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RESEARCHARTICLE

POLYPHENOLS EXCRETION REDUCES LIPIDS PEROXIDATION, HYPERURICEMIA AND HYPERCHOLESTEROLEMIA IN TYPE-2 TUNISIAN DIABETICS

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ABSTRACT

Epidemiologic studies suggest that tea-polyphenols consumption may be beneficial in the prevention and control of type-2 diabetes. The objective of this work was to examine the effect of the absorbed polyphenols, assessed through their plasma and urinary concentrations, on the total antioxidant status, lipids peroxidation and other metabolic parameters in type -2 Tunisian diabetics. A sample of 244 type-2 diabetic patients was enrolled in this study. Green tea consumption, assessed as tea cups/ day was recorded. The malondihaldehyde, as early marker of lipid peroxidation, total antioxidant status, plasma and urinary polyphenols levels and other metabolic parameters were determined by appropriate methods. Uric acid and malondihaldehyde levels were significantly reduced in higher consumers of tea (>3 cups/day) compared to moderate consumers (<3 cups/day) (P=0.02 and P<0.001; respectively). Moreover, the urinary polyphenols was inversely correlated with total cholesterol, LDL-cholesterol andtotal antioxidant status (P=0.011, P=0.008, P =0.028; respectively). These results suggest that the final flavonols metabolites found in urine may be responsible of the hypocholesterolemic; hypouricemic and the reducing of lipid peroxidation observed in type -2 diabetics.

INTRODUCTION

In spite of the considerable progress that has been made in improving the clinical course of type-2 diabetes (T2D), its prevalence continues to dramatically raise worldwide (World Health Organization, 2000). This could be related to rapid change in lifestyles including urbanization, sedentary life, smoking, reduction in physical activity and excess of animal fats and rich sugar foods consumption (Tanasescu *et al.,* 2004; Ben Abid *el al.,* 2007). Implication risk of T2D is extensively associated to oxidative stress which is a link between disease and their complications (Suciu *et al.,* 2004). Recently, a Tunisian epidemiological survey reported that the prevalence of diabetes with dyslipidemia, hypertension and obesity was 31.5 and 30 % in men and women hospitalized for coronary diseases, respectively (Jemaa *et al.,* 2005). On the other hand, it has been demonstrated that consumption of tea, as source of polyphenols, has a great power against oxidative stress (Schroder, 2007). Moreover, green or black tea is a popular beverage in Tunisia as well as in other North African countries like Libya or Algeria. In vitro, animal and some clinical trials, whatever conflicting, have provided evidence that polyphenols derived from tea may possess beneficial effects against pathogenesis of several chronic diseases (Cabrera *et al.,* 2006). The chelating effect of tea polyphenols on iron is beneficial because the high level of iron stored is associated with an increase of myocardial infarction risk, especially in some situations including diabetes mellitus (Cabrera *et al.,* 2006; Klipstein-Grobusch *et al.,* 1999). In addition to the antioxidative and anti-inflammatory properties of tea, epidemiological data show an association between consumption of green and black teas and reduced plasma cholesterol and triglyceride levels (Imai and Nakachi, 1995; Princen *et al.,* 1998; Stensvold *et al.,* 1992; Bornhoeft *et al.,* 2012), which may also explain the reduced cardiovascular mortality associated with tea consumption. However, the health effects of polyphenols depend on their bioavailability. This concept depends on several variables, such as absorption kinetics, intestinal and hepatic metabolisms and excretion

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(D'Archivio *et al.,* 2007). The objective of this work was to examine the polyphenols pathway, assessed through their plasma and urinary concentrations on the total antioxidant status, lipids peroxidation and other metabolic parameters in type -2 T unisian diabetics.

MATERIAL AND METHODS

Our studyinvolved a sample of 244 adultswith T2D recruited randomly fromdifferentconsulting servicesof National Institute of Nutrition of Tunis.Written consent was obtained. Anthropometric, Clinicaland Biochemicalcharacteristics are shown in Table 1. The research protocol described above was approved by the local ethics committee. Patient's data wereidentifiedfrom their updated medical records. Subjects were further interrogating for tea type intake and cups number. Green tea was majorly consumed (up to 80% of cases). Asubdivision was performed according totea intakefrequency: moderate tea drinkers (<3cups/day, n = 144) andgreat consumers(>3 cups/day, n = 100), (mean cup serving 35 ml). Green and black teas are generally consumed in the form of decoction + sugar which is a popular beverage in Tunisia and in other North African countries. It has been demonstrated that Decoction from green or black teas provides high polyphenols content of the extract (Dhaouadi, 2010). Blood and urine samples were obtained. The extraction of total polyphenols from plasma and urine was performed according to the method used by Dhaouadi *et al.* (2010). The total plasmatic (PP) and urinary (UP) polyphenols were estimated spectrometrically by the Folin-Ciocalteu assay described by Singleton and Rossi (1965).TAS was determined by the method of Miller et al. (1993) using Randox Kit (UK). MDA level was determined according to Buege and Aust (1978); Ohkawa *et al.* (1979) methods.Statistical analyses were undertaken using SPSS, version 17 software.

