JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

ARTICLE

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# Cholesterol Metabolism Is Modulated by Quercetin in Rats

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**ABSTRACT:** Quercetin has attracted much attention recently because of its antioxidant capacity and potential in the prevention of chronic degenerative diseases. However, its actions and the mechanisms involved are not completely understood. In this study, male Wistar rats were fed a diet containing 0.5% quercetin for 14 days. Serum samples were collected at the end of the experiment, and the overall serum metabolic profile was investigated by $^{1}$H nuclear magnetic resonance (NMR)-based metabolomic analysis. Remarkable changes in the serum metabolic profile were manifested with the relative increase in the levels of lactate and low-density lipoprotein/very low-density lipoprotein (LDL/VLDL) and reduction in glucose, high-density lipoprotein (HDL), and some amino acids after quercetin exposure. Biochemical measurements confirmed that the serum low-density lipoprotein cholesterol (LDL-C) level was increased significantly after quercetin treatment. Our current findings indicate that quercetin can induce a remarkable change in cholesterol metabolism. Further studies are needed to investigate the molecular mechanisms and the possible links to the health effects or toxic actions of quercetin.

**KEYWORDS:** Quercetin, metabolomics, NMR, cholesterol metabolism, rat

## INTRODUCTION

Dietary consumption of fruits and vegetables has been demonstrated to be inversely related to the occurrence of chronic degenerative diseases.[1−4] Flavonoids are thought to contribute significantly to the beneficial actions of fruits and vegetables.[5−8] Quercetin, one of the most common compounds in the flavonoid family, is ubiquitously present in fruits and vegetables. Numerous *in vitro* studies have suggested that quercetin is a potent antioxidant.[8,9] Our previous studies demonstrated that quercetin could be absorbed extensively in the duodenum, jejunum, ileum, and colon after *in situ* perfusion in rats. Oral administration of quercetin increased plasma antioxidant capacity and protected against hepatic ischemia− reperfusion injury significantly in rats.[10−12] It has also been shown that quercetin plays roles in other biological processes, such as gene expression, cellular proliferation, apoptosis, and platelet aggregation.[13−16] However, many potential actions played by quercetin *in vivo* are still not well-defined.

Nuclear magnetic resonance (NMR) spectroscopy of biofluids has brought a new technique to probing the changes of metabolic processes induced by various stimuli. Typically, NMR spectra of the serum contain abundant signals derived from hundreds of endogenous molecules. The data processing can be simplified by application of multivariate statistical analysis, including data reduction and pattern recognition techniques, such as principal component analysis (PCA) and partial least-squares−discriminant analysis (PLS−DA). The metabolic changes induced by physiological challenge or stimulus, administration of a xenobiotic, genetic modification, or dietary changes can be visualized clearly by the combination of NMR spectroscopy and pattern recognition techniques.[17−19] Several studies have shown that NMR-based metabolomics are useful in investigating the changes in metabolic profiles in many fields. On the basis of NMR analysis, the modulation of energy metabolism by soy isoflavone in humans had been demonstrated by Solanky et al. They also found that epicatechin consumption resulted in reduced urinary concentrations of taurine, citrate, dimethylamine, and 2-oxoglutarate in rats and identified some metabolites of epicatechin in the urine.[20−22] Daykin et al. identified hippuric acid as the major urinary metabolite of black tea using a NMR technique.[23] Van Dorsten et al. found that NMR-based metabolomic analysis could differentiate the metabolic effects induced by green tea, black tea, and caffeine.[24]

In the present study, the overall metabolic profile of the serum sampled after dietary quercetin supplementation in rats were screened by a $^{1}$H NMR-based metabolomic approach. Our objective was to provide insight into the effects of quercetin on internal metabolic processes, which will be helpful in defining the actions played by quercetin *in vivo* and the mechanisms possibly involved.

