Svetol®, green coffee extract, induces weight loss and increases the lean to fat mass ratio in volunteers with overweight problem

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ABSTRACT

In order to test the effects of Svetol®, a green coffee extract rich in chlorogenic acids with specific ratio between 5-caffeoylquinic acid and others caffeoylquinic acid isomers, on weight loss, 50 volunteers with body mass index superior to 25 were selected. They were randomized in two groups, control group (n = 20) receiving placebo, treated group (n = 30) receiving Svetol®. Each volunteer took one capsule of Svetol® or placebo twice a day with main meal, for 60 days. Changes in weight, body mass index (BMI), Muscle Mass/Fat Mass ratio (MM/FM) and self-evaluation of physical aspect were recorded at T0 and T60. After 60 days of treatment, a mean reduction in weight of 4.97 +/- 0.32 kg (5.7%) was observed in the Svetol® group compared to control group in which the mean reduction was 2.45 +/- 0.37 kg (2.9%) ($p < 0.001$). Consequently, body mass index decreased significantly in Svetol® group compared to control group. Moreover, MM/FM ratio was increased significantly in Svetol® group compared to control group: 4.1 +/- 0.7% *vs* 1.6 +/- 0.6 respectively ($p = 0.01$). The significant decrease of weight, boby mass index and fat mass showed that Svetol® is able to exacerbate effect of a bland low caloric diet in volunteers who have overweight. This effect could be explained by increasing the consumption of fatty deposits, as shown by change in the MM/FM ratio, and by preventing them from being accumulated.
To conclude, Svetol® could be used to aid the dietetics prescription in a useful and positive manner.

Key words: chlorogenic acids, Svetol®, weight, muscle mass/fat mass ratio

INTRODUCTION

Hydroxycinnamic acids are one of the major classes of phenolic compounds. They are present in a large variety of fruits and vegetables [1, 2]. The major representative of hydroxycinnamic acids in food is caffeic acid. It largely occurs conjugated with quinic acid as in chlorogenic acid (5-caffeoylquinic acid) **(Figure 1)**. Coffee, one of the most widely consumed beverages in the world, is the major dietary source of chlorogenic acids.
Chlorogenic acid has antioxidant properties showed by its ability to scavenge various free radicals when tested *in vitro* [3-5]. Moreover, chlorogenic acid reduces glucose uptake by favouring the dissipation of the Na^+ electrochemical gradient [6] and inhibits the activity of hepatic glucose-6-phosphatase which is implicated in glucose homeostasis [7, 8].
In vivo, when ingested under coffee form, chlorogenic acid increases the plasma antioxidant capacity [9]. Chlorogenic acid is also able to reverse the prooxidant effects of drugs such as paraquat [10] and have been reported to prevent different cancers and cardiovascular diseases in several experimental studies on animal models [11-15]. Therefore, we hypothesized that chlorogenic acid modulating glucose metabolism and decreasing oxidative stress could limit overweight, obesity development and secondary diseases

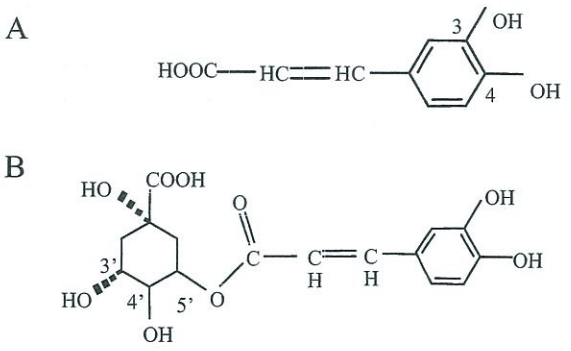

Figure 1: Chemical structure of caffeic (A) and chlorogenic acids (B)

associated with as type 2 diabetes mellitus or cardiovascular problems.

The aim of the present work was to evaluate if Svetol®, a green coffee extract, could decrease overweight in volunteers who had body mass index (BMI) superior to 25.

SUBJECTS AND METHODS

Chemicals

Svetol®, decaffeinated green coffee extract, were purchased from Berkem® SA (Gardonne, France).

Subjects

Fifty volunteers of both sexes, aged from 19 to 75, were assigned at random to the group of 30 in active treatment, and 20 in placebo treatment. The participants of both groups were homogeneous in weight and muscle mass/fat mass (MM/FM) ratio, characterized by an overweight problem, BMI superior to 25, and the acceptance of a bland low caloric diet. Exclusion criteria were as follows: acute or chronic gastro-intestinal pathologies; gastro-intestinal infections and/or parasitosis; severe hyper-tension (P.A. above 120 mm.); gastro-intestinal cancers; serious or chronic metabolic pathologies; big drinkers; assumption of products for the control of weight and glycaemia; a known intolerance to any of the components of the product under examination.

Svetol® supplementation

The product was prepared in jars of 30 capsules absolutely identical. Composition of the product (active capsules) under experimentation was given in **table 1.** Placebo capsules contained the same components as the active capsule, Svetol® was substituted by an identical quantity of maltodextrin (200 mg).

	mg/capsule
Svetol®	200
Starch	0.04
Magnesium stereate	0.015
Silica micronized	0.008
White gelatine	0.087

Table 1: Composition of the Svetol® capsule

Study design

Volunteers took one capsule with each main meal, twice a day, for 60 days. Every participant was given treatment sufficient for 30 days (two jars) when they began the study (T0) and the rest (two jars) at the T30 day.

