ELSEVIER

Soybean isoflavonoids and their metabolic products inhibit in vitro lipoprotein oxidation in serum

Jonathan M. Hodgson, Kevin D. Croft, Ian B. Puddey, Trevor A. Mori, and Lawrie J. Beilin

University of Western Australia Department of Medicine and the Western Australian Heart Research Institute, Royal Perth Hospital, Perth, Western Australia, Australia

Isoflavonoids are compounds present in many legumes, but are derived in the human diet mainly from soybeans and various soybean-based food products. The major isoflavonoids occurring in soy are the glycosides of genistein and daidzein. The metabolic products of genistein metabolism in humans have not been clearly shown. The two main products of daidzein metabolism in humans appear to be equol and O-desmethylangolensin. Increasing evidence suggests that oxidative modification to low-density lipoprotein is involved in atherogenesis, and that natural antioxidants that prevent or inhibit oxidative damage to low-density lipoprotein may beneficially influence atherogenesis. In the present experiments, the effects of genistein and daidzein, and the daidzein metabolites equol and O-desmethylangolensin on Cu^{2+}-induced oxidation of lipoproteins in serum were examined. Three concentrations of each compound (0.1 μM, 1 μM, 10 μM) were tested for antioxidant activity in six individual serum samples. All compounds tested inhibited lipoprotein oxidation. The minimum concentration for significant inhibition was 1 μM for genistein and daidzein ($P < 0.05$), and 0.1 μM equol and O-desmethylangolensin ($P < 0.05$). Equol and O-desmethylangolensin were more potent inhibitors of in vitro lipoprotein oxidation in serum than the two major dietary isoflavonoids. This study has demonstrated that soybean isoflavonoids and metabolic products of daidzein metabolism inhibit lipoprotein oxidation in vitro. Human intervention studies are needed to determine if these compounds can influence oxidation in vivo. © Elsevier Science Inc. 1996 (J. Nutr. Biochem. 7:664–669, 1996.)

Keywords: soybeans; isoflavonoids; lipoprotein oxidation; antioxidants

Introduction

Isoflavonoids are a class of flavonoids which are common constituents of particular legumes. In the human diet, isoflavonoids are derived mainly from soybeans and various soybean-based food products, but are also found in other legumes. Several populations such as the Japanese, and groups such as vegetarians have higher intakes of soybean products, and therefore of isoflavonoids. It has been estimated, for example, that the mean isoflavonoid intake of the Japanese is between 30 and 50 mg per day,[1,2] whereas the mean intake of isoflavonoids in most Western populations is probably less than 3 to 5 mg per day.[1]

The major isoflavonoids occurring in legumes are the glycosides of genistein and daidzein and their 4-methoxy ether derivatives, biochanin A and formononetin (*Figure 1*). When ingested by humans, these isoflavonoids undergo acidic and enzymatic hydrolysis and demethylation to yield the aglycones genistein and daidzein. The aglycones may then be further metabolized by gut flora.[3] The metabolic products of genistein metabolism by the gut flora in humans have not been clearly shown. The two main products of daidzein metabolism in humans appear to be equol and O-desmethylangolensin (O-DMA).[4] The relative proportions of equol and O-DMA produced in the gut may relate to the types of gut flora present.[3]

Isoflavonoid compounds have been shown to have a variety of physiological effects which may be relevant to human health. Genistein, daidzein and equol have been shown to have oestrogenic activity—phytoestrogens[3]—and may therefore influence in vivo estrogen metabolism.[5] In addi-

Address reprint requests to Dr. Jonathan Hodgson at University Department of Medicine, Royal Perth Hospital, GPO Box X2213, Perth, WA 6001 Australia.
Received April 29, 1996; accepted August 29, 1996.