Qualitative Data were compared using Pearson's $\chi 2$ test. The Kolmogorov-Smirnov test was used to assign the normal distribution of the quantitative variables. Data were compared between groups by the unpaired Student's t test and correlated using Spearman's correlation. A $P<0.05$ was considered as statistically significant. Significance was corrected for cofactors (age, sex, BMI, smoking habit, diabetes duration, familial history of the disease, physical activity, treatment type and complication development) using partial correlation.

RESULTS

Comparisons between green tea consumption groups in term of clinical and biological parameters are shown in Table 1. Biological parametersdo not show significant differences between groups, at the exception ofprotein ($P=0.03$) anduric acid (UA) ($P=0.02$) which significantly decreased among great consumers of tea group.The PP, UP, MDA and TAS values are presented in table 2. The PP and UP rates are slightly higher among great consumers compared to moderate consumers. There was a significant decrease in MDA values among tea higher consumers ($P<0.001$) whereas no change was noted in term of TAS between groups. The relations between PP, UP, MDA, UA and tea cups number are shown in Table 3. Tea consumption evaluated as number of cups had a significant direct relationship with PP and UP ($P=0.041$ and $P=0.05$, respectively) and a significant inverse relationship with plasma UA and MDA ($P=0.007$ and$P<0.001$, respectively). The relationships between PP, UP and metabolic parameters are represented in Table 4. Among all studied parameters, only the total cholesterol (TC), LDL-cholesterol (LDL-C) and TAS were negatively correlated with UP ($P=0.011$, $P=0.008$ and $P=0.028$, respectively). For the other serum estimates, only a positive correlation between MDA and UA ($P=0.034$) might be highlighted.

Table 1. Anthropometric, clinicaland biochemicalcharacteristics of subjects and on the basis oftea intake

	Patients (n=244)	Tea consumption		
		n cups <3 (n=144)	n cups >3 (n=100)	*P*
Age (years)	54,59±9,7	54,54±10,17	54,66±9,03	-
Gender (M/F)	106/138	61/83	45/55	-
BMI (kg/m^2)	30,34±5,48	30,41±5,67	30,23±5,22	-
Current smoker				
Yes (%)	16,9	15,4	19,2	-
No (%)	83,1	84,6	80,8	
Duration of diabetes (years)	11±6,98	11,24±6,63	10,66±7,47	-
Physical activity				
Yes (%)	31,1	33,3	28	-
No (%)	68,9	66,7	72	
Treatement				
Insulin (%)	77	79,2	74	-
Oral hypoglycemic drugs (%)	11,5	9	15	
Combination of both (%)	11,5	11,08	11	
Complication				
Yes (%)	30,3	31,2	29	0,7
No (%)	69,7	68,8	71	
Systolic blood pressure (mmHg)	13,3±2,04	13,35±2,07	13,23±2,02	0,66
Diastolic blood pressure (mmHg)	7,92±1,18	8,01±1,13	7,79±1,25	0,14
Protein(g/L)	70,6±9,22	71,67±9,03	69,07±9,30	0,03*
HbA_{1c}(%)	9,22±1,83	9,16±1,88	9,3±1,76	0,55
Glucose (mmol/ L)	11,59±3,17	11,6±3,22	11,56±3,12	0,91
Total cholesterol (mmol/L)	4,89±0,96	4,9±0,84	4,88±1,11	0,85
LDL cholesterol (mmol/L)	2,97±0,78	2,96±0,68	3±0,91	0,73
HDL cholesterol (mmol/L)	1,22±0,43	1,21±0,41	1,24±0,44	0,62
Triglycerides (mmol/L)	1,52±0,84	1,56±0,9	1,46±0,75	0,34
Creatinin (µmol/L)	76,07±20,46	76,3±21,55	75,73±18,9	0,83
Uric acid (µmol/L)	250,71±75,06	260,45±77,17	237,35±70,28	0,02*
Tea consumption (N cups)	1,9±2,31	-	-	-
n<3 (%)	59			
n>3 (%)	41			

*: ($P<0, 05$).