Data collection and parameters of evaluation

In the course of the first check-up, the following data were gathered: age, height, sex, weight, BMI, MM/FM ratio, self-evaluation of physical aspect.
Changes in weight, BMI, MM/FM ratio, self-evaluation of physical aspect were recorded again at T60. An evaluation of compliance and verification of the presence of side effects was undertaken at T30 and T60.
MM/FM ratio was determined by Bioelectric Impedance Analysis. Self-evaluation of physical aspect was done by scale from 0 = very negative to 10 = excellent.

Evaluation of effectiveness, compliance and tolerability

At the end of treatment, effectiveness, compliance and tolerability were verified with regard to the end-points by comparing the changes in the data recorded at T60 to those at T0.
Therefore, changes of weight, BMI, MM/FM ratio, self-evaluation of physical aspect in the active group were compared to those recorded in the placebo group.
The effectiveness was based on those participants who completed the study. Compliance and tolerability were based on all participants.

Statistical analysis

Numerical values are mean +/- SEM (n = 20 for control group or 30 for treated group). Data were entered into Instat statistical analysis program (Instat, San Diego, CA). Student t-test (parametric test) or Mann-Whitney test (non-parametric test) determined the difference between values. Differences with $p \leq 0.05$ were considered significant.

RESULTS

Weight loss and Body Mass Index

After 60 days of treatment, a mean reduction in weight of 4.97 +/- 0.32 kg (-5.7 +/- 0.3%) was observed in the Svetol® group compared to control group in which the mean reduction was 2.45 +/- 0.37 kg (-2.9 +/- 0.4%). These means are significantly different ($p < 0.001$) **(Figure 2A)**. Consequently, body mass index decreased significantly in Svetol® group compared to control group (-1.9 +/- 0.1 kg/m^2 *vs* -0.9 +/- 0.1 kg/m^2 ; $p < 0.001$) **(Figure 2B)**.

Muscle Mass/ Fat Mass ratio

In Svetol® group, MM/FM ratio was increased significantly compared to control group: +4.1 +/- 0.7% *vs* +1.6 +/- 0.6 respectively ($p = 0.01$) **(Figure 3)**.

Self-evaluation of physical aspect

No significant difference about the appearance was observed between both groups at T60 (6.6 +/- 1.05 *vs* 6.5+/- 1.31 for placebo and Svetol® groups respectively) but both groups observed an amelioration of the physical appearance between T0 and T60 ($p < 0.05$ for each group).

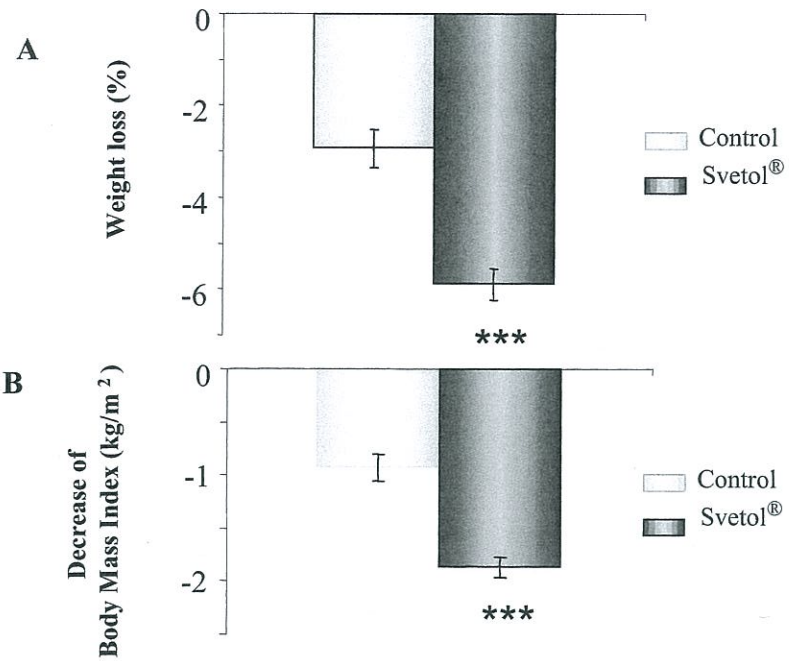

Figure 2: (A) Weight loss (%) and (B) decrease of BMI (kg/m^2) after 60 days treatment. Values are means +/- SEM, n = 20 for control group, n = 30 for Svetol® group. Means are significantly different (*, $p < 0.001$ vs. control group).

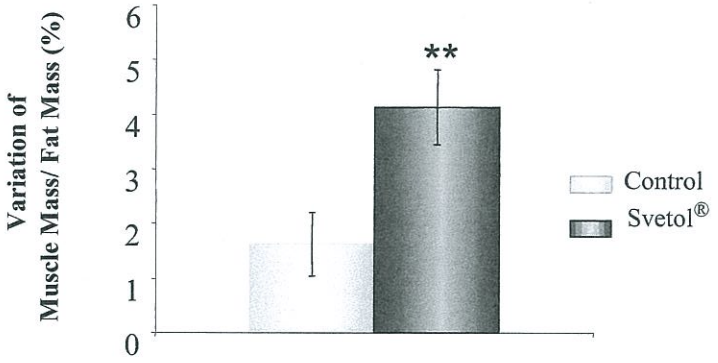

Figure 3: Variation of muscle Mass/ fat Mass ratio after 60 days of treatment (%). Values are means +/- SEM, n = 20 for control group, n = 30 for Svetol® group. Means are significantly different (**, $p = 0.01$ vs. control group).

DISCUSSION

Obesity is a serious public health problem [16]. Overweight and obesity are the cause of health problems of varying degrees of seriousness: asthenia, osteo-arthicular, psychological and cardio-vascular problems.

The reality is that this condition has a negative impact on the quality of life, and in the case of obesity, it can even lead to a reduction in life expectancy.