655 Avenue of the Americas, New York, NY 10010

0955-2863/96/$15.00
PII S0955-2863(96)00133-7

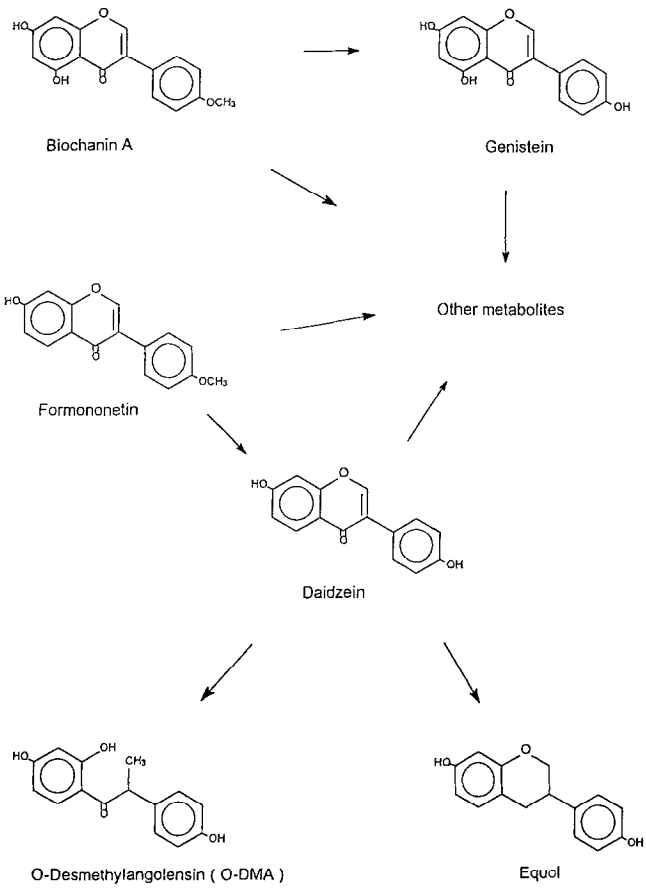

Figure 1 Chemical structures of isoflavonoids and their metabolites, and pathways of isoflavonoid metabolism in humans.

tion, genistein is a potent inhibitor of tyrosine-specific protein kinases (which are important in cell proliferation and transformation)[6] and has been shown to have a variety of effects on the cell cycle and cell functions.[1]

Generally, populations that have higher intake levels of isoflavonoids have lower rates of cardiovascular disease. If a higher intake of these compounds is causally related to reduced risk,[7] the effects are likely to operate through various cardiovascular disease risk factors.

Oxidative damage to cells and tissues is thought to play a role in the development of chronic diseases including atherosclerotic cardiovascular disease and several cancers. Increasing evidence suggests that oxidative modification to low-density lipoprotein (LDL), and possibly other lipoproteins, is involved in atherogenesis.[8] Natural antioxidants that prevent or inhibit oxidative damage to LDL may beneficially influence atherogenesis. Flavonoids have antioxidant activity in a variety of in vitro assay systems,[9] and there is evidence that several flavonoids can inhibit oxidative modification of LDL.[10–12] Isoflavonoids have also been found to have antioxidant activity in vitro,[13–15] and a recent study suggests that genistein can inhibit the oxidative modification of isolated LDL.[16]

The mechanisms by which oxidative damage to LDL and other lipoproteins occurs in vivo, and the role of antioxidants in protecting lipoproteins against oxidation are still not clearly understood.[17] Techniques using Cu^{2+} to induce oxidative damage to isolated LDL particles,[18] have been used to measure in vitro susceptibility to oxidation. A recently developed method to assess Cu^{2+}-induced oxidative damage to lipoproteins in human serum in vitro,[19] has the advantage of measuring lipoprotein oxidation in an environment that may more closely resemble the in vivo situation.[17] In the present experiments, the effects of the isoflavonoids, genistein and daidzein, and the daidzein metabolites equol and O-DMA, on Cu^{2+}-induced oxidation of serum were examined.

Methods and materials

Reagents

Genistein was obtained from ICN Pharmaceuticals Australasia Pty Ltd. (Catalogue number: 152355) through Australian Biosearch (Western Australia), and daidzein was obtained from Sapphire Bioscience Pty. Ltd. (Catalogue number: 320-11600). Equol and O-DMA were obtained from Dr T Hase (University of Helsinki, Department of Chemistry, Finland). The isoflavonoids were dissolved in 100% redistilled methanol. Phosphate-buffered saline (PBS): 138 mM NaCl, 27 mM KCl, 10.1 mM Na_2HPO_4, and 1.8 mM KH_2PO_4. Copper sulphate ($CuSO_4.5H_2O$) was dissolved in deionized distilled water.

Blood collection

Blood samples were obtained from six male and female healthy volunteers aged 25 to 45 years. All subjects gave written consent. A fasting blood sample was drawn from the antecubital fossa vein into evacuated glass tubes, allowed to clot, then centrifuged at 2000 g for 10 minutes. The serum removed was frozen at –80°C until assayed.

