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# Tea Enhances Insulin Activity

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The most widely known health benefits of tea relate to the polyphenols as the principal active ingredients in protection against oxidative damage and in antibacterial, antiviral, anticarcinogenic, and antimutagenic activities, but polyphenols in tea may also increase insulin activity. The objective of this study was to determine the insulin-enhancing properties of tea and its components. Tea, as normally consumed, was shown to increase insulin activity >15-fold in vitro in an epididymal fat cell assay. Black, green, and oolong teas but not herbal teas, which are not teas in the traditional sense because they do not contain leaves of *Camellia senensis*, were all shown to increase insulin activity. High-performance liquid chromatography fractionation of tea extracts utilizing a Waters SymmetryPrep C18 column showed that the majority of the insulin-potentiating activity for green and oolong teas was due to epigallocatechin gallate. For black tea, the activity was present in several regions of the chromatogram corresponding to, in addition to epigallocatechin gallate, tannins, theaflavins, and other undefined compounds. Several known compounds found in tea were shown to enhance insulin with the greatest activity due to epigallocatechin gallate followed by epicatechin gallate, tannins, and theaflavins. Caffeine, catechin, and epicatechin displayed insignificant insulin-enhancing activities. Addition of lemon to the tea did not affect the insulin-potentiating activity. Addition of 5 g of 2% milk per cup decreased the insulin-potentiating activity one-third, and addition of 50 g of milk per cup decreased the insulin-potentiating activity ~90%. Nondairy creamers and soy milk also decreased the insulin-enhancing activity. These data demonstrate that tea contains in vitro insulin-enhancing activity and the predominant active ingredient is epigallocatechin gallate.

**KEYWORDS: Glucose; insulin; diabetes; polyphenols; epigallocatechin gallate; epigallocatechin**

## INTRODUCTION

Tea has a long history as a folk remedy, but the beneficial medicinal properties have mainly been elucidated in the past 20 years. The most convincing evidence for the medicinal properties of tea have been summarized by Mukhtar et al. (*1*) and Hara (*2*). Tea protects against chemically induced tumor initiation and promotion and progression of benign tumors to malignancy (*1*, *2*). The majority of the benefits associated with tea and atherosclerosis (*3*), hypertension (*4*), infectious diseases (*5*), immune response (*6*), and longevity (*7*) are generally attributed to the antioxidant activities of tea.

Folk remedies have also included the antidiabetic properties of tea for decades (*8*, *9*). Xiaoke tea is used as a traditional Chinese treatment for diabetes mellitus (*10*). Cerasee, a wild variety of *Momordica charantia*, is traditionally prepared as a tea for the treatment of diabetes mellitus in the West Indies and Central America (*11*). Aqueous extracts of both of these teas have been shown to reduce the blood glucose of steptozotocin (STZ) diabetic mice (*10*, *11*). A hot water extract of black tea (*Camellia sinensis*) has also been shown to lower blood glucose in STZ-treated rats (*11*). Green tea has also been reported to display antidiabetic properties (*12*−*14*). Administration of bio-tea to mice also leads to decreases in blood glucose (*15*). Human studies have failed to confirm the significant hypoglycemic effects observed in STZ-treated animals, but conclusive studies have not been completed.

The active components are not known, but both catechins and polysaccharides have been postulated as the active antidiabetic components. Epicatechin gallate was shown to have the highest inhibitory capacity of the catechins tested on glucose uptake using Caco-2 cells (*16*) and on the sodium-dependent glucose transporter, SGLT1 (*17*). Epicatechin gallate also inhibited glucose uptake in the brush border membrane vesicles obtained from the rabbit small intestine (*16*). For complex carbohydrates to be absorbed, the individual sugars must be hydrolyzed from the parent molecule prior to absorption. Catechins have been shown to inhibit enzymes that hydrolyze carbohydrates including α-amylase. A mixture of green tea catechins was also shown to suppress increases in blood glucose and insulin following carbohydrate ingestion in rats (*2*).

In this study, we have shown that in addition to its antibacterial, antiviral, anticarcinogenic, and antimutagenic activities, tea also enhances insulin activity with epigallocatechin

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gallate being the most active component. We used an in vitro epididymal assay (*18*), which has been shown to respond to products that improve insulin activity (*19*−*21*), to measure insulin-enhancing activity. The earlier studies reporting antihyperglycemic properties of tea and benefits on risk factors associated with cardiovascular diseases including hypertension may be related in part to the activity of tea components on insulin function.