With the exception of serious neuro-endocrine pathologies the problem is caused mainly by lifestyle. A rational diet in quantity and quality, combined with some physical exercise can help to obtain some loss of weight. A change in lifestyle is not simple so in order to reach the desired goal of controlling weight pharmaceutical products are used as well as nutritional supplements with various compositions, fat burners, all with the aim of contrasting the lack of balance between the number of calories introduced and the number of those consumed which leads to overweight. There is a relationship between the amount of carbohydrates in the diet and the amount of fats in the adipose reserves since the carbohydrates are responsible for most of the calories introduced [17] and the intake of sugars reduces energy consumption. In normal production and activity of insulin, the calories introduced are burnt up without transforming the lipids into stock. On the other hand, if the amount of glucose present in the blood is in excess with regards to its use and to the hepatic glycogenesis, this excess glucose (owing to the insulin which has been increased by the hyperglycemia) enters into the adipocytes where it is stored as fat reserves [18]. The consequences are: (i) the fat reserves are not used to produce energy; (ii) an increase of adipocytes.

In diets the lower quantity of carbohydrates consumed is a way to "force" the organism to burn up the fat which has been deposited in the adipocytes and therefore to lose weight. It is possible to improve the effect of the lower amounts of carbohydrates consumed by exploiting the hepatic activity to regulate the glycemia level. When glucose level in the blood is lower than 1g/L, the liver synthesized glucose-6-phosphate (G6P) by an hexokinase, hydrolysed G6P by means of a glucose-6-phosphatase and released glucose into the bloodstream. It's glycogenolysis. If this sequence is interrupted the fatty deposits do not increase, but are instead used for the production of energy.

The aim of the present work was to evaluate if Svetol®, green coffee extract concentrated in chlorogenic acids with specific ratio between 5-caffeoylquinic acid and others caffeoylquinic acid isomers, could decrease overweight in volunteers by fat burning action as suggested by *in vitro* studies showing inhibition of the activity of hepatic glucose-6-phosphatase by 5-caffeoylquinic acid [7, 8].

The significant decrease of weight and fat mass showed that Svetol® is able to exacerbate effect of a bland low caloric diet in volunteers who had overweight. This effect could be explained by increasing the consumption of fatty deposits, as shown by change in the MM/FM ratio, and by preventing them from being accumulated.

From results presented here and bibliography, Svetol®'s mechanism could be proposed: first of all, associated with the diet, it inhibits glucose absorption in the small intestine[6]. In addition, by inhibiting the activity of glucose-6-phosphatase [7, 8], it limits the release of glucose into the general circulation [19, 20] and therefore limits

insulinemia. This mechanism engenders two results: (i) less fatty deposits in the adipose tissue and a more difficult access into the adipose cells owing to a reduction in insulin activity; (ii) consumption of fat reserves, where there is a lack of glucose, as a source of energy at the disposition of the organism and therefore, as in the previous case, a case of loss of weight.

However, mechanism proposed depends on bioavailability of chlorogenic acid. Recently, fate and metabolism of chlorogenic acid (5-caffeoylquinic acid) in the gastro-intestinal tract of rats were explored to determine the form under which this ester of caffeic acid is absorbed through the different parts of the gut barrier.

After analysis of the different gastro-intestinal contents, it appeared that chlorogenic acid is stable in the stomach and the small intestine but cleaved into caffeic acid in the caecum by the microflora [21]. Consequently, stability of chlorogenic acid in the small intestine is coherent with glucose absorption inhibition in this part of the gut. Moreover, whereas it was shown that chlorogenic acid was hydrolysed into enterocytes before secretion on the serosal side [22], it was absorbed under intact form from the stomach **[21]** and found in gastric vein and aorta without conjugation (glucuronidation, sulfation or methylation). This result suggests that chlorogenic acid is able to rejoin the liver without modification, which is in accordance with its activity of hepatic glucose-6-phosphatase inhibition.

Thus, chlorogenic acid bioavailability studies supported Svetol®'s mechanism proposed.

To conclude, Svetol® has demonstrated its validity and could be used to aid the dietetics prescription in a useful and positive manner.