Table 2. Plasmapolyphenols (PP), urinary polyphenols (UP) and oxidative stress parametersmeasured (MDA and TAS) according totea consumption

	Patients (n=244)	Tea consumption		
		n cups <3 (n=144)	n cups >3 (n=100)	*P*
PP mg/100ml	13,33±,44	12,64±10,53	14,31±12,63	0,26
UP mg/100ml	3,42±2,72	3,23±2,49	3,63±2,98	0,43
MDA mmol/L	4,4±2,16	5,38±1,92	2,95±1,62	<0,001**
TAS mmol/L	1,48±0,67	1,45±0,71	1,52±0,59	0,54

**: P<0, 01.

Table 3. Correlations between PP, UP and tea cups number

Parameter	Rho	P_c
n cups with PP	0,142	0.041
n cups with UP	0,128	0.05
PP with UP	0.363	0.014
n cups with MDA	-0,495	0.001
n cups with UA	-0,210	0.007

P_c: Pvalue corrected for covariates.

Table 4 Correlations among serum and urinary estimates

Parameter	rho	P_c
UP with TC	-0,340	0.011
UP with LDL- C	-0,484	0.008
UP with TAS	-0,330	0.028
PP with TC	0,310	0,625
PP with LDL-C	0,170	0,809
PP with TAS	0,030	0,733
MDA with UA	0,186	0.034

P_c: P value corrected for covariates.

DISCUSSION

Tea-polyphenols are considered to contribute to the prevention of various diseases including diabetes mellitus (Rizvi *et al.,* 2005; Tapiero *et al.,* 2002). In recent years, researchers investigated the potential benefits of green tea and its main catechin epigallocatechin-3-gallate (EGCG). The beneficial effects of EGCG supplementation has been observed in T2D care (Harrisson *et al.,* 2011; Suliburska *et al.,* 2012). In the present work, Tea Polyphenols benefit was further elucidated by looking to total plasmatic and urinary polyphenols effects on T2D biochemical parameters. However, polyphenols are present in most foods and beverages of the human diet besides tea. Furthermore, they are present in different forms, which can affect their intestinal absorption and excretion (Scalbert *et al.,* 2002; Hollman *et al.,* 1997; Manach *et al.,* 2005; Manach *et al.,* 2005). In our study, a significant positive correlation between PP, UP and tea intake was found suggesting that any observed PP or UP effect is linked to tea consumption.Recent studies have reported that tea intake reduced hyperglycemia, hypertriglyceridemia and hypercholesterolemia in vitro and in vivo (Guo *et al.,* 1996; Bryans *et al.,* 2007). In this way, the present study showed that UP, provided by tea consumption, was inversely correlated with TC and LDL-C. Our finding resemble to that of Inami *et al*. (2007) who reported that 500 mg catechin intake which is equivalent to 6 tea cups per day reduced circulating oxidized LDL in healthy adult volunteers. The hypocholesterolaemic effect may be explained through the upregulation of the LDL receptor, receptor mRNA and the reduction of extracellular apo B levels in HepG2 cells by EGCG of green tea (Goto *et al.,* 2012; Bursill and Roach, 2006). Bursill and Roach (2006)explained that the decrease in the cellular cholesterol could have occurred via different mechanisms: an inhibition of cholesterol synthesis, an increase in the efflux of cholesterol from the cell and an increase in the conversion of cholesterol to bile acids.

Many documents report that excess serum accumulation of UA, the final oxidation product of purine catabolism, can lead to various diseases (Becker, 1993; Koenig and Meisinger, 2008). Higher level of serum UA induced oxidative stress is powerfully associated with obesity, metabolic syndromes and mainly with T2D (Baker *et al.,* 2005; Dehghan *et al.,* 2008: Chen *et al.,* 2008). Nakagawa *et al*. (2006)reported that fructose intake is linked to obesity and diabetes due to its ability to raise UA that may lead to endothelial dysfunction by reducing nitric oxide (NO) bioavailability as a mechanism for insulin resistance in rats (Nakagawa *et al.,* 2006; Bhole *et al.,* 2010). In our work, we have observed a significant decrease of UA in higher consumers of tea. In their study, Choi and Curhan (2007) suggested that coffee consumption but not tea is associated with a lower level of UA due to noncaffeine xanthines contained in coffee which may inhibit xanthine oxidase. Indeed, xanthine oxidase inhibition was associated with the decrease of UA and the improvement of insulin sensitivity in rats receiving a high-fructose diet (Nakagawa *et al.,* 2006). Hyperuricemia seems to play a major role in lipid peroxidation as a positive correlation between UA and MDA (lipid peroxidation marker) was observed.