## MATERIALS AND METHODS

Insulin-enhancing properties of tea and its components were assayed in rat epididymal adipocytes according to the method of Anderson et al. (*18*). Briefly, 0.43 $\mu$Ci of [U-$^{14}$C]glucose, 72 $\mu$g of glucose, and adipocytes were incubated with insulin and/or tea or its components in a final reaction volume of 2 mL of Krebs−Ringer phosphate buffer, pH 7.4. Quantitation of $^{14}CO_2$ release by the cells was done using benzethonium hydroxide (Sigma-Aldrich, St. Louis, MO) as a trapping agent, which is a replacement for hyamine hydroxide (*18*). Similar results were obtained by trapping $^{14}CO_2$ and measuring $^{14}$C incorporation into lipids. For the incorporation into lipids, 2 mL of Dole's solution (800 mL of 2-propanol, 200 mL of heptane, and 20 mL of 1 N sulfuric acid) was added to the incubation mixture (*18*), followed by vortexing. After 1 h at room temperature, 1.5 mL of heptane was added, vortexed, and centrifuged for 5 min at 1000 rpm. A 1 mL aliquot of the heptane layer was removed and counted by liquid scintillation. The insulin activity ratio was calculated by dividing the basal counts per mimute into that of the activity due to tea or its components.

Tea extracts were fractionated by high-pressure liquid chromatography (HPLC) using a SymmetryPrep C18, 7 $\mu$m column, 7.8 × 300 mm, equilibrated with 90% 0.05 N acetic acid (flow rate = 4 mL/min) and 10% acetonitrile for 28 min, followed by stepwise increase to 20% acetonitrile at 48 min, 25% at 60 min, and 100% at 70 min. The system was a Waters HPLC chromatograph with Millennium 2100 software and a Waters 996 ultraviolet absorbance detector (Waters Corp., Milford, MA).

Chromatography grade acetonitrile and reagent grade chemicals were purchased from Fisher Scientific Co. (Pittsburgh, PA). Polyphenols and commercially available components of tea were purchased from Sigma Chemical Co., St. Louis, MO. Tea was purchased from local merchants.

Statistical analyses of the samples were determined using analysis of variance followed by the Tukey test to determine differences among several samples (SigmaStat, Jandel Scientific, San Rafael, CA). Samples were assayed at least three times, and values were considered to be significantly different at $p < 0.05$.

## RESULTS

The insulin-enhancing activity of green tea is shown in **Figure 1**. One tea bag, ~2 g of tea, was added to one cup of hot water, 237 mL and steeped for 5 min; the tea was allowed to cool and assayed for insulin-enhancing activity as described under Materials and Methods. The dry weight of the original tea extract was 3.8 mg/mL. The tea was then assayed for insulin-enhancing activity utilizing the insulin-dependent breakdown of glucose to carbon dioxide or the incorporation of glucose into lipids (shown in **Figure 1**). Similar results were obtained using both methods. Assaying 25 $\mu$L directly of green tea, containing <20 ug of dry solids, in the 2 mL assay medium potentiated insulin activity severalfold (**Figure 1**). (The exact value for the potentiation varies depending upon the age of the rats and assay conditions and chemicals.) Dilution of the tea resulted in corresponding decreases in insulin potentiation. Maximal insulin potentiation could be observed in the absence of added insulin. There was often an inhibition of insulin-enhancing activity ratio when higher levels of tea were tested (**Figure 1**). Similar results were observed with black and oolong teas (data not shown). We tested the insulin-potentiating activity of >40 black, green, and oolong teas, and all were shown to enhance insulin activity in the insulin-potentiating epididymal fat cell assay. We were unable to detect insulin-enhancing activity in the instant teas except for one brand that consistently displayed significant activity. Herbal and commercially prepared iced teas displayed minimal insulin-potentiating activity.