BIBLIOGRAPHY

1. Clifford, M.N., Chlorogenic acids and other cinnamates - Nature, occurence and dietary burden. J. Sci. Food. Agric., 1999. 79: p. 362-372.
2. Manach, C., et al., Polyphenols - Food sources and bioavailability. Am. J. Clin. Nutr., 2004. 79(5): p. 727-747.
3. Ohnishi, M., et al., Inhibitory effects of chlorogenic acids on linoleic acid peroxidation and haemolysis. Phytochemistry, 1994. 36(3): p. 579-583.
4. Rice-Evans, C.A., N.J. Miller, and G. Paganga, Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Rad. Biol. Med., 1996. 20(7): p. 933-956.
5. Foley, S., et al., Singlet oxygen quenching and the redox properties of hydroxycinnamic acids. Free Radic Biol Med, 1999. 26(9-10): p. 1202-8.
6. Welsch, C.A., P.A. Lachance, and B.P. Wasserman, Dietary phenolic compounds: inhibition of sodium-dependant D-glucose uptake in rat intestinal brush border membrane vesicles. J. Nutr., 1989. 119(11): p. 1698-1704.
7. Arion, W.J., et al., Chlorogenic acid and hydroxynitrobenzaldehyde: new inhibitors of hepatic glucose 6-phosphatase. Arch Biochem Biophys, 1997. 339(2): p. 315-22.
8. Hemmerle, H., et al., Chlorogenic acid and synthetic chlorogenic acid derivatives: novel inhibitors of hepatic glucose-6-phosphate translocase. J Med Chem, 1997. 40(2): p. 137-45.
9. Natella, F., et al., Coffee drinking influences plasma antioxidant capacity in humans. J Agric Food Chem, 2002. 50(21): p. 6211-6.
10. Tsuchiya, T., O. Suzuki, and K. Igarashi, Protective effects of chlorogenic acid on paraquat-induced oxidative stress in rats. Biosci Biotechnol Biochem, 1996. 60(5): p. 765-8.
11. Mori, H., et al., Inhibitory effect of chlorogenic acid on methylazoxymethanol acetate-induced carcinogenesis in large intestine and liver of hamsters. Cancer Lett, 1986. 30(1): p. 49-54.
12. Tanaka, T., et al., Inhibition of 4-nitroquinoline-1-oxide-induced rat tongue carcinogenesis by the naturally occurring plant phenolics caffeic, ellagic, chlorogenic and ferulic acids. Carcinogenesis, 1993. 14(7): p. 1321-5.
13. Tanaka, T., et al., Inhibitory effects of chlorogenic acid, reserpine, polyprenoic acid (E-5166), or coffee on hepatocarcinogenesis in rats and hamsters. Basic Life Sci, 1990. 52: p. 429-40.
14. Zhou, J., et al., Protective Effect of Chlorogenic Acid on Lipid-Peroxidation Induced in the Liver of Rats by Carbon-Tetrachloride or Co-60-Irradiation. Journal of Clinical Biochemistry and Nutrition, 1993. 15(2): p. 119-125.
15. Suzuki, A., et al., Green coffee bean extract and its metabolites have a hypotensive effect in spontaneously hypertensive rats. Hypertension Research, 2002. 25(1): p. 99-107.
16. Guy-Grand, B., Obésité: génétique, physiopathologie et thérapeutique à l'aube d'une révolution conceptuelle. 2005: Institut Pasteur, Centre d'information scientifique.

17. Liotta, S., Obesita e le adiposità localizzate. 1974, Roma.
18. Anthoni, C. and N. Kolthoff, Fondamenti di anatomia e fisiologia dell'uomo, C.E. Ambrosina, Editor. 1977: Milano. p. 433.
19. Herling, A.W., et al., Pharmacodynamic profile of a novel inhibitor of the hepatic glucose-6- phosphatase system. Am J Physiol, 1998. 274(6 Pt 1): p. G1087-93.
20. Simon, C., et al., Upregulation of hepatic glucose 6-phosphatase gene expression in rats treated with an inhibitor of glucose-6-phosphate translocase. Arch Biochem Biophys, 2000. 373(2): p. 418-28.
21. Lafay, S., et al., Chlorogenic acid is absorbed in its intact form in the stomach of rats. J. Nutr, 2006. 136(5); 1192-1197
22. Lafay, S., et al., Absorption and metabolism of caffeic acid and chlorogenic acid in the small intestine of rats. Br. J. Nutr, 2006. under press.

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Contribution of Chlorogenic Acids to the Inhibition of Human Hepatic Glucose-6-phosphatase Activity in Vitro by Svetol, a Standardized Decaffeinated Green Coffee Extract

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Glucose-6-phosphatase (Glc-6-Pase) is a multicomponent system that exists primarily in the liver and catalyzes the terminal step in gluconeogenesis and glycogenolysis. Several studies have attempted to identify synthetic or natural compounds that inhibit this enzyme complex for therapeutic use in regulating blood glucose and type 2 diabetes. For this paper an in vitro structure−activity relationship study of several natural chlorogenic acids was conducted, and the active components of the natural decaffeinated green coffee extract Svetol were identified. Glucose-6-phosphate (Glc-6-P) hydrolysis was measured in the presence of Svetol or chlorogenic acids in intact human liver microsomes. Svetol significantly inhibited Glc-6-P hydrolysis in intact human liver microsomes in a competitive manner, and it was determined that chlorogenic acids (caffeoylquinic acids and dicaffeoylquinic acids) were the chief compounds mediating this activity. In addition, the structure−activity analysis showed that variation in the position of the caffeoyl residue is an important determinant of inhibition of Glc-6-P hydrolysis. This inhibition by Svetol contributes to its antidiabetic, glucose-lowering effects by reducing hepatic glucose production.

KEYWORDS: Decaffeinated green coffee extract; Svetol; chlorogenic acids; glucose-6-phosphatase

INTRODUCTION

Coffee is one of the world's most popular beverages. The numerous beneficial health effects of coffee consumption have received significant scientific attention recently, because the results of epidemiological and experimental studies suggest that drinking coffee regularly helps prevent several chronic diseases, especially metabolic disorders, such as type 2 diabetes (*1*−*3*). Extensive investigations have revealed that most of these effects are attributed to the chlorogenic acids (CGAs) in coffee (*4*).

Green (or raw) coffee is a significant source of CGAs in nature (5−12 g/100 g) (*5*). In green coffee, the primary CGAs are 3-, 4-, and 5-caffeoylquinic acids (CQA) and 3,4-, 3,5-, and 4,5-dicaffeoylquinic acids (diCQAs). Caffeoylferuloylquinic acids (FQAs) are minor CGA compounds also found in green coffee (*6*).

Weight loss is linked to the capacity of coffee to prevent type 2 diabetes; one prospective epidemiological study found that the consumption of coffee lowered the risk for diabetes, but only in participants who had lost weight (*7*). Two clinical studies distinguished the effects of caffeinated and decaffeinated coffee (*8*, *9*) and suggested that there are noncaffeine compounds in coffee, such as CGAs, that enhance glucose tolerance and insulin sensitivity. In a recent study, Dellalibera et al. showed that chronic consumption of Svetol, a decaffeinated green coffee extract that has high CGA content, decreased weight and increased lean/fat ratios in overweight volunteers (*10*).