Serum oxidation

A modification of the serum oxidation method described by Regnstrom et al.[19] was used. This method provides an indication of diene formation in lipoprotein fatty acids present in serum exposed to Cu^{2+}, assessed by measuring change in absorbance at 234 nM. The formation of dienes in lipoprotein deficient serum exposed to Cu^{2+} is absent, indicating that diene formation in lipoprotein fatty acids is primarily responsible for the increase in absorbance.[19] This finding has been confirmed in our laboratory. Serum was thawed, then diluted to 0.67%—final concentration—in PBS. The test compound was added at three different concentrations (0.1 μM, 1 μM, and 10 μM [final concentrations in diluted serum]). Because the serum is diluted 150 fold, the final concentration of dietary-derived flavonoids and their metabolic products, including the compounds of interest in this present study, in the diluted serum is likely to be low in comparison to the added concentration of each test compound. Control experiments consisted of identical assay conditions but without the test compound added. The concentrations of the test compounds were measured by determining absorbance at the wavelength with maximum absorbance (λmax), using molar extinction coefficients (ε).[20] The following values were used: genistein (λmax = 263 nm, ε = 37154), daidzein (λmax = 250 nm, ε = 20893), equol (λmax = 281 nm, ε = 6761), and O-DMA (λmax = 320 nm, ε = 7586). These measurements were performed in stock solutions before the addition of the test compounds to the diluted serum. The compounds tested for antioxidant activity in this system were genistein and daidzein, and the daidzein metabolites equol and O-DMA. Oxidation was initi-

ated immediately after addition of the test compound by the addition of 12 μM final concentration Cu^{2+} added as $CuSO_4.5H_2O$. Oxidation kinetics were determined by measuring the absorbance at 234 nm using a DU650 UV-Vis spectrophotometer (Beckman Instruments Inc, CA, USA). Absorbance readings were taken every 20 min over 240 min at 37°C. The antioxidant activity of each of the compounds was tested in six individual serum samples.

Statistics

The variables used to describe the differences between the oxidation curves were area under the oxidation curve (AUC), lag time to lipoprotein diene formation in serum (lag time) and slope of the propagation phase of the oxidation curve (slope). AUC, lag time, and slope were calculated by using the Prism statistical analysis software (San Diego, CA, USA). To calculate lag time and slope, a sigmoid regression model was fitted to the oxidation curves obtained.

The SPSS statistical analysis software (Michigan, USA) was used for all other analyses. Comparisons were made individually between each of the three concentrations of the test compounds and the control oxidation curves by using the paired *t* test. Each variable was log transformed before statistical analysis. *P*-values were adjusted for multiple comparisons. Results are presented as the mean paired difference between the log transformed AUC, Lag or slope, for the test compound concentration and the control. This difference in log-transformed values represents the ratio between test compound concentration and control.

Results

The mean oxidation curves for genistein, daidzein, equol and O-DMA are shown in *Figures 2–5*, respectively. The curves presented are the mean of six oxidation curves. These figures provide a visual indication of the antioxidant effects (absorbance values presented in *Figures 2–5* have been adjusted for the initial absorbance reading).

Comparisons of oxidation curves for 0.1 μM, 1 μM, and 10 μM concentrations of each of the test compounds with the control oxidations, using AUC, are presented in *Table 1*. Compared with the control the minimum concentrations of test compounds to produce a significant reduction in AUC were as follows: genistein at 1 μM resulted in 49% decrease in AUC ($P = 0.002$); daidzein at 1 μM resulted in a 19% decrease in AUC ($P = 0.0001$); equol at 0.1 μM resulted in a 41% decrease in AUC ($P = 0.0001$); and O-DMA at 0.1 μM resulted in a 28% decrease in AUC ($P = 0.0001$). Equol and O-DMA at 1 μM, and all compounds at 10 μM resulted in significant inhibition of oxidation (assessed by AUC) compared with respective controls.

The other variables used to examine the effects of the test compounds on oxidation were lag time and slope (*Table 2*). Compared to control: genistein at 1 μM resulted in a 57% increase in lag time ($P = 0.016$) and a 28% decrease in slope ($P = 0.029$); daidzein at 1 μM resulted in a 21% increase in lag time ($P = 0.005$) and a 18% decrease in slope ($P = 0.006$); equol at 0.1 μM resulted in a 37% increase in lag time ($P = 0.001$) and a 32% decrease in slope ($P = 0.003$); and O-DMA at 0.1 μM resulted in a 14% increase in lag time ($P = 0.0001$) and a 24% decrease in slope ($P = 0.014$).

