APPENDICES

APPENDIX A

Progressive Exercise Test (VO_2pk test) on Cycle Ergometer (Method)

Principle

The peak oxygen consumption (VO_2pk) constitutes a distinct measurement of dynamic exercise capacity that has relevance for defining cardiorespiratory fitness and the potential for high level physical performance. The value of VO_2pk is also the definitive laboratory indicator for individual aerobic exercise capacity (Wareham et al., 1998). It is measured as the highest value at the point in a progressive exercise and it is observed to increase no further, despite continual increases in the external work rate or exercise intensity. VO_2pk has shown under conditions of continuously increasing loads, that the highest values are attained when subjects can be brought from a warm-up stage to a maximal effort within an 8-12 minute period. Response criteria accompanying the VO_2pk include: 1) a peak heart rate >90% of the age-adjusted predicted maximal value; 2) appearance of an R value (VCO2/VO2)>1.1-1.2 units; or 3) a rating of perceived exertion (RPE) (Borg Scale (Borg, 1982) to indicate mental fatigue) >18-19. Typical values for VO_2pk are most usefully expressed in relative units such as per kg of body mass = ml/kg/min.

Equipment

Monark cycle ergometer (Monark 818E, Monark, Stockholm, Sweden)

MedGraphics CPX/D VO_2 System (St Paul, MN) automated metabolic cart

ECG system (Quinton: ECG: Models Q5000)

Mouthpieces and noseclip

Borg scale card

Procedure

Before the VO_2pk test, the subject is interviewed to establish an estimated exercise capacity for cycling, so that that the peak oxygen consumption can be achieved within 8-12 min before muscle fatigue.

Before the test, the subject is fitted with a breathing apparatus (mouthpiece, nose clip and three-way breathing tube). One end of the breathing tube is open to room air so the subject can breathe freely and the other end is connected to the MedGraphics machine to analyze expired air during exercise. Software programs that drive the MedGraphics metabolic cart via desktop computer automatically store and record the responses for peak oxygen consumption, maximal pulmonary ventilation, and the respiratory exchange ratio, all of which are used to verify the attainment of the subjects' aerobic exercise capacity. Heart rate is measured by using an ECG system with CC5 lead. This ECG system involves three electrodes: LL = at the fourth left intercostals space, below and lateral to the nipple (positive electrode); RA = at the fourth right intercostals space and lateral to the nipple (negative electrode); and RL = at the lower right quadrant of the anterior thorax.

Subjects warm up on the cycle ergometer for two minutes to familiarize themselves with the cycle ergometer and the speed of 60 rpm. The workload is gradually increased by 30-60 watts every 1-2 min, depending on the subjects' heart rate response and estimated exercise capacity, until the subject is unwilling to continue or unable to maintain 55 rpm. The test is complete after all criteria accompanying the VO_2pk are achieved.

References

Borg, G. A. U. 1982. Psychological bases of physical exertion. Med. Sci. Sports Exer. 14: 377.

Wareham, N. J., S. J. Hennings, C. D. Byrne, C. N. Hales, A. M. Prentice, and N. E. Day. 1998. A quantitative analysis of the relationship between habitual energy expenditure, fitness and the metabolic cardiovascular syndrome. Br. J. Nutr. 80:235.

APPENDIX B

Eighty Percent VO_2pk Exercise on Cycle Ergometer (Method)

Principle

See Appendix 1 for VO_2pk.

In order to let subjects undergo one session of high-intensity exercise, 80%VO2pk exercise on cycle ergometer can lead to such a condition.