One proposed mechanism of such effects is inhibition of glucose-6-phosphatase (Glc-6-Pase; EC 3.1.3.9), which forces lipids to be used as energy to compensate for the decrease in glucose release from glycogenolysis. Liver Glc-6-Pase is a multicomponent system that catalyzes the final step of hepatic glucose production, that is, the hydrolysis of glucose-6-phosphate (Glc-6-P) from glycogen breakdown or gluconeogenesis. The active site of Glc-6-Pase is in the lumen of the endoplasmic reticulum (ER); therefore, transporter proteins are required to shuttle Glc-6-P into this compartment and expel glucose and phosphate (*11*).

Glc-6-P hydrolysis appears to involve a Glc-6-P translocase (Glc-6-PT), which transports Glc-6-P across the ER, and a catalytic subunit, located on the luminal side of the ER (*12*). 5-CQA is a highly specific inhibitor of Glc-6-Pase (*13*), and several analogues of 5-CQA that effect greater inhibition (e.g., S3483) have been synthesized; they increase the latency of Glc-6-Pase by reducing its activity in intact microsomes or in the intact ER in situ (*14*).

The aim of this study was to determine the inhibitory activity of Svetol, a decaffeinated green coffee extract that has a specific ratio between 5-CQA and other CGAs, on Glc-6-P hydrolysis in intact human liver microsomes. In addition, we report the

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Table 1. Chlorogenic Acid Content in Svetol[a]

compound	typical content in Svetol (%)	sample (%)
3-CQA	6.53 ± 0.54	6.61
4-CQA	7.31 ± 0.43	7.66
5-CQA	14.72 ± 1.07	13.83
3,4-diCQA	3.57 ± 0.54	3.34
3,5-diCQA	2.38 ± 0.08	2.38
4,5-diCQA	4.22 ± 0.15	4.15
3-FQA	1.28 ± 0.11	1.30
4-FQA	1.50 ± 0.23	1.87
5-FQA	3.39 ± 0.36	3.39
3,4-caffeoylferuloylquinic acid	0.67 ± 0.06	0.77
3,5-caffeoylferuloylquinic acid	0.30 ± 0.02	0.31
4,5-caffeoylferuloylquinic acid	0.30 ± 0.29	0.81
caffeoyltryptophan	1.00 ± 0.80	1.23

[a] Typical content in five industrial batches (mean ± SD) and the sample used in this study (batch 252/10/A9; Naturex). Data are expressed as 5-CQA equivalents.

inhibitory effects of a series of structurally related compounds in Svetol, such as caffeoylquinic acids and dicaffeoylquinic acids.

MATERIALS AND METHODS

Chemicals. Svetol (ref. GA501071, batch 252/10/A9) was supplied by Naturex (Avignon, France). Ascorbic acid, cacodylic acid, D-glucose 6-phosphate sodium salt, 5-CQA, ammonium molybdate tetrahydrate, potassium phosphate, and sodium dodecyl sulfate were purchased from Sigma (Saint Quentin Fallavier, France). Pooled human liver microsomes were obtained from BD Biosciences (Le Pont le Claix, France) and stored at −80 °C until use. Standards for caffeoylquinic and dicaffeoylquinic acids were supplied by Chengdu Biopurify Phytochemicals LTD (Chengdu, China).

HPLC Analysis of CGAs in Svetol. Analysis of CGAs in Svetol was performed using the HPLC−diode array detector gradient system (Agilent 1100 series). The chromatographic analysis was conducted with a Zorbax Eclipse XDBC$_{18}$ 4.6 × 50 mm column (1.8 μm). The solvents were H_2O/acetic acid (96:4, v/v) as solvent A and methanol/acetonitrile/acetic acid (60:10:2, v/v/v) as solvent B, at a flow rate of 1.2 mL/min with the following gradient: 5% B (0−1 min), 5−15% B (1−4 min), and 15−70% B (4−25 min).

Quantification was performed at optimal wavelengths (330 nm) for the CGAs during chromatographic separation. Samples were filtered (0.45 μm), and 2 μL was injected directly. The standard deviation for three analyses of the same sample was < 5% for all compounds.

Measurement of Glc-6-Pase Activity in Microsomes. Microsomal Glc-6-Pase activity was measured on the basis of the rate of release of phosphate under the assay conditions that were described by Wallert et al. (*15*). The enzyme assays were performed at 37 °C in a final volume of 320 μL, containing 100 mM cacodylic acid, pH 6.5, and concentrations of the substrate Glc-6-P ranging from 2 to 10 mM.

The reaction was started by adding intact microsomes and was stopped with the addition of 3.2 mL of colorimetric reagent [9 volumes of molybdate (0.42% ammonium molybdate in 1 N H_2SO_4), 2 volumes of 5% SDS, and 1 volume of 10% ascorbic acid, freshly prepared and stored on ice for a maximum of 6 h]. All samples were incubated for 30 min at 45 °C, and the absorbance of the phosphate−molybdate complex was measured at 820 nm.

Microsomal intactness was quantified by measuring Man-6-Pase activity (*16*). In a preliminary study, Glc-6-Pase activity in intact human liver microsomes was determined on the basis of microsomal protein concentration and incubation time to obtain optimal experimental conditions, that is, 100 μg of microsomal proteins and 5 min of incubation (data not shown).

Preparation of Test Compounds. Stock solutions of test compounds were prepared in ultrapure water (pH 6.5) and diluted with assay reagent to the final concentrations.

