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Bioavailability of Citrus Polymethoxylated Flavones and Their Biological Role in Metabolic Syndrome and Hyperlipidemia

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1. Introduction

Flavonoids are a group of polyphenolic compounds ubiquitous in many plants including fruits, vegetables, nuts, seeds, grains, tea and wine (Hollman & Katan, 1999). Flavonoids belong to two classes i) Flavanones and ii) Flavones. The most common flavanones are hesperetin from oranges and naringenin from grapefruit. The most common flavones, also known as polymethoxylated flavones, are tangeretin and nobiletin, present in orange and tangerine peel (Kurowska & Manthey, 2004).

Recently, there has been an increasing interest in the health promoting properties of PMFs, which are known to play an important role in a number of biological functions as well as having anti-cancer (Silalahi, 2002), anti-inflammatory (Lin et al., 2003), and neuroprotective properties (Datla et al., 2001). Previously our team had performed pilot studies to investigate the biological properties of both nobiletin and tangeretin in small-animal and human clinical trials. Results of these studies have shown significant decreases in serum triglycerides, total cholesterol, and low density lipoprotein cholesterol and very low density lipoprotein cholesterol levels (Kurowska & Manthey, 2004; Roza et al., 2007) and increases in glucose tolerance (Li et al., 2006).

Metabolic syndrome is a constellation of medical disorders that substantially increases the risk of individuals developing cardiovascular disease and diabetes. Metabolic syndrome has become increasingly common in Western society with a prevalence of 20-25% in the adult US population (Li et al., 2006). Early identification, prevention and treatment of metabolic syndrome portray a major challenge for the healthcare industry. During recent years, dietary supplements have been reported to be promising in reducing hyperglycemia and lipid disorders in individuals with type II diabetes or with predisposition to type II diabetes or hyperlipidemia. To date, little research has been conducted in investigating dietary supplements for their efficacy as hypolipidemic and antidiabetic therapeutic agents.

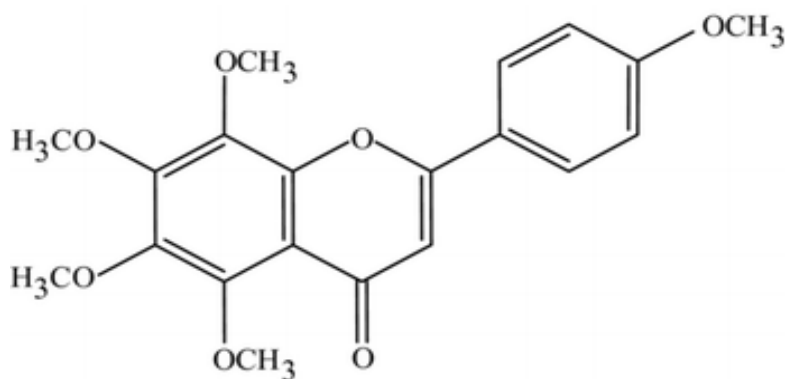
Sufficient absorption of a drug or nutrient is important to obtain a successful therapeutic response. Bioavailability of a nutrient is simply the quantity or fraction of the ingested dose that is absorbed. Since oral bioavailability appears to be a limiting factor in the metabolism of citrus flavonoids and polyphenols, future studies are recommended to further investigate the biochemical factors influencing the bioavailability of polymethoxylated flavones. This

chapter focuses on bioavailability of polymethoxylated flavones and presents results from *in vitro*, *in vivo* and human studies.

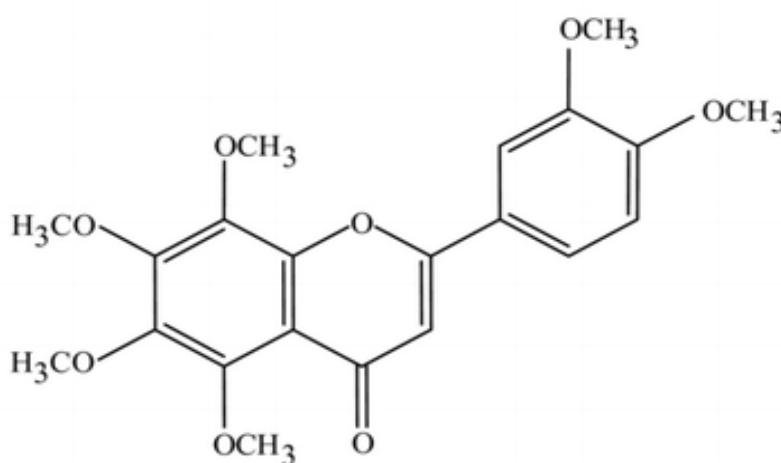
2. Polymethoxylated flavones (PMFs)

PMFs are a group of methoxylated phenolic compounds found exclusively in tissues and peels of *Citrus reticulata* (tangerine), *Citrus paradisi* (grapefruit), *Citrus sinensis* (sweet orange), and *Citrus aurantium* L. (sour orange) (Horowitz & Gentili, 1977; Dugo & McHale, 2002). These various citrus species show very high variability in their content of PMFs.

The main reason for the low oral bioavailability of the dietary flavonoids is the extensive conjugation of the free hydroxyl groups (Walle, et al., 2004; Manach & Donovan, 2004; Walle, et al., 2005). PMFs have a benzo-gamma-pyrone skeleton with a carbonyl group at the C-3 position and methoxy groups in different positions on the benzo-gamma-pyrone skeleton (Fig.1). The exclusive feature in the chemical structure of PMFs is the polymethylation of polyhydroxylated flavonoids, one of the major naturally occurring polyphenolic compounds. This results in increased metabolic stability and membrane transport in the intestine and liver, improving oral bioavailability (Walle, 2007).



Tangeretin



Nobiletin

Fig. 1. Chemical structures of nobiletin and tangeretin (Ishii et al., 2010)

The two most common PMFs are nobiletin and tangeretin. They exhibit distinctive chemical and physical properties in comparison to other plant flavonoids which influence the metabolism and pharmacokinetics of these compounds in animals. Nobiletin (5,6,7,8,3',4'-hexamethoxyflavone) has a molecular formula of $C_{21}H_{22}O_8$. Tangeretin (5,6,7,8,4'-pentamethoxyflavone) has a molecular formula of $C_{20}H_{20}O_7$. The bioavailability of PMFs is considered to be high in comparison to other citrus flavonoids because of the lipophilic nature of the multiple methoxy groups on the PMF structure. The smaller methoxyflavones, sinensetin, eupatorin and rutin, with one to three methoxy groups and without any hydroxyl groups, have been much less studied.

3. Biological role of PMFs

3.1 Metabolic syndrome

Metabolic syndrome refers to a group of risk factors that increase the risk for heart disease and diabetes. The main risk factors of metabolic syndrome include central obesity, insulin resistance, hypertension and cholesterol abnormalities. Obesity contributes to insulin resistance, which has been proposed as the major underlying cause of type II diabetes, dyslipidemia, hypertension, and atherosclerosis (Kopelman, 2000).