## MATERIALS AND METHODS

**Animal Handling and Quercetin Supplementation.** A total of 10 male Wistar rats, weighing 260−300 g, were purchased from the Laboratory Animal Center, Chinese Academy of Military Medical Sciences, Beijing, China, and housed individually in stainless-steel cages in a well-ventilated room. The temperature was controlled between 18 and 24 °C and relative humidity between 40 and 60%. The light/dark cycles were alternated every 12 h. Food and tap water were provided *ad libitum*. All procedures were performed in accordance with the current Chinese legislation on the care and use of laboratory animals and

**Received:** September 13, 2010
**Accepted:** December 20, 2010
**Revised:** November 5, 2010
**Published:** January 20, 2011

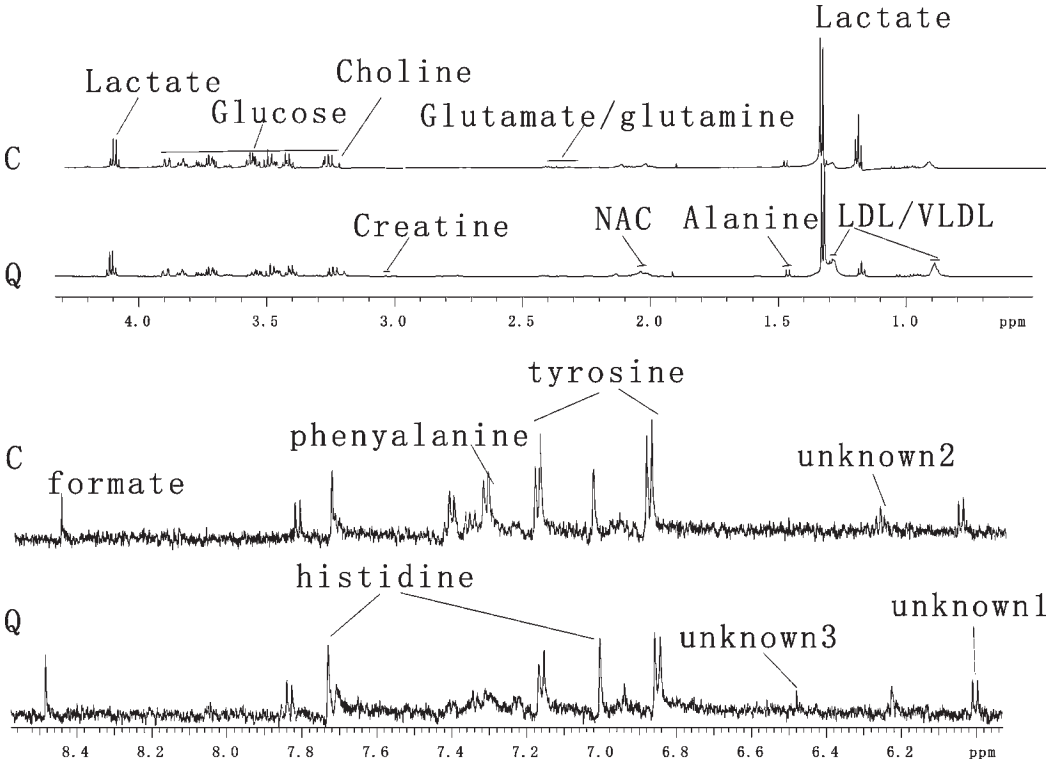


**Figure 1.** Representative 600 MHz CPMG $^{1}$H NMR spectra of serum samples from rats (C, control; Q, quercetin). The vertical scale in the aromatic region (bottom) was magnified 4 times compared to that in the aliphatic region (top).

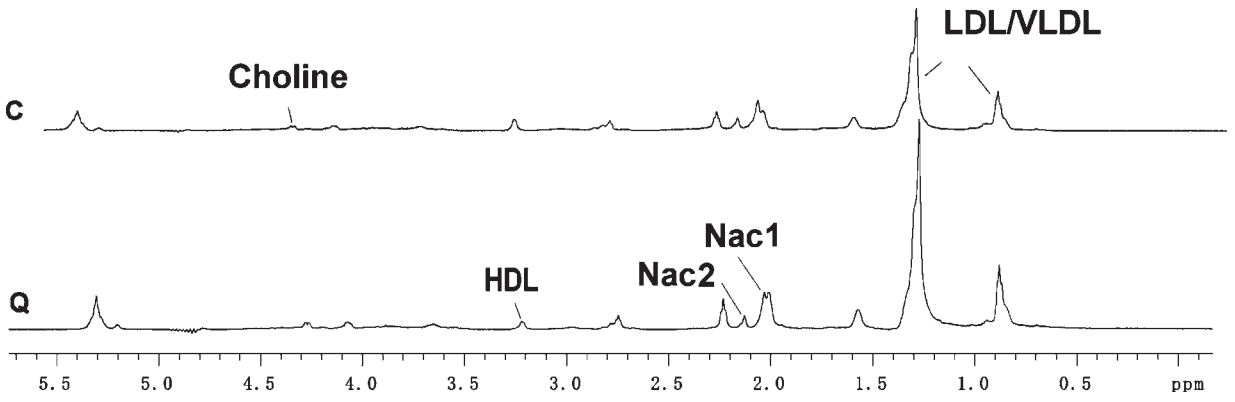


**Figure 2.** Representative 600 MHz LED $^{1}$H NMR spectra of serum samples from rats (C, control; Q, quercetin).

approved by the Department of Scientific Program Management of the college.