Data Analysis. Enzymatic activity was expressed as micromoles of phosphate released per minute per milligram of protein. Results were expressed as means ± standard deviation (SD) of three independent experiments. Percentage of inhibition of Glc-6 Pase activity was calculated by dividing the initial rate of reaction in microsomes that were treated with individual compounds by the initial rate of reaction in untreated microsomes. The contribution of individual CGAs to total inhibition by Svetol was calculated on the basis of their concentrations in Svetol and their own inhibition values by dividing the percentage of inhibition of each CGA by the percentage of inhibition of 0.6 mM total CGAs from Svetol.

Statistical analysis was performed using an ANOVA test followed by a post hoc Tukey test under a normality assumption (Shapiro Wilk) or Kruskall Wallis nonparametric test followed by Bonferroni adjusted Mann−Whitney test otherwise; $p < 0.05$ was considered to be significant.

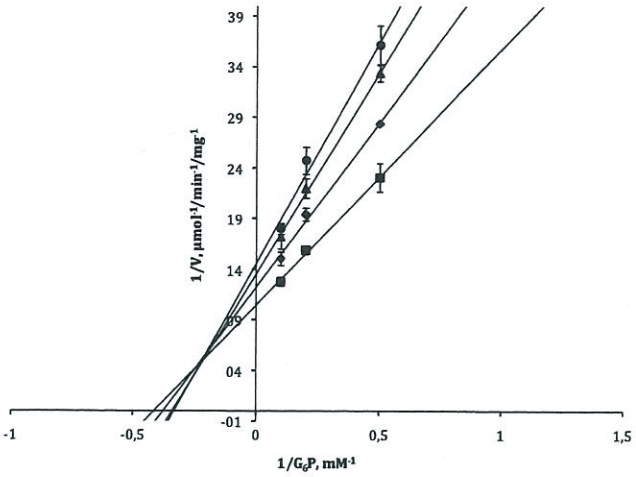


Figure 1. Double-reciprocal or Lineweaver−Burk plot of inhibition of Glc-6-P hydrolysis by Svetol in human liver microsomes. Reaction mixture (pH 6.5) contained 2.0−10.0 mM Glc-6-P with 0 (■), 0.2 (◆), 0.4 (▲), or 0.6 mM (●) Svetol. Each bar represents the mean ± SD of three measurements.

RESULTS

Determination of CGA Composition in Svetol. Svetol is a commercial unroasted and decaffeinated green *Coffea canephora* extract, standardized to contain >45% CGAs and >10% 5-CQA. **Table 1** lists the average contents and standard deviations of CGAs in five industrial batches that have been quantified as 5-CQA equivalents (batches 252/10/A9, H43/17/A8, H37/40/A9, 327/23/A9, and 324/44/A9; Naturex). The sample that we used contained high levels of total CGAs (47.66% of dry weight), with a specific ratio (0.3) between 5-CQA and total CGAs.

Inhibition of Glc-6-Pase Activity by Svetol. We tested whether Svetol could inhibit the hepatic Glc-6-Pase system by measuring enzymatic activity in human liver microsomes. These experiments were conducted with or without Svetol at final CGA concentrations of 0.2, 0.4, and 0.6 mM. The effect of Svetol on Glc-6-Pase activity was tested as a function of Glc-6-P substrate concentration (2−10 mM).

The double-reciprocal plots in **Figure 1** show that Svetol decreased V_m values in a dose-dependent manner, but K_m was unchanged. By Michaelis−Menten kinetics, Svetol inhibited Glc-6-P hydrolysis in human liver microsomes in a significant and competitive manner (**Table 2**), which is consistent with previous studies of 5-CQA in rat liver microsomes (*13*).

Inhibition of Glc-6-Pase Activity by CGAs. Because studies have demonstrated that 5-CQA and its synthetic analogues inhibit Glc-6-Pase (*13*, *14*, *17*), we investigated whether other CGAs in coffee possess the same functions as 5-CQA. Therefore, the selected CQAs and di-CQAs were studied at their respective concentrations in Svetol (0.6 mM total CGAs) with 2 mM Glc-6-P (i.e., below the apparent K_m) to facilitate detection of putative competitive inhibitors.

The percentages of inhibition of Glc-6-P hydrolysis of each compound and its contribution to the inhibitory activity of Svetol are shown in **Table 3**. Of the three CQAs in Svetol, 4-CQA inhibited Glc-6-P hydrolysis to the greatest extent (14% inhibition). In addition, 4-CQA contributed 40% of the inhibitory effect of Svetol.

4,5-diCQA effected similar inhibition as 4-CQA (13% inhibition) and contributed 35% of the inhibitory effect of Svetol.

We also examined inhibition by mixtures of CQAs and diCQAs (at their respective proportions in Svetol). When all CQAs and diCQAs were tested separately, we observed similar inhibition (approximately 20%). Moreover, when combined, the inhibition of Glc-6-P hydrolysis by 0.6 mM total CGAs from Svetol (36%) was recovered (35%), suggesting that no other compounds participate in Svetol-mediated inhibition.

Table 2. Kinetic Parameters of Glc-6-Pase in Human Liver Microsomes[a]

condition	V_{max} (μmol/min/mg of protein)	K_M (mM)
control	0.095 ± 0.002	2.41 ± 0.33
Svetol (0.2 mM)	0.082 ± 0.003*	2.65 ± 0.18
Svetol (0.4 mM)	0.074 ± 0.007*	2.96 ± 0.47
Svetol (0.6 mM)	0.068 ± 0.001*	2.99 ± 0.25

[a] Data are expressed as mean of triplicate ± SD. * indicates values that are significantly different from control ($P < 0.001$).