Results from previous experimental and clinical studies have suggested that citrus PMFs may improve glycemic control and reduce insulin resistance (Kurowska & Manthey, 2004., Miyata et al., 2011; Li et al., 2006; Judy et al., 2010).

It is known that an increase in the number and size of adipocytes in adipose tissues leads to obesity-related insulin resistance. In a recent study, both nobiletin and tangeretin were found to increase the secretion of adiponectin (insulin-sensitizing factor) and decrease the secretion of MCP-1 (insulin-resistance factor) in 3T3-L1 adipocytes demonstrated by lower intracellular triglyceride levels as compared to vehicle-treated adipocytes. Further, nobiletin also decreased the secretion of resistin, which serves as an insulin-resistance factor (Miyata et al., 2011).

Mulvihill & Huff, 2011, reported on the ability of nobiletin to improve whole-body insulin sensitivity and decrease hepatic gluconeogenesis induced in *Ldlr*^{-/-} mice when fed a high-fat Western type diet. This effect of nobiletin was achieved by both an increase in peripheral glucose disposal and enhanced suppression of hepatic glucose production by insulin (Mulvihill & Huff, 2011). Prevention of insulin resistance, glucose intolerance and adiposity required 0.3% nobiletin suggesting that prevention of obesity and improved glucose tolerance by nobiletin are linked (Mulvihill & Huff, 2011).

Furthermore, in *Ldlr*^{-/-} mice, nobiletin supplementation reduced bodyweight, very low density lipoprotein cholesterol-triglyceride secretion and improved dyslipidemia. A study by Lee et al., 2010 suggested that nobiletin improved hyperglycemia and insulin resistance in obese diabetic *ob/ob* mice by regulating expression of glucose transporter (Glut) 4 levels in the whole plasma membrane, white adipose tissue (WAT) and muscle, and by regulating expression of adipokines in WAT. In addition, a mixture of nobiletin and tangeretin were found to regulate glucose metabolism in hamsters with fructose-induced insulin resistance by modulating adipokines (Li et al., 2006). In this study PMF supplementation also reduced triglyceride content in both the liver and heart and was

able to significantly suppress serum tumor necrosis factor- α (TNF- α), interferon- γ (INF- γ), interleukin-1 β (IL-1 β) and interleukin-6 (IL-6) expression and increased serum adiponectin protein in insulin resistant hamsters. Additionally, in a study in hamsters with insulin resistance induced by feeding 60% fructose, the addition of 1% PMFs improved glucose tolerance, as demonstrated by the reduced area under the curve (AUC) measured after the intra-peritoneal injection of glucose (3g/kg body weight)(Kurowska & Manthey, 2004).

In a recent study, the anti-diabetic potential of Diabetinol®, a dietary supplement consisting of >62% PMF extract from dried fruit and orange peel, composed mainly of nobiletin and tangeretin in a 4:1 ratio, was tested in fructose induced insulin resistant male Syrian golden hamsters. The Diabetinol® treatment group and positive control group were fed a fructose-enriched diet for three weeks to induce hypertriglyceridemia and insulin resistance (Taghibiglou et al., 2000). Hamsters ($n=18$) were fed regular chow, 60% fructose or a 60% fructose diet + 1% Diabetinol®. At the end of the study (Day 49), hamsters fed 60% fructose + 1% Diabetinol® demonstrated lower blood glucose, total cholesterol and triacylglycerol levels as compared to the fructose-fed animals (Judy et al., 2010).

3.2 Effect of Diabetinol® on symptoms of metabolic syndrome – A human pilot study

3.2.1 Subjects and study design

The objective of the study was to investigate the efficacy of Diabetinol® (2 × 525 mg/day) vs. Placebo (Cellulose, 2 × 525mg/day) in glycemic control and management of risk factors of metabolic syndrome when supplemented for 12 weeks. Nineteen subjects with impaired fasting glucose (IFG) on stable oral medications were presented with a standard oral glucose challenge for the study.

The study was a randomized, double-blind, placebo-controlled study. Subjects included were between ages of 18-75 years and had a body mass index (BMI) 25.0 to 39.9kg/m², were weight stable (for 3 months prior to study), had a fasting glucose level between 6.1 and 9.0mmol/L (109.8-162.0mg/dL), and a HbA1c level<7%. Subjects were excluded from participating if they were diabetic and required insulin therapy (Judy et al., 2010).

The study included 5 clinic visits, which occurred at screening, baseline (Day 0), day 28, 56 and 84.

At baseline (randomization visit) and at all other visits anthropometric measurements were recorded, and BMI and waist/hip ratio were calculated. Fasting blood was collected for the determination of glucose, insulin and HbA1c. An oral glucose tolerance test (OGTT), where subjects consumed 100g of a glucose beverage over a 10min period was conducted and blood samples collected at 30, 60, 120, 180 and 240min post glucose consumption for analysis of glucose and insulin. On day 84, prior to the OGTT an additional 10mL of blood was collected for serum chemistry, hematology and for the determination of the lipid profile.

Fasting glucose, insulin, HbA1c and AUC glucose and insulin were compared within group and HbA1c levels between the two groups by Student's *t*-test. Statistical significance was established at $p<0.05$.

3.2.2 Results

Analysis of subject demographics demonstrated that baseline fasting glucose was not different between groups (Diabetinol® 130.33 ± 3.64 mg/dL and placebo 129.80 ± 8.52mg/dL) but was above acceptable levels (<100mg/dL) and indicated that subjects in both groups had mild hyperglycemia (100–150mg/dL). Baseline HbA1c levels were not significantly different between subjects on placebo and Diabetinol® (6.53 ± 0.22% and 6.39 ± 0.22%, respectively).

At baseline, 28, 56 and 84 days the peak in the plasma glucose curve following the glucose challenge, occurred between 60 and 120min in subjects on Diabetinol® or placebo (Fig. 2 a and 2 b). After supplementation for 56 and 84 days the glucose response curve of subjects on the Diabetinol® group was blunted as compared to the levels in the baseline OGTT curve.