After the rats were acclimatized on a polyphenol-free semisynthetic diet (AIN-93 formula) [25] for 5 days, they were divided randomly into the control group and quercetin-supplemented group. The rats in the control group continued to be fed the AIN-93 diet, while those in the quercetin-supplemented group were switched to a quercetin (Sigma-Aldrich, Inc., St. Louis, MO)-supplemented AIN-93 diet. We chose 0.5% as the supplemental dose in the present study, because Ameho et al. showed that it was effective in increasing the plasma quercetin concentration in rats.[26] After 14 days of feeding, all rats were fasted overnight and blood samples were collected from the orbital plexus under ether anesthetization. The serum was separated and stored at −20 °C before being subjected to NMR analysis and biochemical measurement.

**Acquisition of $^{1}$H NMR Spectra.** The sample preparation and NMR analysis were performed as described elsewhere,[27] with minor modifications. Briefly, an aliquot of 300 $\mu$L of thawed serum was mixed with 100 $\mu$L of 1 mmol/L sodium 3-trimethylsilyl-propionate-2,2,3,3-d4 (TSP) in $D_2O$. The $D_2O$ and TSP provided the deuterium lock signal for the NMR spectrometer and chemical-shift reference ($\delta$ 0.0), respectively. $^{1}$H NMR spectra were obtained at 599.69 MHz on a Varian INOVA 600 NMR spectrometer. A T2 relaxation-edited Carr−Purcell−Meiboom−Gill (CPMG) spin−echo experiment was used to attenuate broad signals from proteins and lipoproteins, resulting in spectra with the signals mostly from small metabolites because of their long transverse relaxation time. In addition, diffusion-edited experiment was also carried out using a bipolar pulse pair longitudinal eddy current delay (LED) pulse sequence to obtain the spectra with signals mainly from lipoproteins. Water suppression was applied during a relaxation delay of 2 s and a mixing time of 150 ms. A total of 64 free reduction decays were collected into 64 000 data points, with a spectral width of 7002.8 Hz, an acquisition time of 4.68 s, and a total pulse recycle delay of

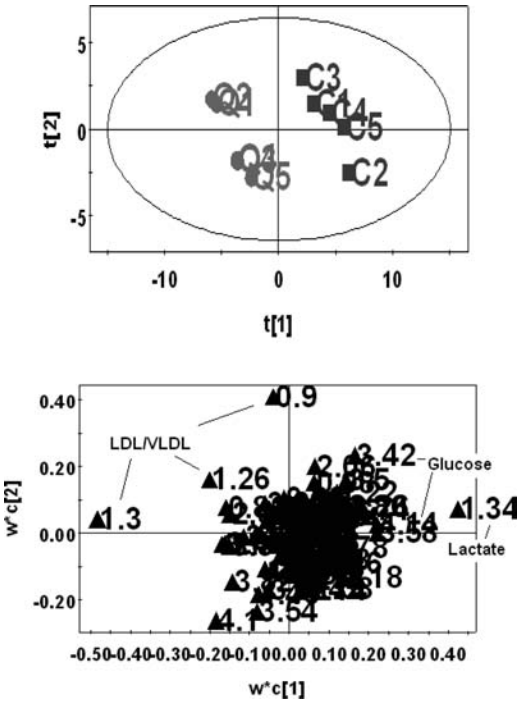


**Figure 3.** Score plot (top) and loading plot (bottom) derived from PLS−DA of the aliphatic region of CPMG $^1$H NMR spectral data (C, control; Q, quercetin).