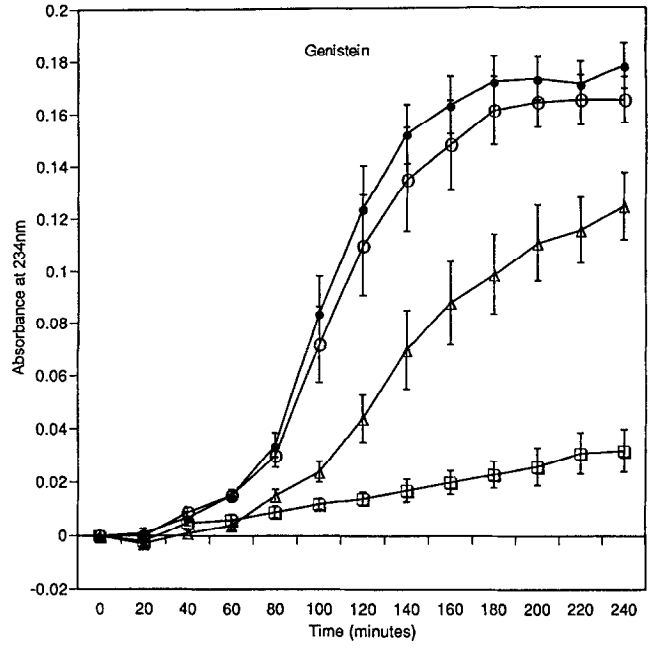

Figure 2 Curves representing the mean of six oxidations for (a) genistein, (b) daidzein, (c) equol and (d) O-DMA. (● = control; ○ = 0.1 μM; △ = 1 μM; □ = 10 μM). Results are presented as mean ± standard error at 20-min intervals up to 240 min.

Discussion

In the studies presented here, the effects of the isoflavonoids genistein and daidzein, and the daidzein metabolites equol and O-DMA, on lipoprotein oxidation in serum were examined. The results show that all four compounds tested in-

Table 1 Area under the oxidation curve (AUC): comparisons between each test concentration and control for each test compound

Isoflavonoid & concentration (μM)	AUC for each test concentration relative to control	95% Confidence interval	*P* Value
Genistein			
0.1 μM	0.914	0.789, 1.057	0.259
1 μM	0.514	0.408, 0.647	0.002
10 μM	0.110	0.063, 0.195	0.0001
Daidzein			
0.1 μM	0.945	0.889, 1.007	0.102
1 μM	0.810	0.759, 0.867	0.0001
10 μM	0.280	0.218, 0.361	0.0001
Equol			
0.1 μM	0.587	0.442, 0.782	0.011
1 μM	0.097	0.054, 0.174	0.0001
10 μM	0	—	—
O-DMA			
0.1 μM	0.72	0.66, 0.79	0.0001
1 μM	0.21	0.10, 0.45	0.005
10 μM	0.07	0.02, 0.24	0.017

Table 2 Lag time to lipoprotein diene formation (Lag) and slope of the propagation phase of the oxidation curve (Slope): comparisons between each test concentration and the control for each test compound

Isoflavonoid & concentration	Lag for each test concentration relative to control	95% Confidence interval	*P* Value	Slope for each test concentration relative to control	95% Confidence interval	*P* Value
Genistein						
0.1 μM	1.05	0.93, 1.19	0.322	0.944	0.796, 1.11	0.639
1 μM	1.57	1.13, 2.17	0.016	0.621	0.435, 0.887	0.029
10 μM	n.d.			n.d.		
Daidzein						
0.1 μM	1.04	1.00, 1.07	0.048	1.01	0.910, 1.12	0.99
1 μM	1.21	1.10, 1.32	0.005	0.822	0.745, 0.908	0.006
10 μM	n.d.			n.d.		
Equol						
0.1 μM	1.37	1.22, 1.52	0.001	0.681	0.579, 0.802	0.003
1 μM	2.61	1.32, 5.17	0.015	0.524	0.350, 0.787	0.014
10 μM	n.d.			n.d.		
O-DMA						
0.1 μM	1.14	1.11, 1.18	0.0001	0.760	0.243, 0.646	0.014
1 μM	2.54	1.36, 4.65	0.014	0.040	0.243, 0.646	0.009
10 μM	n.d.			n.d.		

n.d., not determined

hibited lipoprotein oxidation in serum and, therefore, possess antioxidant activity. Equol and O-DMA were more potent inhibitors of oxidation than their parent compound daidzein, or genistein. Equol was approximately ten times more potent as an antioxidant than daidzein. The variables used to describe the effects of these compounds were area under the oxidation curve, lag time to lipoprotein diene formation in serum and slope of the propagation phase of the oxidation curve. AUC was used to compare differences in the overall oxidation curves without focusing on particular aspects.