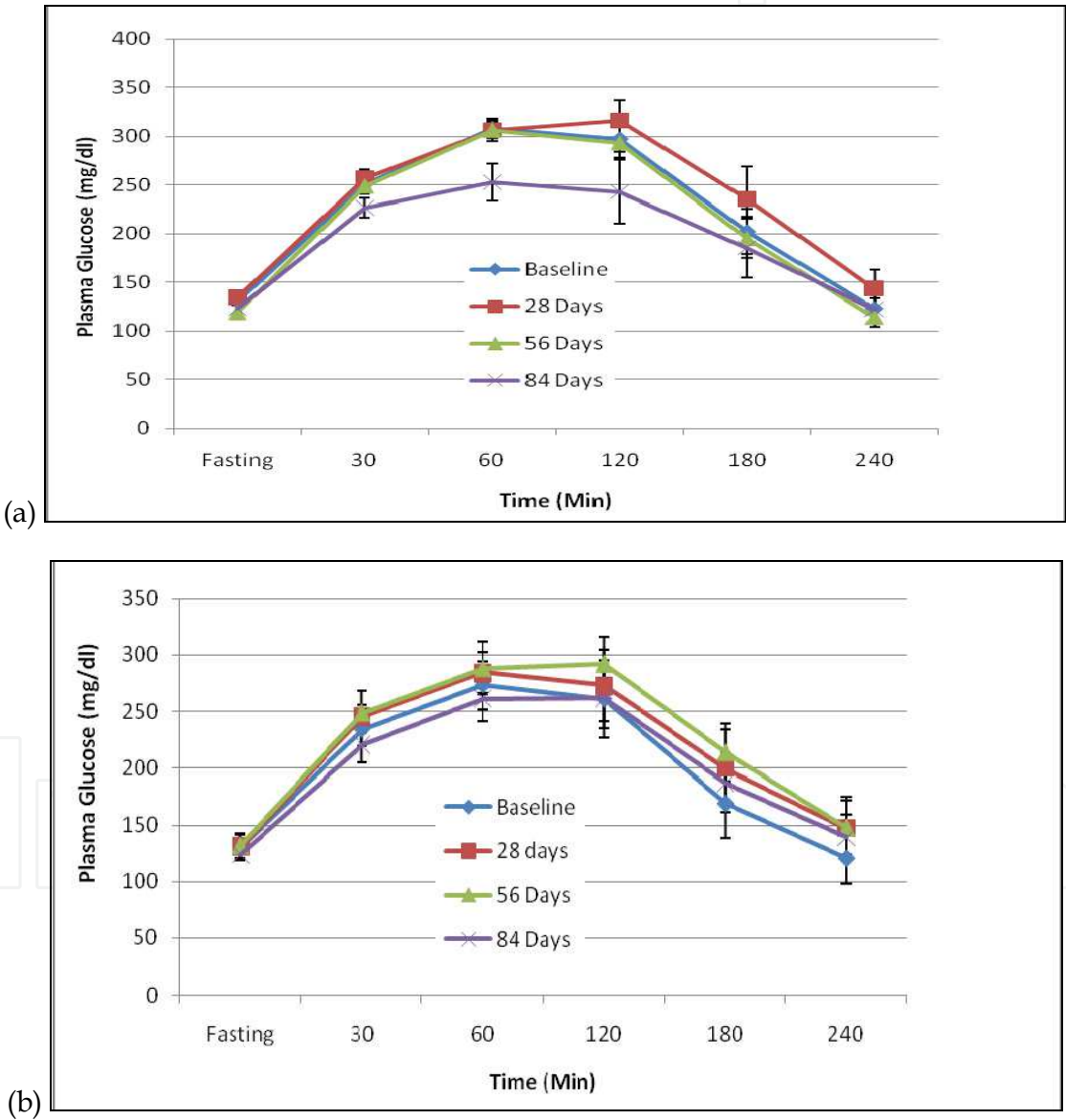


Fig. 2. Plasma glucose response curves to a standard glucose tolerance test before and after 28, 56 and 84 days of (a) Diabetinol® and (b) placebo supplementation. The plasma response curve at 84 days was significantly different ($p<0.01$) from baseline at 30, 60 and 120min for Diabetinol® by Student's t -test. Each data point is represented as mean ± SEM.

Further, after 84 days of supplementation subjects on Diabetinol® demonstrated a significant reduction in plasma glucose at 30, 60 and 120min after the glucose challenge when compared to baseline (Day 0) plasma glucose levels after the OGTT ($p<0.01$). The plasma glucose response curves after a glucose challenge at 28, 56 and 84 days for subjects on the placebo were not significantly different from that of their baseline values (Fig. 2b).

There was no significant difference in mean fasting glucose values at baseline or day 84 between, Diabetinol® (from $130.33 \pm 3.64\text{mg/dL}$ at baseline to $123.33 \pm 6.70\text{mg/dL}$) or placebo (129.80 ± 8.52 to $123.90 \pm 5.04\text{mg/dL}$) (Fig. 2a and b).

There was an increase of 9.0mg/dL in mean plasma glucose on day 28 and a reduction of 9.0mg/dL on Day 56, and a significant reduction of 56.0mg/dL ($p<0.01$) on day 84 from baseline in subjects on Diabetinol® compared to those on placebo (data not shown). A mean reduction in AUC of 127mg/dL/h from baseline to day 84 was demonstrated in the Diabetinol® group while there was a 10mg/dL/h increase in subjects in the placebo group.

There was no difference in plasma HbA1c levels between placebo and Diabetinol® groups at baseline, day 28, 56 or 84 (Fig. 3). HbA1c levels are not expected to change for 3-4 months after an intervention. However, in this study after 84days of supplementation decreasing trends in the HbA1c were observed in subjects supplemented with Diabetinol® compared to subjects on placebo. Fasting plasma insulin levels were not significantly different between subjects on placebo and Diabetinol® at baseline, day 28, 56 or 84 and did not change significantly from baseline to day 28, 56 or 84 in the Diabetinol® or placebo groups.

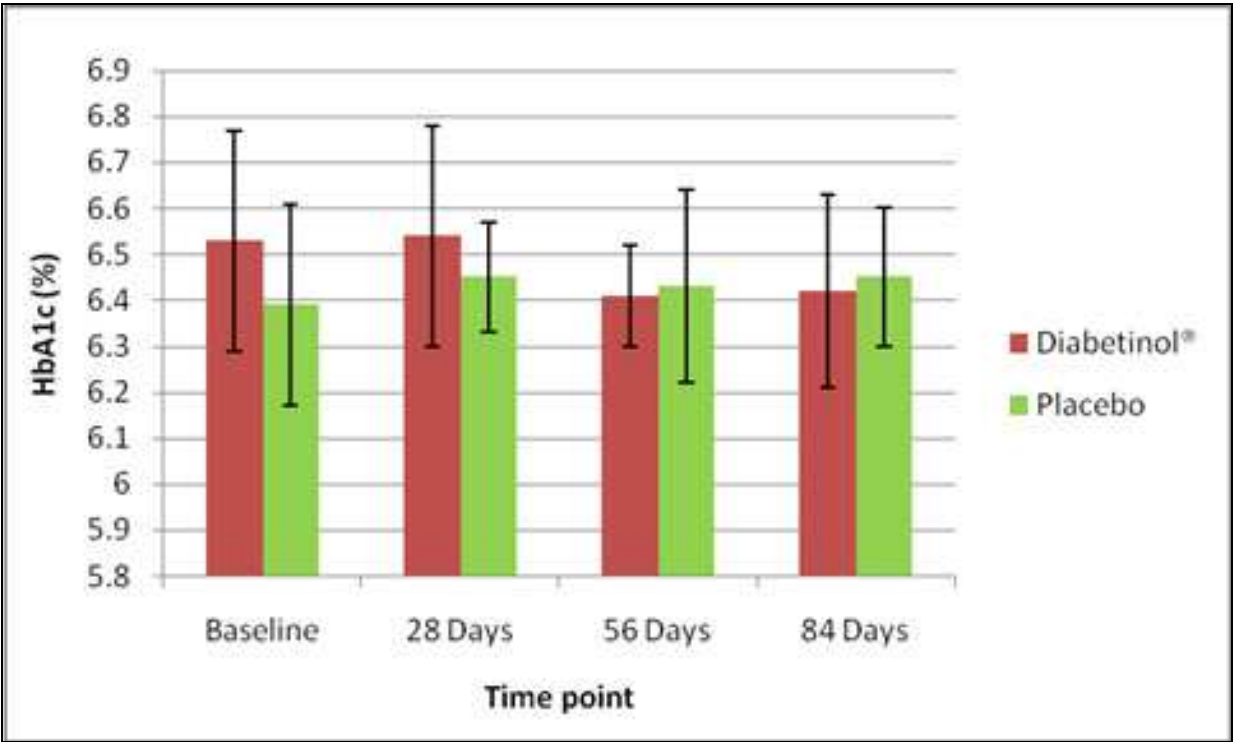


Fig. 3. Plasma HbA1c levels for placebo and Diabetinol® after 28, 56 and 84 days of supplementation. Statistical analysis was determined using Student’s *t*-test. Each data point is represented as mean \pm SEM.