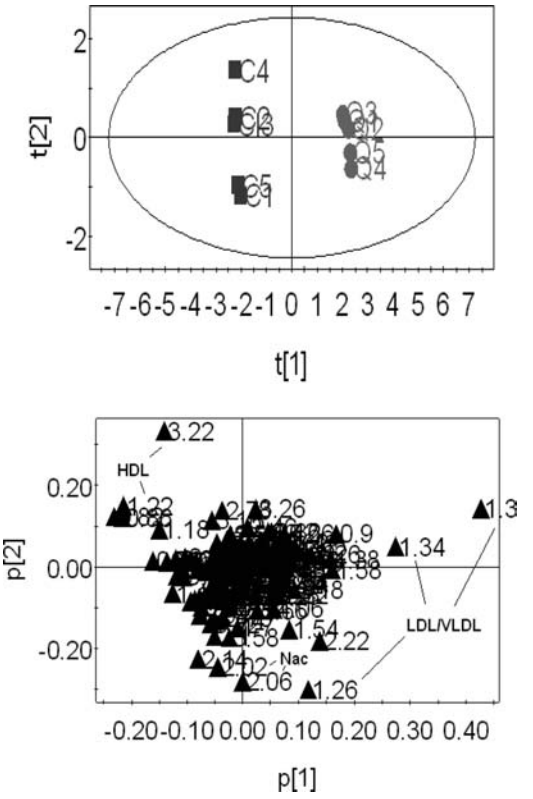


**Figure 4.** Score plot (top) and loading plot (bottom) derived from PCA of LED $^1$H NMR spectral data (C, control; Q, quercetin).

6.68 s. The free reduction decays were multiplied by an exponential weighting function, corresponding to a line broadening of 0.5 Hz before Fourier transformation.

**Data Reduction and Pattern Recognition Analysis of $^1$H NMR Spectra.** All NMR spectra were phased and baseline-corrected, and then, the data were reduced to 225 integrated regions of equal width (0.04 ppm) corresponding to the region from $\delta$ 9.38 to 0.22 using the VNMR 6.1C software package (Varian, Inc., Palo Alto, CA). The region of $\delta$ 6.22−4.62 in the spectra was excluded from the pattern recognition analysis to remove the uncertainty of residual water and urea signals. Each data point was normalized to the sum of its row (i.e., to the total integral for each NMR spectrum), and the values of all variables were mean-centered and Pareto-scaled before PCA or PLS−DA using the SIMCA-P software package (version 10, Umetrics AB, Umea, Sweden). Pareto scaling gives each variable a variance numerically equal to its standard deviation. Score plots of the first two principal components (PCs) were used to visualize the separation of two groups, and loading plots reflected the NMR spectra regions that were altered as a result of quercetin treatment.[28]

**Biochemical Measurement.** The commercial kits supplied by Chemclin Biotech Co., Ltd., Beijing, China, were used to determine fasting serum glucose, lactate, total cholesterol, triglyceride, low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) on a Hitachi 7180 automatic analyzer using corresponding standards and quality-control sera.

**Statistical Analysis.** The data of biochemical measurement were expressed as the mean ± standard deviation and analyzed with Student's $t$ test. Differences were considered significant at $p < 0.05$.

## RESULTS

**$^1$H NMR Spectroscopic Analysis of Serum Samples.** In the aliphatic region of the CPMG $^1$H NMR spectra, the intensity of some endogenous metabolite signals changed in response to quercetin treatment (top of Figure 1). These changes were manifested as a relative increase in the levels of lactate, low-density lipoprotein/very low-density lipoprotein (LDL/VLDL) and a decrease in the level of glucose in comparison to the control. In the aromatic region of the CPMG $^1$H NMR spectra, it was observed that the signal intensity of phenylalanine and tyrosine was decreased. However, there are several peaks remaining to be further identified (bottom of Figure 1).

From the LED $^1$H NMR spectra (Figure 2), it was found that the intensity of signals from lipoproteins (LDL/VLDL) and N-acetyled groups of glycoproteins (Nac1 and Nac2) was clearly changed. The signal intensity of the $CH_3$ groups from fatty acid side chains in LDL/VLDL was remarkably increased in the quercetin-supplemented rats.