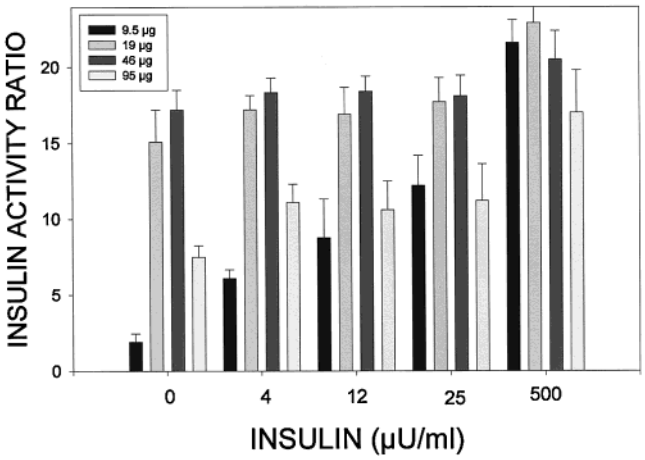


**Figure 1.** Insulin-enhancing activity of green tea at various levels of insulin. One tea bag, ~2 g of tea, was added to one cup of hot water, 237 mL, and steeped for 5 min; the tea was allowed to cool and assayed for insulin-enhancing activity as described under Materials and Methods. Dry weight of original tea extract was 3.8 mg/mL. Values in figure legend refer to the dry weight of the tea sample assayed. Values are for triplicate analyses.

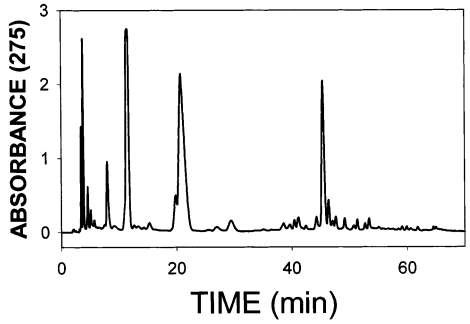


**Figure 2.** HPLC chromatogram of a representative green tea. Five hundred microliters of tea described in **Figure 1** was applied to a SymmetryPrep C18, 7 $\mu$m column, 7.8 × 300 mm, equilibrated with 90% 0.05 N acetic acid and 10% acetonitrile (flow rate = 4 mL/min) for 28 min, followed by stepwise increase to 20% acetonitrile at 48 min, 25% at 60 min, and 100% at 70 min. The system was a Waters HPLC chromatography system with Millennium 2100 software and a Waters 996 ultraviolet absorbance detector (Waters Corp., Milford, MA).

The separation of green tea is shown in **Figure 2**. The majority of the activity of the tea was found in the peak eluting in the region of 20−23 min (**Table 1**). Epicatechin and epigallocatechin gallate both eluted in this region (**Figure 3**). The activity in this region is due to epigallocatechin gallate because epicatechin does not display insulin-potentiating activity (**Table 2**). Separation of these two components also demonstrated that the activity was due to epigallocatechin gallate. Oolong tea displayed a similar HPLC profile (**Figure 4**) and insulin-enhancing activity (**Table 3**). The most active tea component was epigallocatechin gallate, but tannins, theaflavins, and epicatechin gallate also display insulin-enhancing activity and account for the number of fractions of black tea that display insulin-enhancing activity (**Table 4**).

Milk (2%) was shown to inhibit insulin potentiation at 5 g (1 teaspoon) per cup (237 mL), decreasing activity roughly 33%;