There were no statistically significant differences between subjects on placebo and Diabetinol® at baseline for total plasma cholesterol, triacylglycerols, low density lipoprotein cholesterol (LDL-C) and high density lipoprotein cholesterol (HDL-C) levels (Fig. 4). This cohort of subjects had lipid levels which were higher than the clinically acceptable range for these parameters. Subjects in the Diabetinol® group demonstrated a 13.29% reduction in total cholesterol ($p<0.01$) and a 22.79% reduction in LDL-C ($p<0.01$) from baseline to 84 days (Fig. 5a). There were no significant changes in the lipid profiles from baseline to day 84 in subjects on placebo (Fig. 5b).

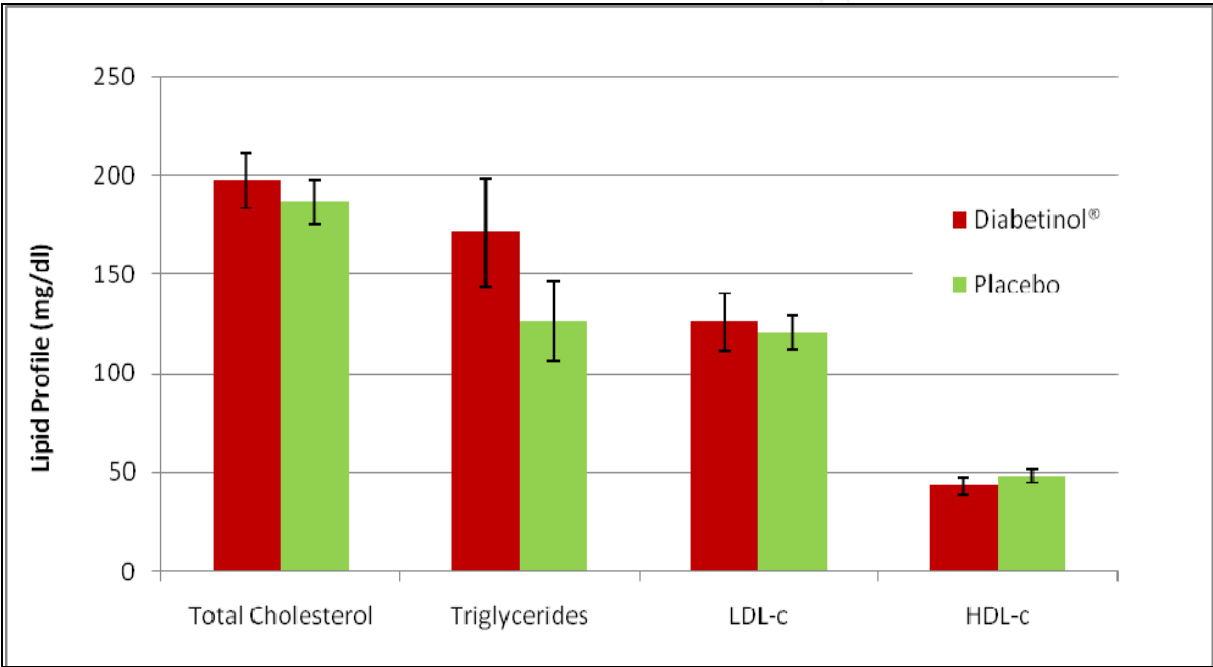


Fig. 4. Baseline (Day 0) plasma lipids for placebo and Diabetinol®. Statistical analysis of plasma lipids were determined using Student's *t*-test. Data points are represented as mean \pm SEM.

3.3 Hyperlipidemia

Elevated blood total cholesterol, higher LDL-C and reduced HDL-C are established risk factors of cardiovascular disease. Interest in PMFs has increased in recent years because of strong evidence showing that PMFs might lower total cholesterol, triglycerides, LDL-C and increase HDL-C in a number of *in-vivo* (Kurowska & Manthey, 2004; Li et al., 2006), *in-vitro* (Kurowska & Manthey, 2002; Kurowska et al., 2004) and human studies (Kurowska et al., 2000; Roza et al., 2007).

Previous studies demonstrated that PMFs from citrus, especially tangeretin and nobletin, produce hypolipidemic responses in cells (Kurowska & Manthey, 2002; Kurowska et al., 2004) and animals (Kurowska & Manthey., 2004), and that they also normalize some metabolic defects associated with experimentally induced insulin resistance (Kurowska & Manthey, 2004).