Increasing evidence suggests that the oxidative modification of lipoproteins, and of LDL in particular, is causally involved in atherogenesis.[8] In vitro biochemical assays which assess oxidation in response to an oxidative stimulus are being employed to examine the relationship between antioxidants and lipoprotein oxidation. In the most widely used assay of this type, conjugated diene formation in the fatty acids of isolated LDL is measured during oxidation with Cu^{2+}.[18] In the in vitro assay utilised in this study, conjugated diene formation in response to oxidation with Cu^{2+} was measured in serum that was diluted 150 fold (0.67%), rather than isolated LDL. There are several reasons why the serum oxidation assay may be more appropriate to the studies performed here, than similar studies with isolated LDL.

There are a large number of natural antioxidants present in plasma. These include the non-enzymatic proteins, enzymatic proteins and small molecule antioxidants.[17] The small molecule antioxidants can be separated into two groups: water soluble and lipid-soluble, lipoprotein-associated molecules. Most of the free isoflavonoids, genistein and daidzein, and the daidzein metabolites, equol and O-DMA absorbed, are reconjugated in the liver to glucuronides and released into the circulation.[3,21] The glucuronides are water-soluble, and are, therefore, unlikely to be incorporated into LDL particles.

The lipid-soluble LDL-associated antioxidants may be important in inhibiting oxidative damage because they are in the immediate proximity to the lipoprotein's lipids. The water-soluble antioxidants may be important in preventing oxidation.[17] Circulating plasma LDL is well protected against oxidative stress,[22] and is probably not oxidised in the plasma because of the relatively high concentrations of antioxidants.[17] The oxidation of LDL probably occurs in the extracellular milieu of the intimal subendothelium of the arterial wall, which appears to be aqueous, and is most likely initiated from the aqueous environment surrounding the lipoprotein.[17] Water-soluble compounds of low molecular weight seem to be freely diffusible, and there is evidence that the concentrations of these compounds in the extracellular subendothelium approximates plasma concentrations. Furthermore, lipoprotein-associated, lipid-soluble antioxidants may be present at considerably lower concentrations in the extracellular subendothelium.[23] In addition to their suggested role in the inhibition of the initiation of oxidation, several water-soluble antioxidants, such as vitamin C, may act as co-antioxidants. Co-antioxidants may indirectly inhibit oxidative damage to LDL by reducing tocopherol-mediated peroxidation, and regenerating α-tocopherol, the major lipid soluble antioxidant in LDL. The result may be that the efficiency of α-tocopherol as an antioxidant is increased.[24] Water-soluble antioxidants may therefore be important in preventing the initiation of lipid peroxidation and oxidative damage to LDL.

Flavonoids can inhibit lipid peroxidation in vitro by acting as free radical scavengers, or as metal-chelating agents.[9] It has been suggested that the antioxidant activity of genis-

tein is due to its free radical scavenging potential, and not due to its chelating properties.[15] The effects observed in the present studies are probably not due to the chelation of Cu^{2+} because significant inhibition of oxidation was observed for all compounds with concentrations of 1 μM or lower, whereas the concentration of Cu^{2+} was 12.5 μM.

In other studies which have examined the relative antioxidant potency of various flavonoids,[16,25] it was found that genistein had moderate antioxidant activity in comparison to other dietary flavonoids. Several flavonoids have shown greater antioxidant activity than genistein, but the observed activity can depend on the assay system used.[16,25] The inhibition of lipid peroxidation by flavonoids appears to be influenced by several other factors. An increased number of hydroxyl groups and a carbonyl group at position 4 of the C ring are related to increased antioxidant activity.[9] Flavonoids with the most potent antioxidant activity usually have many hydroxyl groups.[16] From the structural features of the compounds (*Figure 1*), it could be predicted that the antioxidant activity might be genistein > daidzein > equol/O-DMA. The fact that equol in particular, but also O-DMA, inhibited oxidation to a greater degree than either genistein or daidzein suggests that these compounds may inhibit oxidation via mechanisms other than free radical scavenging alone: possibly acting as co-antioxidants.

