **5**(8): e12399.

Gorinstein S, Leontowicz H, Leontowicz M, *et al.* 2005. Changes in plasma lipid and antioxidant activity in rats as a result of naringin and red grapefruit supplementation. *J Agric Food Chem* **53**: 3223–3228.

Gorinstein S, Yamamoto K, Katrich E, *et al.* 2003. Antioxidative properties of Jaffa sweeties and grapefruit and their influence on lipid metabolism and plasma antioxidative potential in rats. *Biosci Biotechnol Biochem* **67**: 907–910.

Green MS, Heiss G, Rifkind BM, Cooper GR, Williams OD, Tyroler HA. 1985. The ratio of plasma high-density lipoprotein cholesterol to total and low-density lipoprotein cholesterol: age-relates changes and race and sex differences in selected North American populations. The lipid Research Clinics Program Prevalence Study. *Circulation* **72**: 93–104

Kaysen GA, Kotanko P, Zhu F, *et al.* 2009. Relationship between adiposity and cardiovascular risk factors in prevalent hemodialysis patients. *J Ren Nutr* **19**: 357–364.

Kim JA, Park HS, Kang SR, *et al.* 2012. Suppressive effect of flavonoids from Korean *Citrus aurantium* L. on the expression of inflammatory mediators in L6 skeletal muscle cells. *Phytother Res*. **26**(12): 1904–12.

Kou X, Shen K, An Y, Qi S, Dai WX, Yin Z. 2011. Ampelopsin inhibits H_2O_2-induced apoptosis by ERK and Akt signaling pathways and up-regulation of heme oxygenase-1. *Phytother Res* **26**(7):988–994.

Kuppusamy UR, Das NP. 1992. Effects of flavonoids on cyclic AMP phosphodiesterase and lipid mobilization in rat adipocytes. *Biochem Pharmacol* **44**: 1307–1315.

Laughton MJ, Evans PJ, Moroney MA, Hoult JR, Halliwel B. 1991. Inhibition of mammalian 5-lipoxygenase and cyclo-oxygenase by flavonoids and phenolic dietary additives. Relationship to antioxidant activity and to iron ion-reducing ability. *Biochem Pharmacol* **42**: 1673–1681.

Mansoor TA, Ramalho RM, Luo X, Ramalhete C, Rodrigues CM, Ferreira MJ. 2011. Isoflavones as apoptosis inducers in human hepatoma HuH-7 cells. *Phytother Res* **25**(12):1819–1824.

Moghaddam G, Ebrahimi SA, Rahbar-Roshandel N, Foroumadi A. 2012. Antiproliferative activity of flavonoids: influence of the sequential methoxylation state of the flavonoid structure. *Phytother Res* **26**(7):1023–1028.

Moon J, Lee SM, Do HJ, Cho Y, Chung JH, Shin MJ. 2012. Quercetin up-regulates LDL receptor expression in HepG2 cells. *Phytother Res* **26**(11): 1688–1694.

Mulvihill EE, Allister EM, Sutherland BG, *et al.* 2009. Naringenin prevents dyslipidemia, apolipoprotein B overproduction, and hyperinsulinemia in LDL receptor-null mice with diet-induced insulin resistance. *Diabetes* **58**: 2198–2210.

Mushtaq MU, Gull S, Abdullah HM, Shahid U, Shad MA, Akram J. 2011. Waist circumference waist–hip ratio and waist–height ratio percentiles and central obesity among Pakistani children aged five to twelve years. *BMC Pediatr* **11**: 105

Nguyen XM, Lane J, Smith BR, Nguyen NT. 2009. Changes in inflammatory biomarkers across weight classes in a representative US population: a link between obesity and inflammation. *J Gastrointest Surg* **13**: 1205–1212.

Orgogozo JM, Dartigues JF, Lafont S, *et al.* 1997. Wine consumption and dementia in the elderly: a prospective community study in the Bordeaux area. *Rev Neurol (Paris)* **153**: 185–192.

Rodríguez-Rodríguez E, Perea JM, López-Sobaler AM, Ortega RM. 2009. Obesity, insulin resistance and increase in adipokines levels: importance of the diet and physical activity. *Nutr Hosp* **24**: 415–421.

Roza JM, Xian-Liu Z, Guthrie N. 2007. Effect of citrus flavonoids and tocotrienols on serum cholesterol levels in hypercholesterolemic subjects. *Altern Ther Health Med* **13**: 44–48.

Seito LN, Ruiz AL, Vendramini-Costa D, *et al.* 2011. Antiproliferative activity of three methoxylated flavonoids isolated from *Zeyheria montana* Mart. (Bignoniaceae) leaves. *Phytother Res*. **25**(10):1447–1450.

Shen X, Tang Q, Wu J, Feng Y, Huang J, Cai W. 2009. Effect of vitamin E supplementation on oxidative stress in a rat model of diet-induced obesity. *Int J Vitam Nutr Res* **79**: 255–263.

Singh RB, Ghosh S, Beegom R, *et al.* 1998. Prevalence and determinants of central obesity and age-specific waist/hip ratio of people in five cities: the Indian Women's Health Study. *J Cardiovasc Risk* **5** (2): 73–77.

Thomas EL, Collins AL, McCarthy J, Fitzpatrick J, Durighel G, Goldstone AP, Bell JD. 2010. Estimation of abdominal fat compartments by bioelectrical impedance: the validity of the ViSCAN measurement system in comparison with MRI. *Eur J Clin Nutr* **64**: 525–533.

Vaidya H, Prajapati A, Rajani M, Sudarsanam V, Padh H, Goyal RK. 2012. Beneficial effects of swertiamarin on dyslipidaemia in streptozotocin-induced type 2 diabetic rats. *Phytother Res* **26**(8): 1259–1261.

Yang SH, Liao PH, Pan YF, Chen SL, Chou SS, Chou MY. in press. The novel p53-dependent metastatic and apoptotic pathway induced by vitexin in human oral cancer OC2 cells. *Phytother Res*. doi:10.1002/ptr.4841.

Zhang F, Wang H, Wu Q, *et al.* 2012. Resveratrol protects cortical neurons against microglia-mediated neuroinflammation. *Phytother Res* **27**: 344–349.

Zhang H, Zhang C. 2010. Adipose "talks" to distant organs to regulate insulin sensitivity and vascular function. *Obesity* **18**: 2071–2076.

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