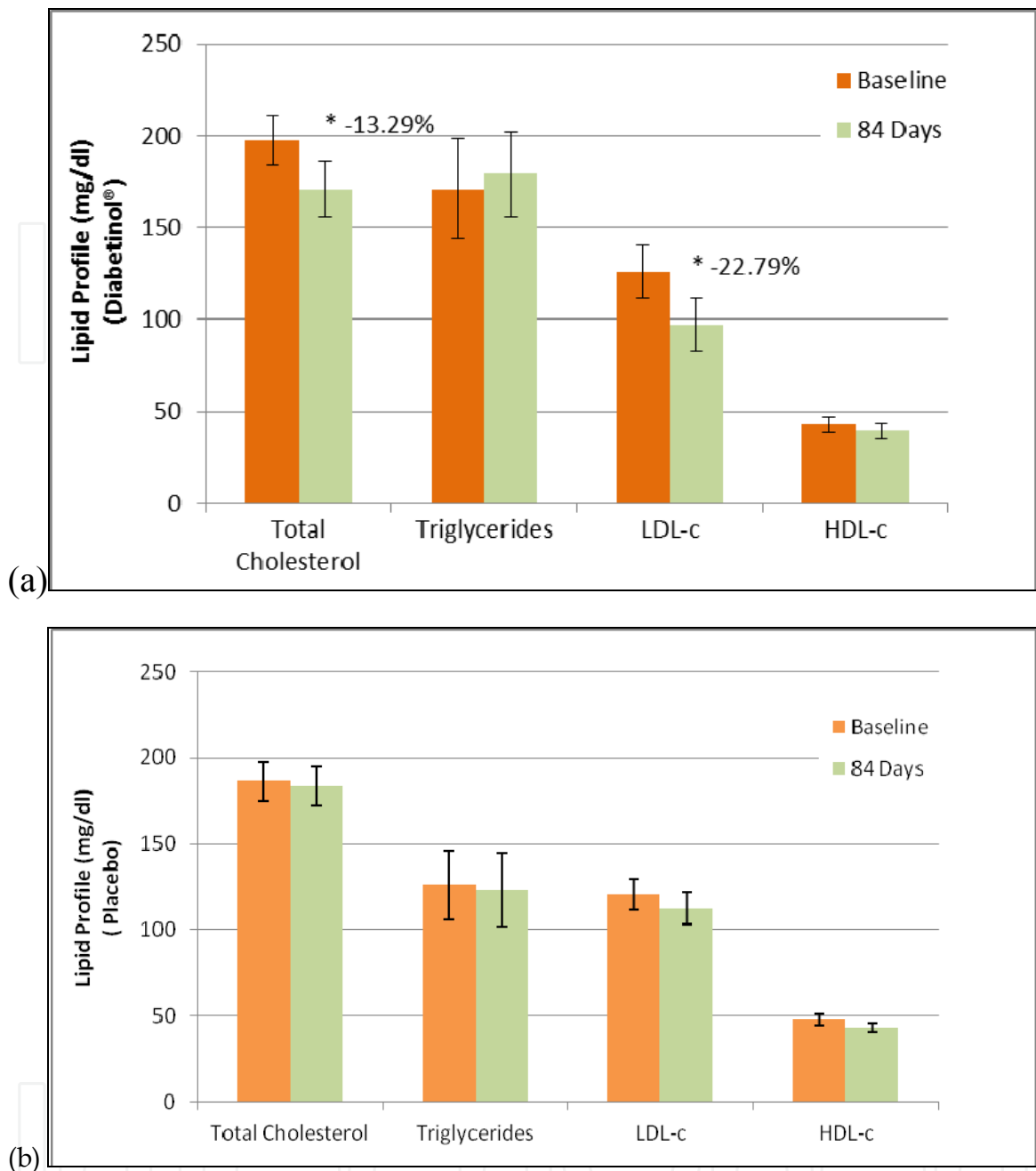


Fig. 5. (a) * Diabetinol® group plasma lipids levels after 84 days of supplementation compared to the baseline (Day 0) levels (b) placebo control group plasma lipids levels after 84 days of supplementation compared to the baseline (Day 0) levels. * $p<0.01$, day 84 values are significantly different from baseline values by Student t -test. Each data point is represented as mean \pm SEM.

Cell culture studies, demonstrated that several natural and synthetic PMFs have the ability to inhibit the net secretion of apolipoprotein B (Apo-B) in human liver cell line HepG2 (Kurowska & Manthey, 2002). In HepG2, tangeretin substantially reduced the secretion of Apo-B and the synthesis of triacylglycerols which was associated with the activation of peroxisome proliferator-activated receptor (PPAR). PPAR is the nuclear transcription factor which is known to possess positive regulatory impact on sugar, fatty acids and lipoprotein

metabolism (Kurowska et al., 2004). Nobiletin has been reported to inhibit macrophage acetylated LDL metabolism, resulting in the blockage of formation of macrophage foam-cells, which are essential to atherosclerotic plaque formation (Whitman et al., 2005).

Results from animal studies demonstrated that dietary supplementation with 1% tangeretin or 1% PMFs (largely tangeretin and nobiletin) significantly reduced serum total cholesterol, very low density lipoprotein cholesterol and LDL-C (by 19–27 and 32–40%, respectively) and also decreased serum and liver triglycerides in hamsters with diet-induced hypercholesterolemia (Kurowska & Manthey, 2004). Positive effects of PMFs were also reported with respect to adipocytokine production and PPAR in insulin resistant hamsters. The PMF supplementation showed a reversal in metabolic defects including a reduction in insulin level and an improvement in glucose tolerance, thus confirming their antiinflammatory and antidiabetic effects (Li et al., 2006). This action is speculated to occur via the inhibition of the synthesis of core protein Apo-B required for LDL synthesis in the liver (Borradaile et al., 1999).

In human studies, the responses to dietary supplements containing citrus PMFs in subjects with moderate hypercholesterolemia and baseline characteristics consistent with metabolic syndrome, demonstrated that a four week period improved blood lipid profiles without causing any adverse effects (Kurowska et al., 2001). Previously lipid lowering effects were also seen in hypercholesterolemic subjects taking orange juice for four weeks (Kurowska et al., 2000). Consistently, in moderately hypercholesterolemic men, mixtures of nobiletin and tangeretin at a dose of 270 mg/day, in combination with tocotrienols (30 mg/day), reduced plasma LDL-C, Apo-B and triglycerides (Roza et al., 2007). This suggests that PMFs have substantial cholesterol and triacylglycerol lowering potential.

4. The importance of pharmacokinetics studies of PMFs

Pharmacokinetics is the study of the mechanisms of absorption, metabolism and routes of excretion of the metabolites of an administered drug or nutrient. Bioavailability of a nutrient is simply the quantity or fraction of the ingested dose that is absorbed. Pharmacokinetic studies on PMFs are important in identifying the composition of metabolites of administered supplements. They may also help in differentiating different supplements on the market which have different levels and ratios of PMFs (i.e. nobiletin, tangeretin). Additionally, these studies may further provide information regarding absorption and metabolism of different formulations (i.e. soft gel capsule, hard shell capsule etc).

There are multiple challenges in the development of PMFs which must be overcome to increase their bioavailability. For example, PMFs exist as aglycones. The solubility of aglycone forms is low, which may lead to slower dissolution rates in addition to the fact that absorbed aglycones are rapidly conjugated to glucuronides and sulfates in the intestine and liver (Li et al., 2007). Therefore, it is important to perform systematic studies to demonstrate how changes in PMF structures affect solubility and dissolution rates.

Previous studies in hamsters showed, that when they were fed a tangeretin-enriched diet for 35 days, the main PMF metabolites identified in serum, urine and in liver tissue included dihydroxytrimethoxyflavone and monohydroxytetramethoxyflavone glucuronides and

aglycones (Kurowska & Manthey, 2004). This demonstrated considerable intestinal absorption of tangeretin based on urinary excretion of several metabolites. However, no unchanged (parental) tangeretin was detected in the circulating plasma (Kurowska & Manthey, 2004).

In a recent study by Manthey et al., 2011, nobiletin and tangeretin were administered to rats by gavage and intraperitoneal (ip) injection. By using high-performance liquid chromatography–electrospray ionization–mass spectrometry (HPLC-ESI-MS), blood serum concentrations of metabolites were monitored. Over 24 hours, two metabolites of tangeretin and eight metabolites of nobiletin were detected with the administered compounds. Results from this study demonstrated that with identical oral doses nearly 10-fold higher absorption of nobiletin occurred in comparison to tangeretin. Further, in the blood serum, maximum levels of glucuronidated metabolites occurred later than nobiletin and tangeretin and occurred at higher concentrations than aglycone metabolites (Manthey et al., 2011). It is also confirmed in previous trials that overall expression of biological actions of nobiletin and tangeretin in animals is due to the biological actions of aglycone metabolites (Eguchi et al., 2007; Li et al., 2007; Lai et al., 2008; Xiao et al., 2009).