**Chemometric Analysis of $^1$H NMR Spectra.** Pattern recognition techniques were used to detect more subtle metabolic changes after quercetin supplementation. In this study, PCA was first used for the aliphatic region (0.22−4.58 ppm) of the CPMG $^1$H NMR spectral data, which contains signals predominantly from small metabolites and provides information about the changes in serum metabolic profiles as induced by quercetin exposure. The results were not satisfactory. Then, PLS−DA models were further calculated. The resulting score plot showed that two clear clusters were formed, indicating that two groups were well-separated because of the differences in their metabolic profiles (top of Figure 3). The loading plot allowed for the determination of the spectral components that were responsible for the separation. It was found that the resonances arising from lactate, glucose, and LDL/VLDL contributed significantly to the observed changes in serum metabolic profile after quercetin treatment (bottom of Figure 3).

PCA were applied to the data set of the LED $^1$H NMR spectra of serum samples. The resulting score plot also revealed clear

**Table 1. Results of Biochemical Measurement**[a]

| group | glucose (mmol/L) | lactate (mmol/L) | TC (mmol/L) | TG (mmol/L) | LDL-C (mmol/L) | HDL-C (mmol/L) |
|---|---|---|---|---|---|---|
| control | 5.95 ± 0.54 | 0.80 ± 0.57 | 1.84 ± 0.15 | 1.38 ± 0.17 | 1.24 ± 0.43 | 0.60 ± 0.21 |
| quercetin | 5.33 ± 0.74 | 0.94 ± 0.49 | 1.93 ± 0.13 | 1.22 ± 0.33 | 1.88 ± 0.34[b] | 0.53 ± 0.01 |

[a] Data are shown as the mean ± standard deviation ($n$ = 5). TC, total cholesterol; TG, total triglyceride; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol. [b] Student $t$ test, $p < 0.05$ compared to the control.

separation of two groups (top of Figure 4). The loading plot showed that LDL/VLDL, HDL, and Nac were among the components that contributed mostly to the separation of two groups (bottom of Figure 4).

**Results of Biochemical Measurement.** The differences in serum glucose, lactate, total cholesterol (TC), total triglyceride (TG), and HDL-C levels between two groups were without statistical significance. However, the level of LDL-C was increased significantly in response to the quercetin treatment (Table 1).

## DISCUSSION

Previously, we demonstrated that dietary supplementation of 0.5% quercetin for 14 days could increase serum antioxidant capacity and the contents of ascorbic acid and vitamin E in rats.[29] In the present study, the results of $^1$H NMR-based metabolomic analysis of serum samples indicated that dietary 0.5% quercetin supplementation had a clear impact on the internal metabolism of rats. With chemometric analysis of the CPMG $^1$H NMR spectral data, it was found that the level of lactate increased, whereas glucose decreased after quercetin supplementation. It suggests an overall increase in anaerobic glycolysis, which may be associated with the actions played by quercetin on mitochondrial function. Dorta et al. and Trumbeckaite et al. reported that the function of isolated rat liver and heart mitochondria was inhibited in the presence of quercetin, manifested by uncoupling and release of mitochondria-accumulated $Ca^{2+}$. In consequence, the activity of tricarboxylic acid cycle was decreased.[30,31] To compensate for energy inadequacy, the anaerobic glycolysis has to be increased.

LDL, VLDL, and HDL are lipoproteins responsible for lipid transportation *in vivo*. LDL, converted from VLDL in the bloodstream, transports cholesterol and triglycerides from the liver to other tissues or organs, while HDL transports cholesterol back mostly to the liver or the steroidogenic organs, such as adrenals, ovaries, and testes. We found that the level of LDL/VLDL was increased, while the level of HDL was decreased in rats after quercetin treatment based on $^1$H NMR analysis. It is implied that quercetin treatment induces a significant change in cholesterol metabolism, indicating that more LDL-C is excreted into the bloodstream or the elimination of LDL-C is possibly slowed down. Because the increase in serum LDL-C is a potential risk of cardiovascular diseases, we should perform more studies to investigate its impact on the cardiovascular system. Egert et al. also found that quercetin administration resulted in an increased LDL-C/HDL-C ratio in overweight patients with the apoE4 genotype.[32] They hypothesized that altered expression or activities of enzymes associated with the apoE genetype, such as cholesterol ester transfer protein and lecithin cholesterol acyltransferase or hepatic lipase, were involved.