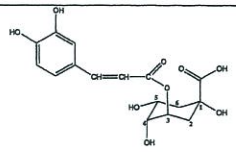
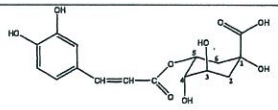
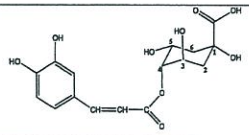
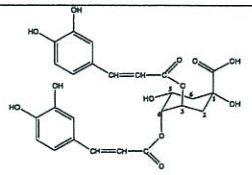
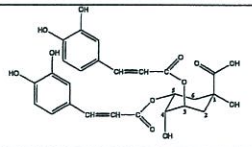
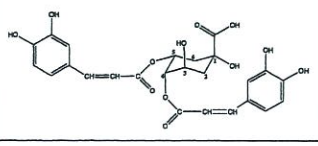
DISCUSSION

Starvation and diabetes cause a 2−3-fold increase in Glc-6-Pase activity in the liver (*18*, *19*), making this enzyme system a potential target for nutritional compounds that are intended, for example, to suppress hepatic glucose production to ameliorate diabetic hyperglycemia. Our study details the inhibition of Glc-6-P hydrolysis in intact human liver microsomes by Svetol; Svetol was found to be a competitive inhibitor of Glc-6-Pase in a dose-dependent manner.

Svetol is a decaffeinated green coffee extract that has a high CGA content and a specific ratio between CQAs and diCQAs. In this study, we showed that CQAs and diCQAs, at their respective concentrations in Svetol, have inhibitory effects similar to those of Svetol, suggesting that they are the compounds that are solely responsible for Svetol activity.

Our structure−activity analysis showed that variation in the position of the caffeoyl residue is important for the inhibition of Glc-6-P hydrolysis. Notably, two compounds (3-CQA and

Table 3. Structure, Percentage of Inhibition, and Contribution of Chlorogenic Acids to Glc-6-Pase Inhibition by Svetol[a]

Compound	Structure	Concentration tested (μM)	Percentage inhibition of G6Pase	Contribution (%)
3-CQA		110	0	0
5-CQA		160	9.2 ± 1.4	25
4-CQA		120	14.4 ± 1.2	40
3,4-diCQA		33	6.9 ± 4.3	19
3,5-diCQA		20	0	0
4,5-diCQA		38	12.8 ± 2.6	35
All CQA			18.1 ± 5.5	50
All di-CQA			22.7 ± 1.5	62
All CQA + all di-CQA			34.8 ± 4.0	96

[a] Each compound was tested at its naturally occurring concentration in 0.6 mM Svetol.

3,5-diCQA) were apparently ineffective in suppressing Glc-6-P hydrolysis, and greater inhibition was achieved with 4-CQA and 4,5-diCQA. This result suggests that the caffeoyl residue at position 3 has an unfavorable effect, whereas at position 4, it appears to be beneficial.

The observed 36% inhibition by Svetol should contribute to its antidiabetic, glucose-lowering effects by reducing hepatic glucose production. On the basis of these and other published results (*13*, *14*, *17*), we propose a mechanism by which Svetol acts. In combination with diet, it inhibits glucose absorption from the small intestine (*20*). Furthermore, by inhibiting Glc-6-Pase activity, Svetol could limit the release of glucose from glycogen into general circulation and prevent insulinemia, as reported in vivo with the chlorogenic acid derivative S3483 (*21*, *22*).

This mechanism, however, depends on the bioavailability of chlorogenic acid and its isomers. In rats, Lafay et al. showed that 5-CQA is not hydrolyzed in the stomach or small intestine but is absorbed in the stomach in its intact form and as caffeic and (iso)ferulic acids in the small intestine (*23*). Recently, Farah et al. (*24*) confirmed that CQA and diCQA are differentially absorbed and metabolized throughout the entire gastrointestinal tract. In addition, Farah et al. (*24*) also provide evidence that urination is not a major excretion pathway of intact CGA compounds and their metabolites.

In conclusion, our findings engender new research opportunities because they demonstrate the importance of the position of the caffeoyl residue in the inhibition of Glc-6-Pase by CGAs. Because few structure−activity relationship studies on the inhibition of Glc-6-Pase by synthetic analogues of 3-CQA exist (*14*, *17*), additional complementary studies will provide new tools for investigating the molecular structure and function of the Glc-6 Pase system.

ABBREVIATIONS USED

CGAs, chlorogenic acids; CQAs, caffeoylquinic acids; diCQAs, dicaffeoylquinic acids; FQAs, feruloylquinic acids; Glc-6-Pase, glucose-6-phosphatase; Glc-6-P, glucose-6-phosphate; ER, endoplasmic reticulum; Glc-6-PT, glucose 6-phosphate translocase.

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LITERATURE CITED

(1) Salazar-Martinez, E.; Willett, W. C.; Ascherio, A.; Manson, J. E.; Leitzmann, M. F.; Stampfer, M. J.; Hu, F. B. Coffee consumption and risk for type 2 diabetes mellitus. *Ann. Intern. Med.* **2004**, *140*, 1–8.

(2) Agardh, E.; Carlsson, S.; Ahlbom, A.; Efendic, S.; Grill, V.; Hammar, N.; Hilding, A.; Östenson, C. G. Coffee consumption, type 2 diabetes and impaired glucose tolerance in Swedish men and women. *J. Intern. Med.* **2004**, *255*, 645–652.

(3) Van Dam, R. M.; Feskens, E. J. M. Coffee consumption and risk of type 2 diabetes mellitus. *Lancet* **2002**, *360*, 1477–1478.