Equipment

Monark cycle ergometer (Monark 818E, Monark, Stockholm, Sweden)

MedGraphics CPX/D VO_2 System (St Paul, MN) automated metabolic cart

ECG system (Quinton: ECG: Models Q5000)

Mouthpieces and noseclip

Borg scale card

Timer

Procedure

The subject is instructed not to participate in any exercise or sport two days before the submaximal exercise. Upon reporting to the laboratory, the subject rests for 10 minutes before a venous blood sample is drawn from an antecubital vein. Subsequently, the subject warms up to reach his own 80% VO_2pk on cycle ergometer and then exercises for another 30 minutes. During the submaximal exercise, heart rate and rate of perceived exertion are monitored continuously throughout the exercise. In addition, oxygen consumption is measured every five minutes of the exercise in order to keep the subject exercised at 80% VO_2pk. In the meantime, the workload on the cycle ergometer is adjusted according to subject's 80% VO_2pk; and oxygen consumptions are recorded. The average exercise intensity throughout the submaximal exercise is calculated by the recorded oxygen consumptions of the subject.

APPENDIX C

Total Glutathione and Oxidized Glutathione Analyses in Blood (Method)

Principle

Total glutathione is conveniently assayed by an enzymatic recycling procedure in which GSH is sequentially oxidized by 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) and reduced by NAPDH in the presence of glutathione reductase (GR). The rate of 2-nitro-5-thiobenzoic acid formation from DTNB is monitored at 412 nm and the glutathione present is evaluated by comparison of that result with a standard curve created using different amounts of GSH.

In order to measure GSSG, GSH is masked with 2-vinylpyridine, which does not inhibit GR activity. Acidic solution must be at least partially neutralized before GSH and 2-vinylpyridine will react. After neutralization and derivatization of GSH, the procedure for the determination of GSSG is the same as the one for total glutathione determination.

Reagents

Stock buffer: 143 mM Na_3PO4 and 6.3 mM Na_4EDTA, pH 7.5

10 % SSA (5-Sulfosalicyclic acid) + 1 mM bathophenanthrolinedisulfonic acid (BPDS)

0.3 mM NADPH made with the stock buffer

6 mM DTNB made with stock buffer

10 % SSA

266 U/ml GR

2-vinylpyridine

GSH standards: 200, 400, 600, and 800 nmol/ml

Procedure

Immediately after blood is drawn, 1 ml of blood is mixed with 2 ml of 10% 5-sµlfosalicyclic acid containing BPDS for further total glutathione measurement. In addition, 2 ml of blood is mixed with 1 ml of 10% 5-sµlfosalicyclic acid containing BPDS for further oxidized glutathione measurement. Both mixtures are kept in ice; and they are centrifuged at 10,000 x g at 4^oC for 15 minutes within 30 minutes from the preparation.

Total glutathione measurement

Five microliters of supernatant processed from whole blood are pipetted into a cuvette with 1-cm light path. Then, 700 µl of 0.3 mM NADPH made fresh daily using stock buffer and 100 µl of 6 mM DTNB prepared from stock buffer are added into the cuvette. Subsequently, 20 microliters of 10% 5-sulfosalicylic acid and 175 µl of distilled water are added to bring the volume to 1 ml and then the solution is mixed. The mixtures are equilibrated at 30^{o}C for 12-15 minutes. The absorbance at 412 nm is recorded for 70 seconds immediately after glutathione reductase is added. Unknown concentration of glutathione in sample is calculated from the standard curve created by using 200, 400, 600, and 800 nmol/ml of glutathione.

Oxidized glutathione measurement

An aliquot of 900 µl supernatant processed from whole blood is placed in a 10 ml tube and neutralized by adding 0.1 N NaOH (pH 7-7.5). Then 2 µl of 2-vinylpyridine is added into 100 µl neutralized supernatant to derivatize glutathione and prevent its oxidation. After the tube is mixed vigorously for 1-5 minutes, the mixtures are incubated at room temperature for 2 hours. The derivatized sample is assayed as described above in the DTNB-oxidized glutathione reductase recycling assay. Unknown amount of oxidized glutathione is also calcµlated from the standard curve created by using 200, 400, 600, and 800 nmol/ml of glutathione.

Reference

Griffith, W. 1980. Determination of glutathione and glutathione disulfide using glutathione reductase and 2-vinylpyridine. Anal Biochem. 15:207.