Almost all of the genistein and daidzein, and much of the equol and O-DMA seem to be present in the plasma as glucuronides.[21] The presence of a sugar moiety may reduce the effectiveness of flavonoids,[9] and possibly isoflavonoids, to inhibit oxidation. This could be of relevance to the in vivo situation. Preliminary studies (unpublished) looking at the effects of extracts of foods high in isoflavonoids (where the isoflavonoids are present predominantly as glycosides) on in vitro lipoprotein oxidation in serum, show that the antioxidant activity of the extracts is closely related to isoflavonoid concentrations. However, other antioxidant compounds are also present in these extracts.

There is no clear evidence to date of free radical scavenging antioxidant effects of isoflavonoids or their metabolites in vivo, but it is useful to relate the in vitro assay conditions in this study to what may be occurring in vivo.

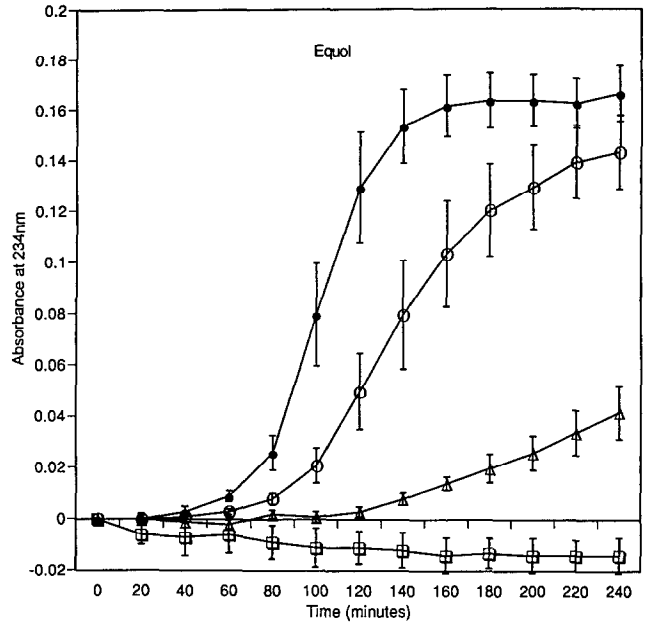

Figure 4 Curves representing the mean of six oxidations for equal (● = control; ○ = 0.1 μM; △ = 1 μM; □ = 10 μM). Results are presented as mean ± standard error at 20 min intervals up to 240 min.

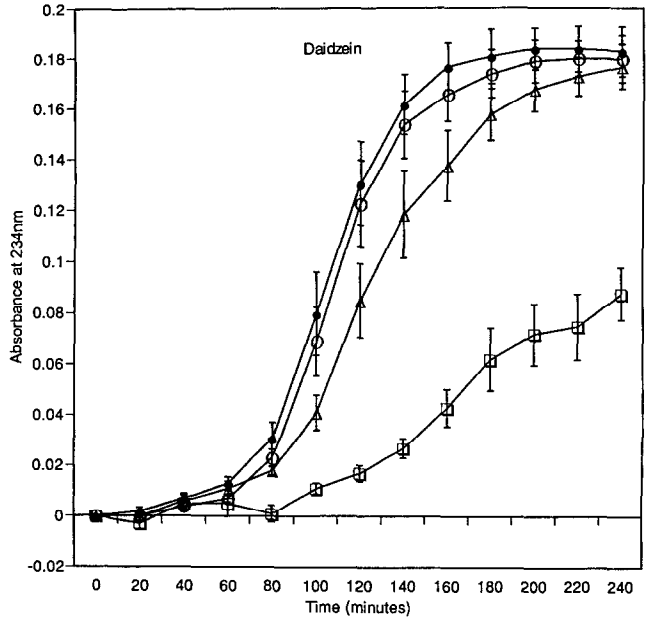

Figure 3 Curves representing the mean of six oxidations for daidzein (● = control; ○ = 0.1 μM; △ = 1 μM; □ = 10 μM). Results are presented as mean ± standard error at 20 min intervals up to 240 min.