This finding was already described by Al-Rawi (2011) who found a significant increase and direct relationship between UA and MDA among T2D and suggested that this previously serum antioxidant (UA) can paradoxically become pro-oxidant. Several studies have reported an increased susceptibility to lipid peroxidation in patients with diabetes mellitus (Gallu *et al.,* 1993). Mahboob *et al*. (2005) have observed that MDA levels were significantly elevated in diabetic patients (Rietveld and Wiseman, 2003). In our sample, the consumption of more than 3 cups of green tea significantly decreased MDA levels. These results are consistent with those of Erba *et al.* (1999) who showed that supplementation of the Jurkat T-cell line with green tea extract significantly decreased MDA production and DNA damage after Fe^{2+} oxidative treatment compared to control. The cytoprotective effects of tea polyphenols against lipid peroxidation can be attributed to either rapid reaction of phenolic compounds with free radicals or the fall in UA levels. Furthermore, the inverse correlation seen between the TAS and UP might simply be a result of tea intake related hypouricemia. Indeed, even UA can act as a pro-oxidant at increased concentrations (Strazzullo and Ping, 2007; Becker, 1991), it is generally held that it is the major provider to the antioxidant defense in plasma (UA contributes to 60 % of plasma total antioxidant capacity) (Duplancic *et al.,* 2011). However, no direct correlation between UP and UA was detected, probably because of its double biological role (antioxidant/pro-oxidant). This finding was also described in the study of Bahorun *et al*. (2012) in which the plasma total antioxidant activity was significantly reduced by tea intake in normal population. However, other data reported that the plasma antioxidant capacity is improved by the increase of

polyphenols intake, higher concentration of vitamin E, vitamin C, β-carotene and uric acid (Princen *et al.,* 1998; Manach *et al.,* 2005; Bahorun *et al.,* 2012).

Interestingly, the biological correlations observed here were in particular with UP at the opposite of tea cups number per day and PP. There were no similar findings in the literature. However, Medina-Remon *et al*. (2011) also reported that UP was inversely associated with systolic and diastolic pressures. In one hand, the biological effects of tea intake depend on its polyphenols bioavailability, absorption and exposure time related to tea intake frequency per day. Furthermore, high inter-individual variability of absorption and metabolism efficiency should be considered. The influence of tea intake in human, assessed as number of tea cups consumed per day, on metabolic parameters, lipid peroxidation and antioxidant status is controversial. This may be ascribed to several confounding factors including dietary habits, behavior, physiological and lifestyle (Henson *et al.,* 2012; Polychronopoulos *et al.,* 2008; Mackenzie *et al.,* 2007). In the other hand, Plasma concentration in total polyphenols may not be considered as an objective measurement of polyphenols intake, since it is highly influenced by repeated ingestion (Williamson and Manach, 2005). Indeed, plasma pharmacokinetic of polyphenols depends on its concentration and exposure time (Scalbert *et al.,* 2002). Total polyphenols excretion is widely accepted as biomarker of total polyphenols intake. Ito *et al.* (2005) quantified polyphenols in human urine to estimate polyphenols recovery after ingestion of six different polyphenol-rich beverages including tea and found that urinary excretion of epicatechin can be selected as potential and specific biomarkers of tea intake.Mennen *et al.* (2006), Krogholm *et al.* (2004) and Medina-Remon *et al*. (2009) also reported an association between polyphenol-rich beverages consumption and the concentration of polyphenols in urine samples, suggesting that total polyphenols in urine can be considered as a nutritional biomarker.

The most bioactive polyphenol compounds and metabolites determination may be of great interest (Manach *et al.,* 2010). Indeed, different polyphenols catabolites can be seen throughout dietary polyphenols routes in human body (Salbert *et al.,* 2002). Polyphenols are converted by methylation, sulfation, or glucuronidation (Del Rio *et al.,* 2010). The biotransformation of catechins begins at the intestinal mucosa, where they are transformed by colonic bacteria. The liver plays a secondary role to further modify the conjugated polyphenols (Del Rio *et al.,* 2010; Chow and Hakim, 2011). The final stage is occurring in kidney where the recovery of polyphenol is excreted in urine. In the study of Del Rio *et al.* (2010) who investigated the catabolism of green tea flavanols, plasma pharmacokinetic and urinary excretion in a sample of volunteers consuming 400 ml of ready-to-drink green tea, they found that EGCG was the major polyphenol in plasma, present in its non-metabolized form.The main epigallocatechin metabolites excreted in urine were epigallocatechin-O-glucuronide and its methoxy counterpart methyl-O-epigallocatechin-O-glucuronide. The main epicatechin metabolite was methyl-epicatechin-sulfate. The major class of colonic catabolites is represented by M6 and M60 valerolactones. The authors reported that colonic catabolites are by far the main excreted metabolites and their urinary concentration is 10 times higher than that of flavanol conjugates. On the basis of our data and those of Del Rio *et al.* (2010) , we can speculate that the final flavonols metabolites found in urine of T2D are those responsible of the observed beneficial effects, since only UP showed correlation with biological parameters. Further investigations are still needed to confirm this hypothesis.

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