Wan & Walle, 2006, demonstrated that the flavonoid chrysin (5,7-dihydroxyflavone) has poor bioavailability, but when two hydroxyl groups in chrysin were methylated, as in 5,7-dimethoxyflavone (5,7-DMF), using cofactors for glucuronidation, sulfation and oxidation, chrysin was rapidly metabolized within 20 minutes of incubation showing no parent compound at the end. In contrast, over the whole 60-min time-course studied, 5,7-DMF was found to be metabolically stable (Wen & Walle, 2006). Further, there is evidence of the role of different cytochrome P450 isozymes, in oxidative demethylation in PMFs metabolism (Koga et al., 2007; Breinholt et al., 2003).

Results from these studies on oral bioavailability thus indicated that the methoxylated flavones have a great advantage over the nonmethylyated flavones. Future studies are needed to investigate the biochemical factors influencing the pharmacokinetics of PMFs.

5. Pharmacokinetics of PMFs in Sytrinol®

5.1 Sytrinol®: A proprietary supplement consisting of PMFs and Tocotrienols

Sytrinol® is a proprietary supplement developed by KGK Synergize Inc and consists of PMFs, mainly tangeretin and nobiletin (1:1 v/v) and palm oil tocotrienols. Tocotrienols are a group of dietary constituents, which are analogues of tocopherol (vitamin E), found mainly in palm oil and cereal grains. Tocotrienol-rich fractions from palm oil usually contain tocotrienols α , λ and Δ , as well as 15-40% α -tocopherol (Guthrie & Carroll, 1998). The cholesterol-lowering potential of PMFs and tocotrienols has been investigated in preclinical studies and in clinical trials (Kurowska et al., 2004; Kurowska & Manthey, 2004; Roza et al., 2007).

5.2 Human bioavailability study of three Sytrinol® formulations in healthy subjects

5.2.1 Subjects & study design

The objective of this study was to determine whether PMFs may be detected and quantified in serum obtained from healthy adults after a single dose administration of Sytrinol® capsules (containing 1053 mg of total PMFs). The study compared oral bioavailability of

three Sytrinol® formulations in healthy subjects. The endpoints were the determination of various pharmacokinetic parameters, specifically area under the concentration-time curve (AUC_{0-48h}), time at maximum concentration (T_{max}) and maximum serum concentration (C_{max}) for each of tangeretin and nobiletin.

The study was conducted as a randomized crossover trial. Ten healthy subjects, five men and five women, age 23 ± 3 years, were recruited for the study. Prior to the start of the study, subjects had blood drawn for routine tests to confirm eligibility. All subjects were asked to avoid caffeine-containing products 12 h prior to the study and during the study.

Subjects were administered a single dose of the first Sytrinol® product (either Sytrinol® soft gel capsules (Product A), Sytrinol® powder hard shell capsules (Product B) or Sytrinol® hard shell capsules with added lecithin (Product C), containing 1053 mg of PMFs, largely tangeretin and nobiletin (1:1 v/v) and tocotrienols. Standard citrus-free meals (breakfast, lunch and dinner) were provided on each of the days of the multiple blood sampling. Participants took the second PMF product 14 days later and the third product after another 14 day washout period.

Peripheral blood was taken by venipuncture at time 0 (baseline), 0.25, 0.5, 1, 2, 4, 6, 8, 24 and 48 hours after ingestion of Sytrinol® capsules. Quantitation of tangeretin and nobiletin in serum was done by Liquid chromatography/Mass spectrometry (Varian 1200 L LC/MS/MS system equipped with ESI and APCI sources). The identities of these PMFs were verified by comparing fragment ion mass spectra of authentic tangeretin and nobiletin standards.

5.2.2 Results

The serum samples obtained from healthy subjects following oral administration of three different Sytrinol® formulations demonstrated detectable amounts of parental tangeretin and nobiletin. Tangeretin and nobiletin peaks were identified and quantitated in all serum samples collected after administration of Sytrinol® formulations A, B or C.

Results demonstrated that for both tangeretin and nobiletin, the AUC_{0-48h} and C_{max} values were significantly higher ($P < 0.001$) after administration of formulation A than after treatment with products B or C, suggesting greater bioavailability of formulation A vs. B or C (Tables 1 & 2). For all three formulations, the AUC_{0-48h} values were higher for nobiletin than for tangeretin indicating that nobiletin might be more bioavailable than tangeretin. The time-concentration curves and pharmacokinetic results demonstrated that for both tangeretin and nobiletin, significantly higher AUC_{0-48h} and C_{max} values were obtained following the administration of formulation A than following the administration of either B or C (Figure 6 & 7). The effects of the Sytrinol® formulations A, B and C on AUC_{0-48h} and C_{max} of tangeretin and nobiletin derivatives are depicted in Figures 8 & 9. For nobiletin, the AUC_{0-48h} values were 2.7-3.0 times higher for supplement A than for B or C whereas for tangeretin, the AUC_{0-48h} values were 1.7-2.3 times higher for A than for B or C. Changes in mean serum concentrations of tangeretin and nobiletin products after a single-dose administration of Sytrinol® A, B or C formulations are shown in Figures 6 and 7. The T_{max} values were not affected by the type of treatment and also were similar for tangeretin and nobiletin peaks (1.3-1.4 h).

	A	B	C	P value
Initial conc. (µg/L)	49.5 ± 10.6	63.5 ± 51.6	28.5 ± 20.8	
AUC _{0-48h} (µg x min/L)	4654.4 ± 1929.3	1538.0 ± 249.3***	1715.0 ± 914.2***	<0.0001
C _{max} (µg /L)	1006.4 ± 555.3	51.8 ± 18.8***	63.7 ± 33.2***	<0.0001
T _{max} (h)	1.4 ± 0.5	1.48 ± 0.8	1.4 ± 0.6	

***A vs. B, C are significantly different (p<0.001) by ANOVA followed by Tukey’s test.

Table 1. Pharmacokinetics of serum nobiletin (means ± SD).