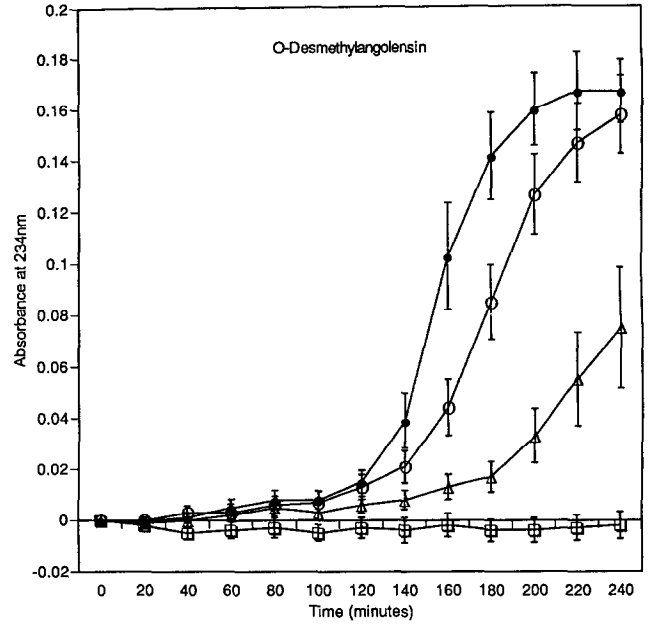

Figure 5 Curves representing the mean of six oxidations for O-DMA (● = control; ○ = 0.1 μM; △ = 1 μM; □ = 10 μM). Results are presented as mean ± standard error at 20 min intervals up to 240 min.

The assay conditions themselves and their possible relationships to in vivo lipid peroxidation have been discussed. The concentrations of the compounds used in these studies (0.1 μM, 1 μM, and 10 μM) were chosen to relate to concentrations which may be achieved in circulating plasma. For example, the mean plasma concentrations of genistein and daidzein observed in a group of Japanese subjects were 0.27 μM and 0.11 μM, respectively,[21] and in subjects who were fed isoflavonoids as soymilk plasma concentrations ranged from a mean of 0.74 μM to 2.24 μM 6.5 hr after feeding isoflavonoids at 0.7 to 2 mg/kg body weight.[26] When attempting to relate the in vitro assay to the in vivo situation it is important to note that the serum used in the in vitro assay has been diluted 150 fold. Although lipoproteins are probably not oxidised in circulating plasma,[17] the relative molar concentrations of the lipoproteins and the test compounds in the in vitro assay may not provide a good indication of relative in vivo concentrations in the intimal subendothelium of the arterial wall where it has been suggested that lipoprotein oxidation can occur.[17] Intervention studies in human subjects are needed to determine whether physiological levels of these compounds can influence oxidation in vivo.

Acknowledgments

Jonathan Hodgson was supported by an Athelstan and Amy Saw Medical Research Fellowship from the University of Western Australia. The authors wish to thank Julie Proudfoot for her help with the serum oxidation assay.

References

1 Barnes, S., Peterson, T.G., and Coward, L. (1995). Rationale for the use of genistein-containing soy matrices in chemoprevention trials for breast and prostate cancer. *J. Cellular Biochem.* **22(Suppl),** 181–187

2 Messina, M. (1995). Isoflavone intakes by Japanese were overestimated. Am. J. Clin. Nutr. **62,** 645. Comment on: *Am. J. Clin. Nutr.* **60,** 333–340

3 Setchell, K.D.R. and Adlercreutz, H. (1988). Mammalian lignans and phyto-oestrogens: recent studies on their formation, metabolism and biological role in health and disease. In *Role of gut flora in toxicity and cancer* (I.R. Rowland, ed), pp. 315–345, Academic Press Limited, San Diego, CA USA

4 Kelly, G.E., Nelson, C., Waring, M.A., Joannou, G.E., and Reeder, A.Y. (1993). Metabolites of dietary (soya) isoflavones in human urine. *Clin. Chim. Acta.* **223,** 9–22

5 Cassidy, A., Bingham, S., and Setchell, K.D.R. (1994). Biological effects of a diet of soy protein rich in isoflavones on the menstrual cycle of premenopausal women. *Am. J. Clin. Nutr.* **60,** 333–340

6 Akiyama, T., Ishida, J., Nakagawa, S., Nakagawa, S., Ogawara, H., Watenabe, S., Itoh, N., Shibuya, M., and Fukami, Y. (1987). Genistein, a specific inhibitor of tyrosine specific protein kinases. *J. Biol. Chem.* **262,** 5592–5595

7 Adlercreutz, H. (1990). Western diet and western diseases: some hormonal and biochemical mechanisms and associations. *Scand. J. Clin. Lab. Invest.* **50(Suppl 201),** 3–23