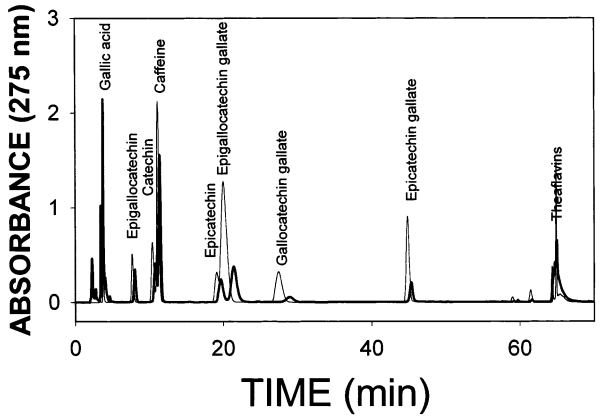


**Figure 3.** HPLC chromatogram of tea standards ± 2% milk. The lighter line represents the tea standards and the darker line the same tea standards plus 1 teaspoon (5 g) of 2% milk per cup (237 mL). The amount of tea standards added to the column was similar to that representative of extracts of tea; gallic acid, 20 $\mu$g; epigallocatechin, 150 $\mu$g; catechin, 100 $\mu$g; caffeine, 50 $\mu$g; epicatechin, 75; epigallocatechin gallate, 200 $\mu$g; gallocatechin gallate, 50 $\mu$g; epicatechin gallate, 50; and theaflavins, 45 $\mu$g. Tannins gave a mixture of peaks and were not added to the test mixture. Conditions were the same as those described in **Figure 2**.

**Table 1.** Insulin Activity Ratios of Fractions from Green Tea[a]

| fraction time (min) | insulin activity ratio | fraction time (min) | insulin activity ratio |
|---|---|---|---|
| 2.5–6 | 1.1 | 38–44 | 1.2 |
| 6–13 | 1.3 | 44–47 | 1.1 |
| 13–20 | 2.4 | 47–52 | 1.1 |
| 20–23 | 6.1 | 52–60 | 0.9 |
| 23–38 | 1.0 | 60–68 | 1.0 |

[a] Fractions were collected as described from the chromatogram shown in **Figure 2**. Individual fractions were concentrated by rotoevaporation to 0.5 mL and diluted 5-fold in water prior to assay.

**Table 2.** Insulin Activity Ratio of Tea Components

| tea component | insulin activity ratio[a] |
|---|---|
| caffeine | 0.9 ± 0.1d |
| catechin | 1.0 ± 0.1d |
| epicatechin | 1.0 ± 0.1d |
| epicatechin gallate | 5.0 ± 2.5b |
| epigallocatechin | 1.7 ± 0.2bc |
| epigallocatechin gallate | 17.5 ± 2.3a |
| gallic acid | 1.7 ± 0.1bc |
| gallocatechin gallate | 1.3 ± 0.4cd |
| tannins | 5.0 ± 0.2b |
| theaflavins | 2.4 ± 0.4b |

[a] Values are insulin enhancing ratio using an epididymal fat cell assay system (*18*). A ratio of 1 indicates no increase in activity. Values are the mean ± SEM of six determinations. Values with different letters are significantly different at $p < 0.05$ by Tukey's test (Sigmastat, Jandel Scientific).

50 g of milk decreased the activity >90%. Water was added to the control sample to correct for dilution of the sample to which was added 50 g of milk. Results were similar for whole and skim milks. We also tested several nondairy creamers and soy milk, and they also inhibited insulin-enhancing activity. Lemon juice had no significant effects on insulin-enhancing activity.

Addition of milk, 5 g per 237 mL, to a mixture of tea components led to precipitation of primarily epigallocatechin gallate, gallocatechin gallate, and epicatechin gallate (**Figure 3**, heavy line). Addition of 50 g of milk per 237 mL led to essentially complete loss of these components (data not shown).

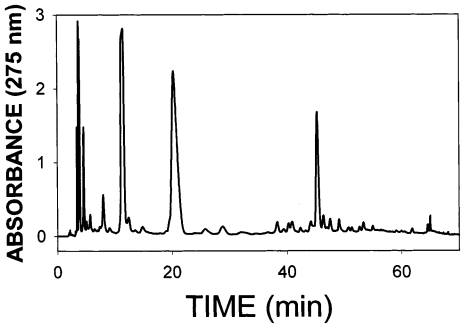


**Figure 4.** HPLC chromatogram of a representative oolong tea. Conditions were as described in **Figure 2**. Dry weight of tea extract was 3.4 mg/mL.

**Table 3.** Insulin Activity Ratios of Fractions from Oolong Tea[a]

| fraction time (min) | insulin activity ratio | fraction time (min) | insulin activity ratio |
|---|---|---|---|
| 2.5–6 | 1.5 | 38–44 | 1.1 |
| 6–13 | 1.2 | 44–47 | 0.9 |
| 13–20 | 1.3 | 47–52 | 1.3 |
| 20–23 | 4.5 | 52–60 | 1.4 |
| 23–38 | 1.0 | 60–68 | 1.3 |

[a] Fractions were collected as described for the chromatogram shown in **Figure 4**. Individual fractions were concentrated by rotoevaporation to 0.5 mL and diluted 5-fold in water prior to assay.