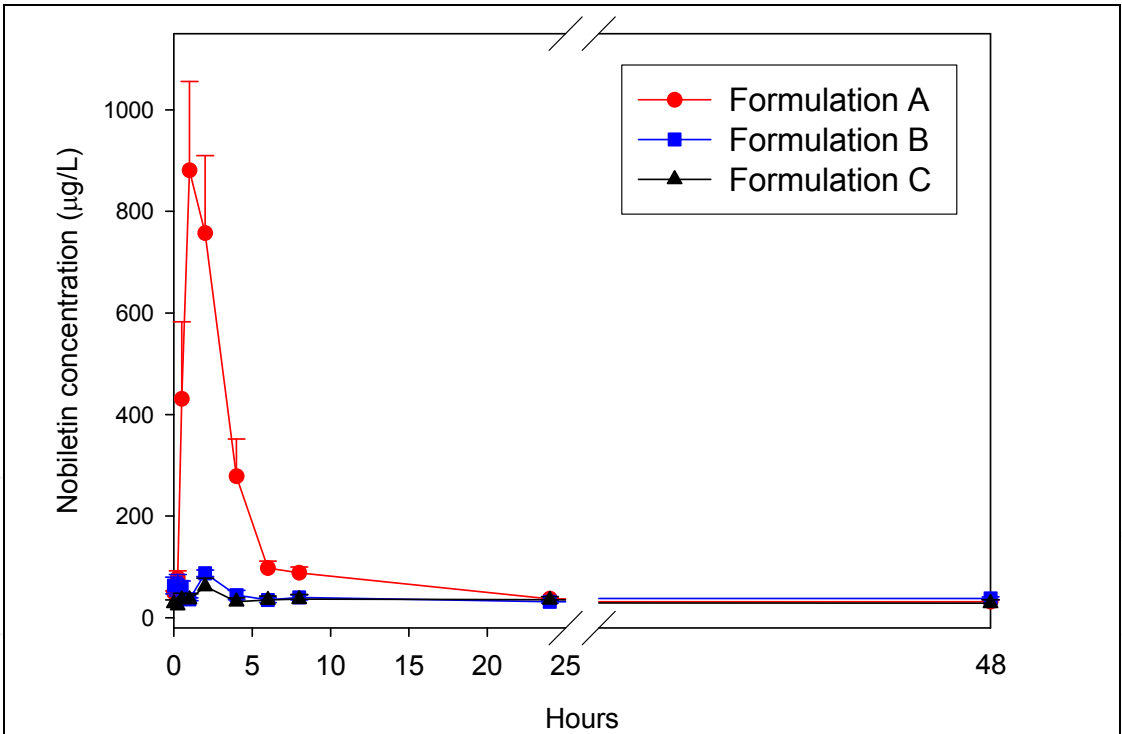


Fig. 6. Changes in serum concentrations of nobiletin after a single dose administration of Sytrinol® formulations A, B and C (1053 mg PMFs per product) (means ± SEM).

Capsules contained both PMFs in equal amounts, however, more nobiletin than tangeretin was generally found in the blood. The differences were particularly striking in blood samples collected after administration of supplements A and B (with the AUC_{0-48h} ratios of nobiletin to tangeretin 1.85 and 1.42, respectively) but also tended to occur after the administration of C (with the nobiletin to tangeretin ratio 1.14). The results suggest that in

healthy human subjects, nobiletin might be more bioavailable than tangeretin. The pharmacokinetic results showed that in healthy human subjects, Sytrinol® product A (Soft Gel) was significantly more bioavailable than the remaining two products, B (hard shell) and C (hard shell + lecithin). Formulation A differed from B and C in respect to AUC_{0-48h} and C_{max} but not in respect to T_{max} , which was not affected by treatments. Our data also suggest that nobiletin present in Sytrinol® formulations is more bioavailable than tangeretin.

	A	B	C	P value
Initial conc. (µg /L)	31.4 ± 5.7	37.2 ± 25.6	29.9 ± 21.6	
AUC_{0-48h} (µg x min/L)	2509.6 ± 1092.8	1085.8 ± 198.8**	1499.3 ± 864.0*	0.0041
C_{max} (µg /L)	532.3 ± 335.7	76.4 ± 49.9***	48.0 ± 30.1***	<0.0001
T_{max} (h)	1.3 ± 0.5	1.3 ± 0.8	1.2 ± 0.6	

*A vs. C is $p<0.05$, **A vs. B is $p<0.01$,
***A vs. B, C are $p<0.001$ by ANOVA followed by Tukey’s test.

Table 2. Pharmacokinetics of serum tangeretin (means ± SD)

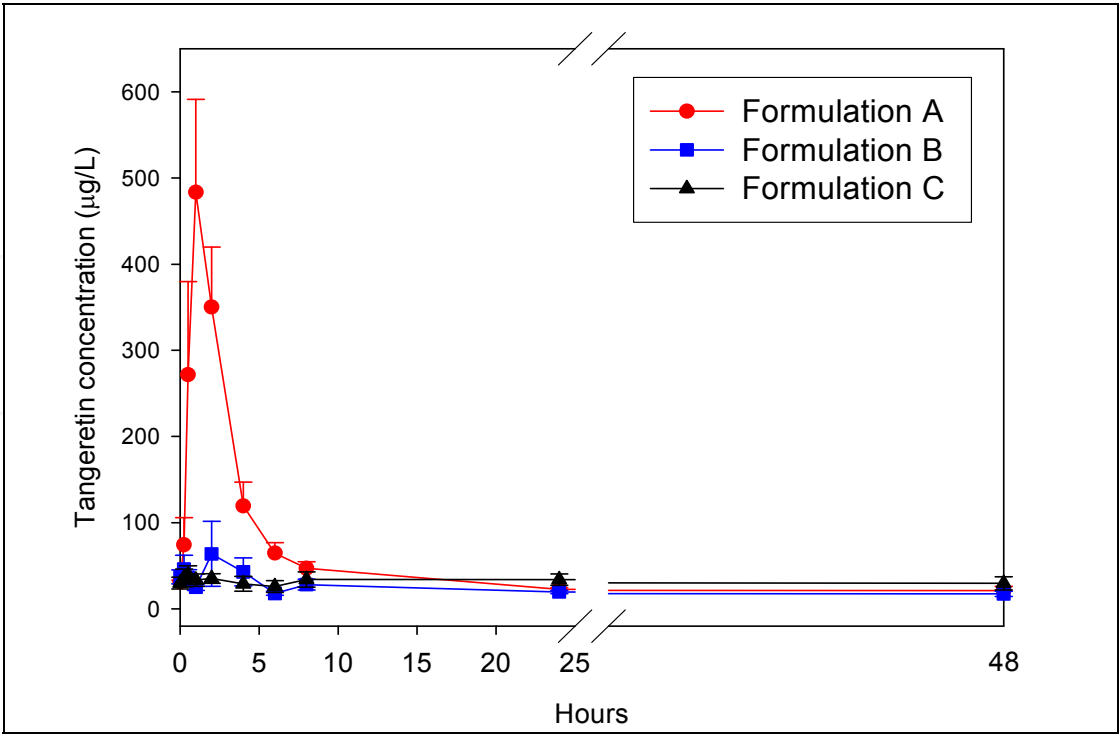


Fig. 7. Changes in serum concentrations of tangeretin after a single-dose administration of Sytrinol® formulations A, B and C (1053 mg PMFs per each dose (means ± SEM).