To evaluate the reliability of the results of $^1$H NMR-based metabolomic analysis, we measured some serum biochemical indexes related to the energy and lipid metabolism. Although the lowering level of serum glucose or the increasing level of serum lactate were shown as a result of quercetin supplementation, both of them did not show statistical significance between two groups. It is suggested that the $^1$H NMR technique may be too sensitive in detecting metabolic changes induced by quercetin treatment. However, the level of serum LDL-C was increased significantly in response to quercetin treatment, which is in line with the results of $^1$H NMR analysis. It is confirmed that quercetin can exert a profound impact on cholesterol metabolism. The precise mechanisms behind this action need to be further investigated.

It should be noted that, because quercetin is extensively metabolized in the gut and liver after absorption and occurs in the bloodstream mainly as conjugated metabolites, quercetin is active *in vivo* possibly not only in the form of its aglycone but also its metabolites. The major metabolites of quercetin identified include glucuronide, sulfoglucuronide, and sulfate- and methylate-conjugated derivatives.[33−36] However, part of deconjugated quercetin could be recovered in some organs of rats and pigs.[37,38] We also detected a significant amount of quercetin aglycone and methyl quercetin, quercetin sulfate, quercetin monoglucuronide, and methyl quercetin monoglucuronide in the serum sampled from the portal vein after quercetin gavage in rats.[39] Terao et al. reported that one of the quercetin metabolites, quercetin 3-*O*-β-D-glucuronide, retained considerable antioxidant activity and could accumulate significantly in human atherosclerotic lesions.[8] Therefore, the effects of quercetin *in vivo* may come from the concerted actions of quercetin aglycone and its metabolites. It is rather interesting to further explore the roles played by different quercetin metabolites *in vivo*.

In conclusion, this study showed that quercetin induced a significant increase in serum LDL-C in rats, which should be paid more attention because it may be closely associated with the cardiovascular effects of quercetin. Further studies are warranted to probe into the underlying mechanisms and possible links to the health effects or toxic actions of quercetin.

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### Funding Sources

This work was supported by a grant from the Science and Technology Program for the Eleventh Five Years of China (2008BAI58B06).

## ACKNOWLEDGMENT

We thank Dr. Qi Zhang and Xianzhong Yan from the NMR Laboratory, National Center of Biomedical Analysis, Beijing, China, for expert assistance in $^1$H NMR analysis and data processing.

## ABBREVIATIONS USED

CPMG, Carr−Purcell−Meiboom−Gill; HDL, high-density lipoprotein; LDL, low-density lipoprotein; LED, longitudinal eddy current delay; Nac, *N*-acetyl glycoprotein; NMR, nuclear magnetic resonance; PCA, principal component analysis; PLS−DA, partial least-squares−discriminant analysis; TSP, sodium 3-trimethylsilyl-propionate-2,2,3,3-d4; VLDL, very low-density lipoprotein

## REFERENCES


(1) Dauchet, L.; Amouyel, P.; Hercberg, S.; Dallongeville, J. Fruit and vegetable consumption and risk of coronary heart disease, a meta-analysis of cohort studies. *J. Nutr.* **2006**, *136*, 2588–2593.

(2) He, F. J.; Nowson, C. A.; Lucas, M.; MacGregor, G. A. Increased consumption of fruits and vegetables is related to a reduced risk of coronary heart disease, meta-analysis of cohort studies. *J. Hum. Hypertens.* **2007**, *21*, 717–728.

(3) Pavia, M.; Pileggi, C.; Nobile, C. G.; Angelillo, I. F. Association between fruit and vegetable consumption and oral cancer: A meta-analysis of observational studies. *Am. J. Clin. Nutr.* **2006**, *83*, 1126–1134.

(4) Hansen, L.; Dragsted, L. O.; Olsen, A.; Christensen, J.; Tjønneland, A.; Schmidt, E. B.; Overvad, K. Fruit and vegetable intake and risk of acute coronary syndrome. *Br. J. Nutr.* **2010**, *24*, 1–8.

(5) Le Marchand, L. Cancer preventive effects of flavonoids—A review. *Biomed. Pharmacother.* **2002**, *56*, 296–301.

(6) Hooper, L.; Kroon, P. A.; Rimm, E. B.; Cohn, J. S.; Harvey, I.; Le Cornu, K. A.; Ryder, J. J.; Hall, W. L.; Cassidy, A. Flavonoids, flavonoid-rich foods, and cardiovascular risk, a meta-analysis of randomized controlled trials. *Am. J. Clin. Nutr.* **2008**, *88*, 38–50.