**Table 4.** Insulin Activity Ratios of Fractions for Black Tea[a]

| fraction time (min) | insulin activity ratio | fraction time (min) | insulin activity ratio |
|---|---|---|---|
| 2.5–6 | 2.8 | 38–44 | 1.7 |
| 6–13 | 4.2 | 44–47 | 1.5 |
| 13–20 | 1.6 | 47–52 | 2.4 |
| 20–23 | 6.5 | 52–60 | 4.9 |
| 23–38 | 4.3 | 60–68 | 0.9 |

[a] Fractions were collected as described for the chromatogram shown in **Figure 2**. Individual fractions were concentrated by rotoevaporation to 0.5 mL and diluted 5-fold in water prior to assay.

## DISCUSSION

Tea was shown to have insulin-potentiating activity, and the primary active component was shown to be epigallocatechin gallate. Other components in tea including epicatechin gallate, tannins, and theaflavins were also shown to enhance insulin activity. The presence of caffeine, which has been shown to induce a rise in blood glucose that is insulin independent (*22*), did not have a significant effect on insulin-enhancing activity; both caffeinated and decaffeinated teas potentiated insulin similarly, and caffeine alone was devoid of insulin-enhancing activity (**Table 2**).

The mechanism of the antidiabetic activity of the black and green tea extracts in the STZ-treated animals is both preventive and curative (*23*). In the STZ-treated animals, there are many surviving B-cells and the effects of the tea on cell regeneration cannot be ignored. Tea extracts were also shown to protect the B-cells from the toxic effects of STZ (*23*). Tea polyphenols also inhibit 2-amylase, a type of digestive enzyme that works on the starch present in saliva or pancreatic juice, and may have an indirect effect on glucose and insulin levels. In human trials, when tea catechins were ingested (200−500 mg) prior to the ingestion of 50 g of starch, there was a suppression of the elevation of glucose and corresponding insulin levels (*2*). Intestinal glucose uptake is markedly inhibited by green tea

polyphenols (*17*), with the inhibitory activity being most pronounced in polyphenols having galloyl residues. This is consistent with our results demonstrating that polyphenols with galloyl residues have the greatest effects on insulin-enhancing activity (**Table 2**). Similarly, epigallocatechin gallate was also shown to have greater effects than related catechins on insulin-related variables in rats (*24*).

In this in vitro study, there was a direct effect of epigallocatechin gallate and epicatechin gallate on insulin activity. This suggests that effects would also be present in vivo because upon ingestion of tea, polyphenols are spread throughout the body and can be detected in blood, urine, and feces (*25*). They probably exert their actions directly at the cellular level rather than indirectly via intestinal effects (*25*).

Addition of milk and nondairy creamers led to a decrease in insulin-enhancing activity due to precipitation of the epigallocatechin, gallocatechin gallate, and epicatechin gallate. However, consumption of the mixture of milk and green or black tea by humans does not impair the bioavailability of the tea catechins (*26*). Leenen et al. (*27*) reported that addition of milk to tea did not abolish the increase in plasma antioxidant activity due to tea determined using plasma ferric reducing activity. It is possible that when the precipitated mixture of milk and tea catechins is consumed, the catechin−milk complexes dissociate and the tea catechins are absorbed. However, Tewari et al. (*28*) observed that the antioxidant potentials of tea alone, or tea plus lemon, were greater than those observed when milk was added to the tea. This is consistent with our results, where tea alone and tea plus lemon juice displayed greater in vitro insulin-enhancing activity than tea plus milk. Similarly, consumption of black tea without milk but not with milk also increased the antioxidant potential of plasma (*29*).

If there is a direct effect of primarily epigallocatechin gallate and epicatechin gallate on insulin function, why have previous human studies failed to detect consistent changes in blood glucose? The answers to this may involve measurements of insulin rather than glucose, which is more likely to illicit an effect. A more effective insulin may lead to lower levels of insulin with no change in glucose. Therefore, measuring changes in glucose following tea consumption would lead to no effect when indeed there would be an effect on insulin function. In one of our recent studies involving chromium, which also increases insulin activity (*30*, *31*), we observed no changes in glucose clearance but a very significant effect on circulating insulin (*31*). Polyphenols also clear rapidly from the blood, and measuring the effects of tea following an overnight fast would likely yield no effect of tea consumption because the half-life in humans for epigallocatechin gallate is $<6$ h, and those for epigallocatechin and epicatechin are $<4$ h (*32*).