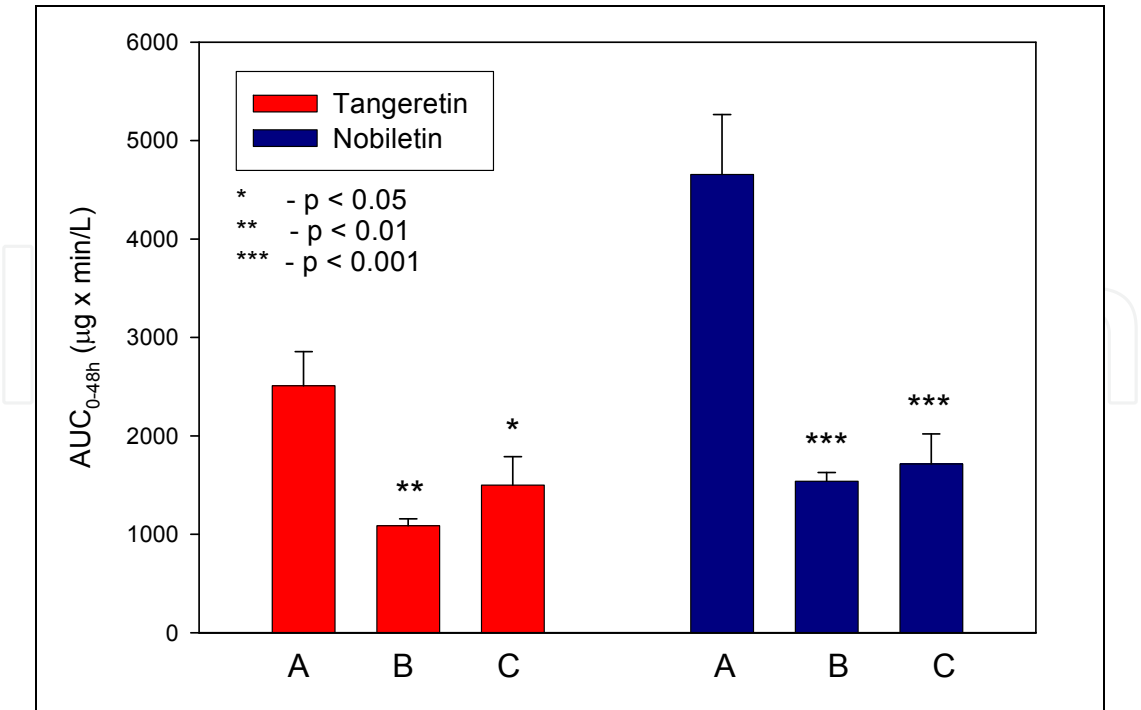


Fig. 8. Effects of Sytrinol® formulations A, B and C on AUC_{0-48h} of tangeretin and nobiletin (means \pm SEM). Values significantly different by ANOVA followed by Tukey’s test. *A vs. C is $p < 0.05$, **A vs. B is $p < 0.01$, ***A vs. B, C are $p < 0.001$.

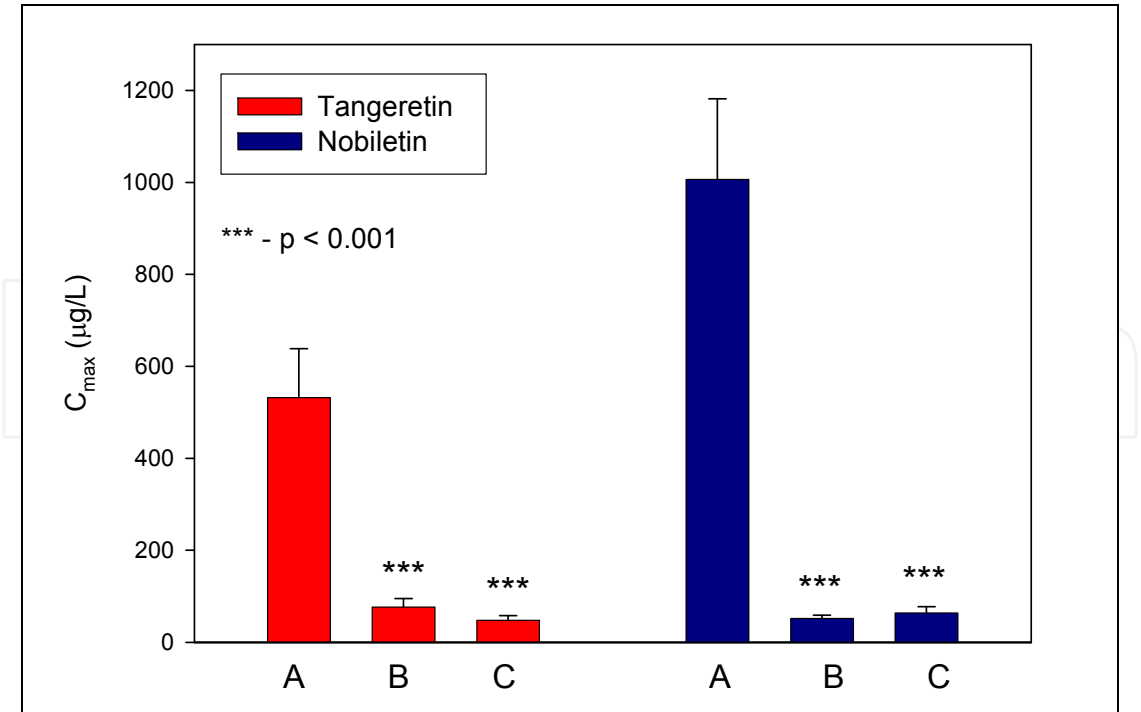


Fig. 9. Effects of Sytrinol® formulations A, B and C on C_{max} of tangeretin and nobiletin (means \pm SEM). Values significantly different by ANOVA followed by Tukey’s test. ***A vs. B, C are significantly different ($p < 0.001$).

6. Conclusion

Bioavailability of a drug or nutrient supplement is largely determined by the properties of the dosage form. Yet, further characterizations of the metabolism and the biochemical factors influencing these compounds' pharmacokinetics are essential, especially as oral bioavailability appears to be a significant limiting factor to the efficacy of these and other citrus PMFs.

Both nobiletin and tangeretin have demonstrated efficacy in metabolic syndrome and hyperlipidemia, but vary in their pharmacokinetic properties. Research has demonstrated that nobiletin has greater bioavailability and efficacy as compared to tangeretin. Diabetinol® was developed with this concept in mind in order to address metabolic syndrome and hyperlipidemia.

The pharmacokinetic profiles of nobiletin and tangeretin were previously based on the detection and quantification of their metabolites. In this chapter we have presented data on the detection of the parental compound nobiletin and tangeretin and suggest that in future research it is important to determine these compounds in human pharmacokinetic studies.

Differences in bioavailability among formulations of a given supplement may have clinical significance; thus, knowing whether nutrient or drug formulations are equivalent is essential in making reasonable conclusions.

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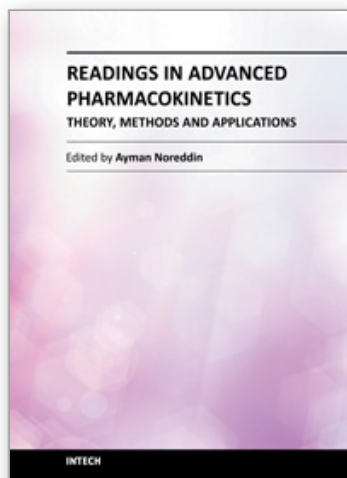
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