(4) Van Dijk, A. E.; Olthof, M. R.; Meeuse, J. C.; Seebus, E.; Heine, R. J.; Van Dam, R. M. Acute effects of decaffeinated coffee and the major coffee components chlorogenic acid and trigonelline on glucose tolerance. *Diabetes Care* **2009**, *32*, 1023–1025.

(5) Farah, A.; Donangelo, C. M. Phenolic compounds in coffee. *Braz. J. Plant Physiol.* **2006**, *18*, 23–36.

(6) Clifford, M. N. Chlorogenic acids and other cinnamates − nature, occurrence, dietary burden, absorption and metabolism. *J. Sci. Food Agric.* **2000**, *80*, 1033–1043.

(7) Greenberg, J. A.; Axen, K. V.; Schnoll, R.; Boozer, C. N. Coffee, tea and diabetes: the role of weight loss and cafeine. *Int. J. Obes.* **2005**, *29*, 1121–1129.

(8) Naismith, D. J.; Akinyanju, P. A.; Szanto, S.; Yudkin, J. The effect, in volunteers, of coffee and decaffeinated coffee on blood glucose, insulin, plasma lipids and some factors involved in blood clotting. *Nutr. Metab.* **1970**, *12*, 144–151.

(9) Battram, D. S.; Arthur, R.; Weekes, A.; Graham, T. E. The glucose intolerance induced by caffeinated coffee ingestion is less pronounced than that due to alkaloid caffeine in men. *J. Nutr.* **2006**, *136*, 1276–1280.

(10) Dellalibera, S. O.; Lemaire, B.; Lafay, S. Le Svétol, un extrait de café vert décaféiné, induit une perte de poids et augmente le ratio masse maigre sur masse grasse chez des volontaires en surcharge pondérale. *Phytotherapie* **2006**, *4*, 1–4.

(11) Csala, M.; Banhegyi, G.; Benedetti, A. Endoplasmic reticulum: a metabolic compartment. *FEBS Lett.* **2006**, *580*, 2160–2165.

(12) Mithieux, G. New knowledge regarding glucose-6 phosphatase gene and protein and their roles in the regulation of glucose metabolism. *Eur. J. Endocrinol.* **1997**, *136*, 137–145.

(13) Arion, W. J.; Canfield, W. K.; Ramos, F. C.; Schindler, P. W.; Burger, H. J.; Hemmerle, H.; Schubert, G.; Below, P.; Herling, A. W. Chlorogenic acid and hydroxynitrobenzaldehyde: new inhibitors of hepatic glucose 6-phosphatase. *Arch. Biochem. Biophys.* **1997**, *339*, 315–322.

(14) Hemmerle, H.; Burger, H. J.; Below, P.; Schubert, G.; Rippel, R.; Schindler, P. W.; Paulus, E.; Herling, A. W. Chlorogenic acid and synthetic chlorogenic acid derivatives: novel inhibitors of hepatic glucose-6-phosphate translocase. *J. Med. Chem.* **1997**, *40*, 137–145.

(15) Wallert, M. A.; Foster, J. D.; Scholnick, D. A.; Olmschenk, S. M.; Kuehn, B. J.; Provost, J. J. Kinetic analysis of glucose-6-phosphatase: an investigative approach to carbohydrate metabolism and kinetics. *Biochem. Mol. Biol. Educ.* **2001**, *29*, 199–203.

(16) Arion, W. J. Measurement of intactness of rat liver endoplasmic reticulum. *Methods Enzymol.* **1989**, *174*, 58–67.

(17) Arion, W. J.; Canfield, W. K.; Ramos, F. C.; Su, M. L.; Burger, H. J.; Hemmerle, H.; Schubert, G.; Below, P.; Herling, W. Chlorogenic acid analog S3483: a potent competitive inhibitor of the hepatic and renal glucose-6-phosphatase systems. *Arch. Biochem. Biophys.* **1998**, *351*, 279–285.

(18) Segal, H. L.; Washko, M. E. Studies of liver glucose 6-phosphatase. III. Solubilization and properties of the enzyme from normal and diabetic rats. *J. Biol. Chem.* **1959**, *234*, 1937–1941.

(19) Arion, W. J.; Nordlie, R. C. Liver glucose-6-phosphatase and pyrophosphate-glucose phosphotransferase: effects of fasting. *Biochem. Biophys. Res. Commun.* **1965**, *20*, 606–610.

(20) Welsch, C. A.; Lachance, P. A.; Wasserman, B. P. Dietary phenolic compounds: inhibition of Na^+-dependent D-glucose uptake in rat intestinal brush border membrane vesicles. *J. Nutr.* **1989**, *19*, 1698–1704.

(21) Herling, A. W.; Burger, H. J.; Schwab, D.; Hemmerle, H.; Below, P.; Schubert, G. Pharmacodynamic profile of a novel inhibitor of the hepatic glucose-6-phosphatase system. *Am. J. Physiol.* **1998**, *274*, 1087–1093.

(22) Simon, C.; Herling, A. W.; Preibisch, G.; Burger, H. J. Upregulation of hepatic glucose 6-phosphatase gene expression in rats treated with an inhibitor of glucose-6-phosphate translocase. *Arch. Biochem. Biophys.* **2000**, *373*, 418–428.

(23) Lafay, S.; Gil-Izquierdo, A.; Manach, C.; Morand, C.; Besson, C.; Scalbert, A. Chlorogenic acid is absorbed in its intact form in the stomach of rats. *J. Nutr.* **2006**, *136*, 1192–1197.

(24) Farah, A.; Monteiro, M.; Donangelo, C. M.; Lafay, S. Chlorogenic acids from green coffee extract are highly bioavailable in humans. *J. Nutr.* **2008**, *138*, 2309–2315.

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