8 Witztum, J.L. and Steinberg, D. (1991). Role of oxidised low density lipoprotein in atherogenesis. *J. Clin. Invest.* **88,** 1785–1792

9 Cook, N.C. and Samman, S. (1996). Flavonoids—chemistry, metabolism, cardioprotective effects and dietary sources. *J. Nutr. Biochem.* **7,** 66–76

10 DeWhalley, C.V., Rankin, S.M., Hoult, J.R.S., Jessup, W., and Leake, D.S. (1990). Flavonoids inhibit the oxidative modification of low density lipoproteins by macrophages. *Biochem. Pharmacol.* **39,** 1743–1750

11 Frankel, E.N., Kanner, J., German, J.B., Parks, E., and Kinsella, J.E. (1993). Inhibition of oxidation of human low density lipoprotein by phenolic substances in red wine. *Lancet* **341,** 454–457

12 Teissedre, P.L., Frankel, E.N., Waterhouse, A.L., Peleg, H., and German, J.B. (1996). Inhibition of in vitro human LDL oxidation by phenolic antioxidants from grapes and wines. *J. Sci. Food Agric.* **70,** 55–61

13 Fleury, Y., Welti, D.H., Philippossian, G., and Magnoloto, D. (1992). Soybean (malonyl) isoflavones: characterization and antioxidant properties. *In Phenolic compounds in food and their effects on health II: antioxidants and cancer prevention* (M.T. Huang, C.T. Ho, C.Y. Lee, eds), pp. 99–113, American Chemical Society, Washington, DC USA

14 Pratt, D.E. and Birac, P.M. (1979). Source of antioxidant activity of soybeans and soy products. *J. Food Sci.* **44,** 1720–1722

15 Record, I.R., Dreosti, I.E., and McInerney, J.K. (1995). The antioxidant activity of genistein in vitro. *J. Nutr. Biochem.* **6,** 481–485

16 Vinson, J.A., Dabbagh, Y.A., Serry, M.M., and Jang, J. (1995). Plant flavonoids, especially tea flavonols, are powerful antioxidants using an in vitro oxidation model for heart disease. *J. Agric. Food Chem.* **43,** 2800–2802

17 Frei, B. (1995). Cardiovascular disease and nutrient antioxidants: role of low-density lipoprotein oxidation. *Crit. Rev. Food Sci. Nutr.* **35,** 83–98

18. Esterbauer, H., Striegl, G., Puhl, H., and Rothender, M. (1989). Continuous monitoring of in vitro oxidation of human low density lipoprotein. *Free Radic. Res.* **15,** 67–75

19 Regnstrom, J., Strom, K., Moldeus, P., and Nilsson, J. (1993). Analysis of lipoprotein diene formation in human serum exposed to copper. *Free Radic. Res.* **19,** 267–278

20 Franke, A.A., Custer, L.J., Cerna, C.M., and Narla, K. (1995). Rapid HPLC analysis of dietary phytoestrogens from legumes and human urine. *Proc. Soc. Exp. Biol. Med.* **208,** 18–26

21 Adlercreutz, H., Markkanen, H., and Watanabe, S. (1993). Plasma concentrations of phyto-oestrogens in Japanese men. *Lancet* **342,** 1209–1210

22 Frei, B., Stocker, R., and Ames, B.N. (1988). Antioxidant defences and lipid peroxidation in human blood plasma. *Proc. Natl. Acad. Sci. USA* **85,** 9748–9752

23 Dabbagh, A.J. and Frei, B. (1995). Human suction blister interstitial fluid prevents metal ion-dependent oxidation of low density lipoprotein by macrophages and in cell free systems. *J. Clin. Invest.* **96,** 1958–1966

24 Thomas, S.R., Neuzil, J., Mahr, D., and Stocker, R. (1995). Coantioxidants make α-tocopherol an efficient antioxidant for low density lipoprotein. *Am. J. Clin. Nutr.* **62(Suppl),** 1357S–1364S

25 Rice-Evans, C.A., Miller, N.J., Bolwell, P.G., Bramley, P.M., Pridham, J.B. (1995). The relative antioxidant activities of plant derived polyphenolic flavonoids. *Free Radic. Res.* **22,** 375–383

26 Xu, X., Wang, H., Murphy, P.A., Cook, L., and Hendrich, S. (1994). Daidzein is a more bioavailable soymilk isoflavone than is genistein in adult women. *J. Nutr.* **124,** 825–832