(7) Grassi, D.; Desideri, G.; Croce, G.; Tiberti, S.; Aggio, A.; Ferri, C. Flavonoids, vascular function and cardiovascular protection. *Curr. Pharm. Des.* **2009**, *15*, 1072–1084.

(8) Terao, J. Dietary flavonoids as antioxidants. *Forum Nutr.* **2009**, *61*, 87–94.

(9) Rice-Evans, C. A.; Miller, N. J.; Paganga, G.. Structure−antioxidant activity relationship of flavonoids and phenolic acids. *Free Radical Biol. Med.* **1996**, *20*, 933–956.

(10) Su, J. F.; Guo, C. J.; Wei, J. Y.; Yang, J. J.; Jiang, Y. G.; Li, Y. F. Study on the absorption of quercetin and rutin at different segments of intestine. *J. Hyg. Res.* **2002**, *31*, 55–57.

(11) Su, J. F.; Guo, C. J.; Wei, J. Y.; Yang, J. J. Antioxidant capacity of quercetin in vitro and in vivo. *Chin. J. Appl. Physiol.* **2002**, *18*, 382–386.

(12) Su, J. F.; Guo, C. J.; Wei, J. Y.; Yang, J. J.; Jiang, Y. G.; Li, Y. F. Protection against hepatic ischemia−reperfusion injury in rats by oral pretreatment with quercetin. *Biomed. Environ. Sci.* **2003**, *16*, 1–8.

(13) Middleton, E.; Kandaswami, C., Jr.; Theoharides, T. C. The effects of plant flavonoids on mammalian cells, implications for inflammation, heart disease, and cancer. *Pharmacol. Rev.* **2000**, *52*, 673–751.

(14) Angeloni, C.; Leoncini, E.; Malaguti, M.; Angelini, S.; Hrelia, P.; Hrelia, S. Role of quercetin in modulating rat cardiomyocyte gene expression profile. *Am. J. Physiol. Heart Circ. Physiol.* **2008**, *294*, H1233–H1243.

(15) Tanigawa, S.; Fujii, M.; Hou, D. Stabilization of p53 is involved in quercetin-induced cell cycle arrest and apoptosis in HepG2 cells. *Biosci., Biotechnol., Biochem.* **2008**, *72*, 797–804.

(16) Hubbard, G. P.; Wolffram, S.; Lovegrove, J. A.; Gibbins, J. M. Ingestion of quercetin inhibits platelet aggregation and essential components of the collagen-stimulated platelet activation pathway in humans. *J. Thromb. Haemostasis* **2004**, *2*, 2138–2145.

(17) Nicholson, J. K.; Lindon, J. C.; Holmes, E. `Metabonomics', understanding the metabolic responses of living systems to pathophysiological stimuli via multivariate statistical analysis of biological NMR spectroscopic data. *Xenobiotica* **1999**, *29*, 1181–1189.

(18) Holmes, E.; Nicholls, A. W.; Lindon, J. C.; Connor, S. C.; Connelly, J. C.; Haselden, J. N.; Damment, S. J. P.; Spraul, M.; Neidig, P.; Nicholson, J. K. Chemometric models for toxicity classification based on NMR spectra of biofluids. *Chem. Res. Toxicol.* **2000**, *13*, 471–478.

(19) Dunn, W. B.; Ellis, D. I. Metabolomics: Current analytical platforms and methodologies. *Trends Anal. Chem.* **2005**, *24*, 285–294.

(20) Solanky, K. S.; Bailey, N. J. C.; Holmes, E.; Lindon, J. C.; Davis, A. L.; Mulder, T. P. J.; Van Duynhoven, J. P. M.; Nicholson, J. K. NMR-based metabonomic studies on the biochemical effects of epicatechin in the rat. *J. Agric. Food Chem.* **2003**, *51*, 4139–4145.

(21) Solanky, K. S.; Bailey, N. J.; Beckwith-Hall, B. M.; Davis, A.; Bingham, S.; Holmes, E.; Nicholson, J. K.; Cassidy, A. Application of biofluid $^{1}$H nuclear magnetic resonance-based metabonomic techniques for the analysis of biochemical effects of dietary isoflavones on human plasma profile. *Anal. Biochem.* **2003**, *323*, 197–204.