In summary, green, black, and oolong, but not herbal, teas were shown to significantly enhance in vitro insulin activity. The primary active component in tea was shown to be epigallocatechin gallate.

## LITERATURE CITED


(1) Mukhtar, H.; Katiyar, S. K.; Agarwal, R. Green tea and skin−anticarcinogenic effects. *J. Invest. Dermatol.* **1994**, *102*, 3−7.

(2) Hara, Y. *Green Tea, Health Benefits and Applications*; Dekker: New York, 2001.

(3) Kono, S.; Shinchi, K.; Ikeda, N.; Kanai, F.; Imanishi, K. Green tea consumption and serum lipid profiles: A cross-sectional study in northern Kyoshu, Japan. *Prev. Med.* **1992**, *21*, 526−531.

(4) Abe, Y.; Umemura, S.; Sugimoto, K.; Hirawa, N.; Kato, Y.; Yokoyama, N.; Yokoyama, T.; Iwai, J.; Ishii, M. Effect of green tea rich in $\gamma$-aminobutyric acid on blood pressure of Dahl salt-sensitive rats. *Am. J. Hyperten.* **1995**, *8*, 74−79.

(5) Nakayama, M.; Suzuki, K.; Toda, M.; Aqaba, S.; Hard, Y.; Shimamura, T. Inhibition of the infectivity of influenza virus by tea polyphenols. *Antiviral Res.* **1993**, *21*, 289−299.

(6) Hu, Z. Q.; Toda, M.; Okubo, S.; Hara, Y.; Shimamura, T. Mitogenic activity of epigallocatechin gallate on B-cells and investigation of its structure−function relationship. *Int. J. Immunopharmacol.* **1992**, *14*, 1399−1407.

(7) Sadakata, S.; Fukao, A.; Hisamichi, S. Mortality among female practitioners of Chanyou (Japanese "tea-ceremony"). *Tohoku J. Exp. Med.* **1992**, *166*, 475−477.

(8) Konayagi, S.; Minowada, M. On the effect of green tea for diabetes mellitus. *Study of Physiology*; Kyoto University: Kyoto, Japan, 1933; Vol. 10, pp 449−454.

(9) Konayagi, S.; Minowada, M. A medicine for diabetes mellitus, minowarin. *Rinshou to Yakubatsu* **1935**, *4*, 1140−1142.

(10) Hale, P. J.; Horrocks, P. M.; Wright, A. D.; Fitzgerald, M. G.; Nattrass, M.; Bailey, C. J. Xiaoke tea a Chinese herbal treatment for diabetes mellitus. *Diabetic Med.* **1989**, *6*, 675−676.

(11) Bailey, C. J.; Day, C.; Turner, S. L.; Leatherdale, B. A. Cerasee, a traditional treatment for diabetes. Studies in normal and steptozotocin diabetic mice. *Diabetes Res.* **1985**, *2*, 81−84.

(12) Shimizu, M.; Wada, S.; Hayashi, T.; Ikegaya, K.; Ogaku, S.; Ynano, S.; Mortia, N. Studies on hypoglycemic constituent of Japanese tea. *Ykugaku Zasshi* **1988**, *108*, 964−970.

(13) Isigaki, K.; Takakuwa, T.; Takeo, T. Anti-diabetes mellitus effect of water-soluble tea polysaccharide. *Proceedings of the International Symposium on Tea Science*; Yamanishi, T., Ed.; 1991; pp 240−242.

(14) Matsumoto, N.; Ishigaki, F.; Ishigaki, A.; Iwashina, H.; Hard, Y. Reduction of blood glucose levels by tea catechin. *Biosci., Biotechnol., Biochem.* **1993**, *32*, 525−527.

(15) Shenoy, C. Hypoglycemic activity of bio-tea in mice. *Indian J. Exp. Biol.* **2000**, *38*, 278−279.