APPENDIX D

Ascorbic Acid and Dehydroascorbic Acid Analyses in Human Plasma (Method)

Principle

Vitamin C has two forms, ascorbic acid (AA) and dehydroascorbic acid (DHAA). Ascorbic acid concentration in the sample is directly measured with ultraviolet light detection (254 nm). However, DHAA is found in only very low levels compared with ascorbate. DHAA is indirectly measured by converting it to AA by dithiothreitol (DTT). After the conversion, total AA is measured, and then DHAA amount is equal to TAA minus AA.

Reagents

10% perchloric acid containing 1% metaphosphoric acid

Mobile phase (20 mM ammonium dihydrogen phosphate containing 0.015% metaphosphoric acid), pH 3.5

10 mM dithiothreitol in mobile phase containing 0.5 mM K_2HPO_4

Standards: 1.25, 2.50, 5.00, 10, 15 and 20 mg/l ascorbic acid

Procedure

One tube of blood is immediately covered with aluminum wrap and kept in ice after it is drawn. Plasma is separated from blood by centrifugation at 2000 x g for 20 minutes at 4^{o}C within 30 minutes. Two aliquots (0.5 ml) of plasma (one used for ascorbic acid and the other for total ascorbic acid) are added to an equal volume of precooled acid solution (10% perchloric acid containing 1% metaphosphoric acid). The resulting mixture is held at 4^{o}C for 1 hour for deproteinization and then centrifuged at 12,000 x g at 4^{o}C for 5 minutes. The supernatant is placed in a brown microcentrifuge vial and is snap frozen in liquid nitrogen and stored at –80^{o}C for further ascorbic acid, total ascorbic acid, and uric acid analyses by high performance liquid chromatography (HPLC).

<u>Ascorbic acid determination</u>

Frozen acidic supernatant is gently thawed and kept on ice. One hundred microliters of supernatant is mixed with 150 µl mobile phase and then centrifuged at 12,000 x g at 4^{o}C for 2 minutes. Subsequently, 40 µl of supernatant is injected into HPLC for ascorbic acid and uric acid determinations. Unknown concentrations of ascorbic acid and uric acid in plasma are calculated from the standard curves.

Total ascorbic acid determination

Frozen acidic plasma supernatant is gently thawed and kept on ice at all times. One hundred microliters of plasma is mixed with 150 µl of 10 mM dithiothreitol, which is freshly made every day. After all dehydroascorbic acid is reduced by DTT at 25^{o}C for 30 minutes in the dark, the mixture is centrifuged at 12,000 x g at 4^{o}C for 2 minutes. Subsequently, 40 µl of supernatant is injected into HPLC for total ascorbic acid determination. Unknown concentration of total ascorbic acid in plasma is calculated from the standard curve.

Reference

Rümelin, A., Fauth, U. and Halmagyi, M. 1999. Determination of ascorbic acid in plasma and urine by high performance liquid chromatography with ultraviolet detection. Clin Chem Lab Med. 37:533-536.

APPENDIX E

LDL Separation from Plasma (Method)

Principle

Human LDL can be isolated from plasma by ultracentrifugation within a density gradient of 1.019-1.063 g/ml (Esterbauer and Ramos, 1996).

Reagents

Potassium bromide

0.154 M NaCl

Procedure

The density of the plasma is adjusted to 1.063 g/ml by addition of 0.3265 g potassium bromide to one ml of plasma. One and one-half ml of density adjusted plasma is overlaid with 3 ml of 0.154 M NaCl in a Beckman centrifuge tube. Subsequently, all tubes are centrifuged at 800,000 rpm (Optima L-90K, Beckman) at 7°C for 45 minutes. After centrifugation, approximately 1 ml LDL fraction, seen in the upper 1/3 of tube, is carefully isolated by means of a glass pipette. This fraction is retained for further measurement of ex vivo and in vivo LDL MDA by a HPLC method.