(22) Solanky, K. S.; Bailey, N. J.; Beckwith-Hall, B. M.; Bingham, S.; Davies, A.; Holmes, E.; Nicholson, J. K.; Cassidy, A. Biofluid $^{1}$H NMR-based metabonomic techniques in nutrition research—Metabolic effects of dietary isoflavones in humans. *J. Nutr. Biochem.* **2005**, *16*, 236–244.

(23) Daykin, C. A.; Van Duynhoven, J. P. M.; Groenewegen, A.; Dachtler, M.; Van Amelsvoort, J. M. M.; Mulder, T. P. J. Nuclear magnetic resonance spectroscopic based studies of the metabolism of black tea polyphenols in humans. *J. Agric. Food Chem.* **2005**, *53*, 1428–1434.

(24) Van Dorsten, F. A.; Daykin, C. A.; Mulder, T. P. J.; Van Duynhoven, J. P. M. Metabonomics approach to determine metabolic differences between green tea and black tea consumption. *J. Agric. Food Chem.* **2006**, *54*, 6929–6938.

(25) Reeves, P. G.; Nielsen, F. H.; Fahey, G. C. AIN-93 purified diets for laboratory rodents, final report of the American Institute of Nutrition Ad Hoc Writing Committee on the reformulation of the AIN-76A rodent diet. *J. Nutr.* **1993**, *123*, 1939–1951.

(26) Ameho, C. K.; Chen, C. Y. O.; Smith, D.; Sanchez-Moreno, C.; Milbury, P. E.; Blumberg, J. B. Antioxidant activity and metabolite profile of quercetin in vitamin E-depleted rats. *J. Nutr. Biochem.* **2008**, *19*, 467–474.

(27) Lenz, E. M.; Bright, J.; Wilson, I. D.; Morgan, S. R.; Nash, A. F. P. A $^{1}$H NMR-based metabonomic study of urine and plasma samples obtained from healthy human subjects. *J. Pharm. Biomed. Anal.* **2003**, *33*, 1103–1115.

(28) Lindon, J. C.; Holmes, E.; Nicholson, J. K. Pattern recognition methods and applications in biomedical magnetic resonance. *Prog. NMR Spectrosc.* **2001**, *39*, 1–40.

(29) Zhao, L. T.; Guo, C. J.; Cai, D. L.; Wu, J. Q.; Yang, J. J.; Wei, J. Y. Effect of quercetin supplementation on the peripheral blood antioxidant system in rats. *Acta Acad. Med. CPAF* **2009**, *18*, 269–270.

(30) Dorta, D. J.; Pigoso, A. A.; Mingaytto, F. E.; Rodrigues, T.; Prado, I. M. R.; Helena, A. F. C.; Uyemura, S. A.; Santos, A. C.; Curti, C. The interaction of flavonoids with mitochondria: Effects on energetic processes. *Chem.-Biol. Interact.* **2005**, *152*, 67–78.

(31) Trumbeckaite, S.; Bernatoniene, J.; Majiene, D.; Jakštas, V.; Savickas, A.; Toleikis, A. The effect of flavonoids on rat heart mitochondrial function. *Biomed. Pharmacother.* **2006**, *60*, 245–248.

(32) Egert, S.; Boesch-Saadatmandi, C.; Wolffram, S.; Rimbach, G.; Müller, M. J. Serum lipid and blood pressure responses to quercetin vary in overweight patients by apolipoprotein E genotype. *J. Nutr.* **2010**, *140*, 278–284.

(33) Ader, P.; Wessmann, A.; Wolffram, S. Bioavailability and metabolism of the flavonol quercetin in the pig. *Free Radical Biol. Med.* **2000**, *28*, 1056–1067.

(34) Day, A. J.; Mellon, F.; Barron, D.; Sarrazin, G.; Morgan, M. R. A.; Williamson, G. Human metabolism of dietary flavonoids: Identification of plasma metabolites of quercetin. *Free Radical Res.* **2001**, *35*, 941–952.

(35) Spencer, J. P. E.; Kuhnle, G. G. C.; Williams, R. J.; Rice-Evans, C. Intracellular metabolism and bioactivity of quercetin and its in vivo metabolites. *Biochem. J.* **2003**, *372*, 173–181.

(36) Justino, G. C.; Santos, M. R.; Canario, S.; Borges, C.; Florencio, M. H.; Mira, L. Plasma quercetin metabolites: Structure−antioxidant activity relationships. *Arch. Biochem. Biophys.* **2004**, *32*, 109–121.