(16) Shimizu, M.; Kobayashi, Y.; Suzuki, M.; Satsu, H.; Miyamamoto, Y. Regulation of intestinal glucose transport by tea catechins. *Biofactors* **2000**, *13*, 61−65.

(17) Kobayashi, Y.; Suzuki, M.; Satsu, H.; Arai, S.; Hard, Y.; Suzuki, K.; Miyamoto, Y.; Shimizu, M. Green tea polyphenols inhibit the sodium dependent glucose transporter of intestinal epithelial cells by a competitive mechanism. *J. Agric. Food Chem.* **2000**, *48*, 5618−5623.

(18) Anderson, R. A.; Brantner, J. H.; Polansky, M. M. An improved assay for biologically active chromium. *J. Agric. Food Chem.* **1978**, *26*, 1219−1221.

(19) Khan, A.; Bryden, A.; Polansky, M. M.; Anderson, R. A. Insulin potentiating factor and chromium content of selected foods and spices. *Biol. Trace Elem. Res.* **1990**, *24*, 183−188.

(20) Berrio, L. F.; Polansky, M. M.; Anderson, R. A. Insulin activity: stimulatory effects of cinnamon and brewer's yeast as influenced by albumin. *Hormone Res.* **1992**, *37*, 225−229.

(21) Broadhurst, C. L.; Polansky, M. M.; Anderson, R. A. Insulin-like biological activity of culinary and medicinal plant aqueous extracts in vitro. *J. Agric. Food Chem.* **2000**, *48*, 849−852.

(22) Pizziol, A.; Tikhonoff, V.; Paleari, C. D.; Russo, E.; Mazza, A.; Ginocchio, G.; Onesto, C.; Pavan, L.; Casiglia, E.; Pessina, A. C. Effects of caffeine on glucose tolerance: a placebo controlled study. *Eur. J. Clin Nutr.* **1998**, *52*, 846−849.

(23) Gomes, A.; Vedasiromoni, J. R.; Das, M.; Sharma, R. M.; Ganguly, D. K. Anti-hyperglycemic effect of black tea (*Camellia sinesis*) in rat. *J Ethnopharmacol.* **1995**, *45*, 223−226.

(24) Kao, Y. H.; Hiipakka, R. A.; Liao, S. Modulation of the endocrine systems and food intake by green tea epigallocatechin gallate. *Endocrinology* **2000**, *141*, 980−987.

(25) He, Y. H.; Kies, C. Green and black tea consumption by humans: Impact on polyphenol concentrations in feces, blood and urine. *Plant Foods Hum. Nutr.* **1994**, *46*, 221−229.

(26) van het Hof, K. H.; Kivits, G. A.; Westgate, J. A.; Tijburg, L. B. Bioavailability of catechins from tea: the effect of milk. *Eur. J. Clin. Nutr.* **1998**, *52*, 356−359.

(27) Leenen, R.; Rordenburg, A. J.; Tijburg, L. B.; Wiseman, S. A. A single dose of tea with or without milk increases plasma antioxidant activity in humans. *Eur. J. Clin. Nutr.* **2000**, *54*, 87−92.

(28) Tewari, S.; Gupta, V.; Bhattacharya, S. Comparative study of antioxidant potential of tea with and without additives. *Indian J. Physiol. Pharmacol.* **2000**, *44*, 215−219.

(29) Langley-Evans, S. C. Consumption of black tea elicits an increase in plasma antioxidant potential in humans. *Int. J. Food Sci. Nutr.* **2000**, *51*, 309−315.

(30) Anderson, R. A. Chromium, glucose intolerance and diabetes. *J. Am. Coll. Nutr.* **1998**, *17*, 548−555.

(31) Striffler, J. S.; Polansky, M. M.; Anderson, R. A. Overproduction of insulin in the chromium-deficient rat. *Metabolism* **1999**, *48*, 1063−1068.

(32) Yang, C. S.; Chen. L.; Lee, M.-J.; Balentine, D.; Kuo, M. C.; Schantz, S. P. Blood and urine levels of tea catechins after ingestion of different amounts of green tea by human volunteers. *Cancer Epidemiol. Biomarkers Prev.* **1998**, *7*, 351−354.

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