References

Esterbauer, H., Rotheneder, M. D., Striegl, G., and Wage, G. 1991. Role of vitamin E in prevention of LDL oxidation. Am. J. Clin. Nutr. 53:3145.

Havel, R., Eder, H. and Bragdon, J. 1995. The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. J. Clin. Invest. 34:1354

APPENDIX F

Erythrocyte Superoxide Dismutase Assay (Method)

Principle

Superoxide dismutase (SOD) reduces superoxide to hydrogen peroxide. The theory of this method is based on the competition between SOD activity and iodonitrotetrazolium violet in reacting with superoxide, which is generated by xanthine oxidase (XOD) reaction. The reactions are demonstrated below:

a) Xanthine + O_2 $\longrightarrow$ uric acid + $O_2^{\cdot -}$

b) $O_2^{\cdot -} + O_2^{\cdot -} + 2H^+ \xrightarrow{XOD}$ hydrogen peroxide + O_2

c) $O_2^{\cdot -}$ + violet $\xrightarrow{SOD}$ oxidized detector (generate color)

With increasing SOD concentration, the competition between reaction b and c measured as a decrease of the rate of the detector reaction. The SOD activity measured is related to 50% inhibition of the detector reaction.

Reagents

Phosphate buffer-10 mM KH_2PO_4, pH 7

Cocktail buffer, pH 10.2, made with the solutions below

69.4 ml of 0.1 M Na_2CO_3

30.4 ml of 0.1 M $NaHCO_3$

1 ml of 40 mM xanthine in 0.1 N NaOH

4 ml of 10 mM iodonitrotetrazolium violet in 11% ethanol

Xanthine oxidase, 0.3 units/ml

Superoxide dismutase standards: 20.8, 10.4, 5.2, 2.6, and 1.3 U/ml

Procedure

One hundred microliters of RBC is mixed with 900 microliters of distilled water. Then, 60 microliters of RBC lysate is further diluted with 940 µl of 10 mM phosphate buffer, and this diluted RBC lysate is used for the superoxide dismutase assay. Ninety-four microliters of superoxide dismutase standard or diluted RBC lysate sample and 1.27 ml of carbonate buffer are added into a cuvette. Just before reading the change of absorbance, 75 µl of xanthine oxidase is added. Absorbance is read every 20 seconds continuously for 3 minutes on a spectrophotometer at 500 nm at room temperature. The changing rate of absorbance is used to determine superoxide dismutase activity.

Concentration of superoxide dismutase in samples is determined by the comparison with the calibration curve from SOD standards.

Reference

Xin, Z., Waterman, D. F., Hemken, R. M. and Harmon, R. J. 1991. Effects of copper status on neutrophil function, superoxide dismutase, and copper distribution in steers. J. Dairy Sci. 74: 3078.

APPENDIX G

Erythrocyte Catalase Assay (Method)

Principle

The ultraviolet absorption of hydrogen peroxide can be easily measured at 240 nm. On the decomposition of hydrogen peroxide with catalase, the absorption decreases with time and from this decrease catalase activity can be measured.

Reagents

Phosphate buffer, pH 7

3.522 g KH_2PO_4 and 7.268 gm $Na_2HPO_4.2H_2O$ in 1000 ml water

Hydrogen peroxide solution

0.085 ml of 30% hydrogen peroxide in 25 ml phosphate buffer

Procedure

Red blood cell lysate is prepared by adding 1.2 ml of distilled water to 0.2 ml of RBC. Then five hundred-fold dilution of RBC lysate by phosphate buffer is made before the determination of catalase activity. Immediately following the addition of 1 ml phosphate buffer (blank) or hydroxide peroxide solution into 2 ml RBC diluted lysate, the change of absorbance of RBC against blank at 240 nm is recorded every 15 seconds for 1 minute on a spectrophotometer. The activity of catalase is calculated by using the following the equation: catalytic concentration (unit/l) = (0.23*log A1/A2)/0.00693, where A1 is A240 at t = 0; A2 is A240 at t = 15 second.

Reference

Aebi, H. E. 1983. Catalase. In: Methods of enzymatic analysis. 3rd ed. (Bergmeyer, H. U., et al. Ed.) pp.273-285. Weinheim. Deerfield Beach, FL.

APPENDIX H

Erythrocyte Glutathione Peroxidase Assay (Method)

Principle

Glutathione peroxidase catalyzes hydrogen peroxide by the oxidation of GSH according to the following reaction:

$$2GSH + H_2O_2 \xrightarrow{GPx} GSSG + H_2O$$

$$GSSG + NADPH \xrightarrow{GR} 2GSH + NAPD$$

Rather than measuring the progressive loss of GSH, however, this substrate is maintained at a constant concentration by the addition of exogenous GR and NADPH, which immediately convert any GSSG produced to GSH. The rate of GSSG formation, representing the GPx activity, is then measured by following the decrease of NADPH in absorbance of the reaction mixture at 340 nm.

Reagents

0.1 % $NaHCO_3$

KH_2PO_4 buffer, pH 7.2

5.444 gm KH_2PO_4 plus 0.596 gm EDTA in 100 ml water

GSH

0.0154 gm in 1 ml water

Glutathione reductase, 2 units/ml

Beta-NADPH

0.0025 gm NAPDH in 1 ml of 0.1 % $NaHCO_3$

t-butyl hydroperoxide (TBH)

40 µl of TBH in 50 ml water

Cyanodilution

0.137 gm KCN, 0.052 gm $K_3Fe(CN)_6$, and 0.2653 gm $NaHCO_3$in 500 ml water

Procedure

Fifty microliters of RBC is mixed with 1 ml cyanodilution mixture and then shaken. To 0.02 ml of diluted RBC are added five hundred microliters of potassium

buffer, 0.2 ml of glutathione, 0.05 ml of GR, 0.48 ml of distilled water, and 0.2 ml of NADPH. After 10 minutes, 0.55 ml of tertiary butylhydroperoxide is added into the mixture. Following mixing, the absorbance of the solution is immediately monitored at 340 nm for 100 seconds. The activity of glutathione peroxidase is determined by the change of absorbance and calculated as follows: mmole/l/s=2813* (A_{sample} - A_{blank}).

Reference

Agergaard, N. and Jensen, P. T. 1982. Procedure for blood glutathione peroxidase determination in cattle and swine. Acta. Vet. Scand. 23: 515.

APPENDIX I

Plasma Genistein and Daidzein Assay (Method)

Principle

Isoflavones are lipophilic, thus, they can be extracted by methanol. Before the isoflavone detection by the HPLC system, two steps are needed: condensation and glucuronidase digestion. With the range from 10-2000 ng/ml, the condensation step is needed to enhance the chance of isoflavone detection by HPLC. In addition, most isoflavones in the body are in the glucuronidated forms. Therefore, the linkage between glucuronide and isoflavone must be cleaved before isoflavones can be detected by HPLC.

Reagents

Methanol

1 M sodium acetate buffer, pH 5.5

Beta-glucuronidase/sulfatase

80% acetonitrile

Fluorescein, 199.541 ng/µl (internal standard)

Standards

Genistein: 12.5, 62.5, 125, 250, and 500 ng

Daidzein: 12.5, 62.5, 125, 250, and 500 ng

Fluorescein: 140, 700, 1400, 2800, and 5600 ng

Procedure

The determination of plasma genistein and daidzein by HPLC, modified from methods of Wang and Murphy (1994) and Xu et al. (1994). Half ml plasma and 19 µl fluorescein are mixed with 10 ml of methanol and then the mixture is centrifuged at 5000 x g for 10 minutes at 10°C. The methanol supernatant is decanted into a rotary evaporated flask. The plasma residue is redissolved twice in 5 ml methanol and centrifuged at 5000 x g for 10 minutes at 10°C. Total 20 ml methanol supernatant, harvested from the extractions, is evaporated to almost complete dryness, using a rotary evaporator (Buchi Model 461, Brinkmann, Westbury, NY) at 37°C. Total seven ml of 1 M sodium acetate buffer are used to rinse the sides of the flask. The hydrolyzate is transferred to a glass test tube with a cap and 50 µl beta-glucuronidase/sulfatase is added. The mixture is incubated at 37°C overnight (16-20 hours) in a dry heating block. After incubation, the hydrolyzate is filtered by a solid phase extraction cartridge (J&W

Scientific, Folsum, CA) before proceeding with HPLC analysis. The filtrate is dried under nitrogen. The residue is re-dissolved in 170 µl of 80% acetonitrile. Then, 100 µl of extract is injected into HPLC for genistein and daidzein determination. The recovery rate of plasma isoflavone from all extraction steps is calculated according to the amount of fluorescein, added in the beginning of extraction and detected from HPLC analysis. Unknown concentrations of plasma genistein and daidzein and internal standard, fluorescein, are calculated from the standard curves of genistein, daidzein, fluorescein.

References

Wang, H. and Murphy, P.A. 1994. Isoflavone content in commercial soybean foods. J. Agric. Food Chem. 42: 1666-1673.

Xu, X., Wang, H., Murphy, P.A., Cook, L., and Hendrick, S. 1994. Daidzein is a more bioavailable soy milk isoflavone than is genistein in adult women. J. Nutr. 124: 825-832.

APPENDIX J

Malondialdehyde by TBARS-HPLC Assay (Method)

Principle

Lipid peroxides are formed from lipids oxidized by free radicals. The product of the decomposition of lipid peroxide is malondialdehyde (MDA), which reacts with thiobarbituric acid (TBA). The amount of TBA-MDA adduct can be measured by the fluorescence detector with excitation at 515 nm and emission at 553 nm, indicating the extent of lipid peroxidation in vivo and ex vivo.

Reagents

100 um CuSO4

0.1 M sodium acetate buffer

0.2% (W/V) TBA, pH 3.5

Standards using 1,1,2,2-tetraethoxypropane

Ex vivo LDL-MDA: 1, 5, 10, 20, 40 μM

In vivo LDL-MDA; 0.001, 0.005, 0.01, 0.05, and 0.1 μM

Plasma: 0.5, 0.75, 1, 2.5, and 5 μM

Procedure

LDL oxidation

To test LDL resistance to lipid peroxidation, the following steps are applied. First, LDL is dialyzed with 10 μM EDTA phosphate buffer solution at 4^{o}C for 24 hours. Then, 200 μl of 100 μM $CuSO_4$ and 600 ml distilled water are added to the tube containing 200 μl dialyzed LDL. The mixture is incubated at 37°C for 24 hours. Seventy-five μl of oxidized LDL is mixed with 1 ml TBA in a 1.5 ml polypropylene microtube. The microtube is capped and heated in a water bath at 95^{o}C for 60 minutes. After cooling with tap water, the reaction mixture is centrifuged at 10,000 x g at ambient temperature for 15 minutes. Five μl of supernatant is injected into HPLC for MDA determination. The TBA-malondialdehyde adduct is monitored by the fluorescence detector with excitation at 515 nm and emission at 553 nm. The concentration of MDA is calculated from the peak area, based on a calibration curve prepared by standards.

Plasma and LDL MDA (in vivo)

An aliquot of 50 µl plasma or standards is added to 1 ml TBA in a 1.5 ml polypropylene microtube and mixed by vortex mixer. In order to increase the sensitivity in the determination of in vivo LDL MDA, 50 µl dialyzed LDL is mixed with 100 µl 0.2% TBA solution. The following steps for measuring plasma and LDL MDA are the same as those described above for ex vivo LDL MDA determination by HPLC.

References

Jialal, I. and Grundy, S. 1991. Preservation of the endogenous antioxidants in low density lipoprotein by ascorbate but not probucol during oxidative modification. J. Clin. Invest. 87: 597.

Natio, C., Mitsunobo, M. and Yamamoto, Y. 1993. Lipid peroxides as the initiating factor of atherosclerosis. Ann. N. Y. Acad. Sci. 673: 27.

Fukunaga, K., Yoshida, M. and Nakazono, N. 1998. A simple, rapid, highly sensitive and reproducible quantification method for plasma malondialdehyde by high-performance liquid chromatography. Biomed. Chromatogr. 12(5):300-303.

APPENDIX K

Ferric Reducing/Antioxidant Ability of Plasma (FRAP) (Method)

Principle

The assay which measures the combined antioxidant effect of the non-enzymatic defenses in biological fluids is useful in providing an index of ability to resist oxidative damage. At low pH, when a ferric-tripyridyltriazine complex is reduced to the ferrous form by reductants (antioxidants) in the mixture, an intense blue color with an absorption at 593 nm develops. In this assay, excess ferric ion is used, and the rate-limiting factor of ferrous-tripyridyltriazine formation, and hence color, is the reducing/antioxidant of the plasma.

Reagents

40 mM HCl

300 mM acetate buffer, pH 3.6

3.1 gm $C_2H_3NaO_2.3$ H2O plus 16 ml acetate added up 1 liter with water.

10 mM 2,4,6-tripyridyl-s-triazine (TPTZ)

20 mM $FeCl_3 \cdot 6H_20$

Cocktail solution

All three above solution mixed together in the ratio 10 : 1 : 1

Standards using $FeSO_4 \cdot 7H2O$

250, 500, 1000, 1500, and 2500 μM

Procedure

The FRAP assay is performed using the cocktail solution preheated to 37°C. To 30 μl sample or standard are added 900 μl cocktail solution and 90 μl water. The mixture is incubated at 37°C for 4 minutes. Absorbance at 593 nm is determined relative to a reagent blank that is also incubated at 37°C. Antioxidant power of plasma is determined against a standard curve of ferrous sulphate.

References

Benzie, I. F. F. and Strain, J. J. 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP Assay. Anal. Biochem. 239: 70-76.

Langley-Evans, S. C. 2000. Consumption of black tea elicits an increase in plasma antioxidant potential in humans. Inter. J. Food Sci. Nutri. 51: 309-315.

APPENDIX L

Hematocrit (Method)

Principle

Hematocrit is used to account for plasma volume change after exercise and is analyzed by microcentrifugation. Plasma volume change (% ΔPV) is calculated according to the equation below (Van Beaumont et al., 1973):

$$\% \Delta PV = 100/(100-Hct_{pre})*100*(Hct_{pre}-Hct_{post})/Hct_{post}$$

Procedure

Two capillary tubes are filled with whole blood. After 15 minutes of spin, the percentage of hematocrit is read on the ruler. Plasma volume change between pre- and post-exercise is calculated by the above equation.

Reference

Van Beaumont, W., Strand, J. C., Petrofsky, J. S., Hipskind, S. G. and Greenleaf, J. E. 1973. Changes in total plasma content of electrolytes and proteins with maximal exercise. J. Appl. Physiol. Jan. 34(1):102-106.

APPENDIX M

Protein Assay-Sigma 5656 (St. Louis, MO) (Method)

Principle

An alkaline cupric tartrate reagent complexes with the peptide bonds and forms a purple-color when the phenol reagent is added. Absorbance is read at a suitable wavelength between 500 nm and 800 nm. The protein concentration is determined from a calibration curve.

Regents

Lowery Reagent, modified

Trichloroacetic acid solution, 72% (W/V)

Folin & Ciocalteu's phenol reagent

Protein standard, prepared from bovine serum albumin.

Procedure

Dialyzed LDL is diluted 20-fold with distilled water. One ml of diluted LDL, standard, and blank (water) is added to test tubes, respectively. Then, one ml of Lowry reagent is added to all tubes. All tubes are mixed well and incubated at room temperature for 20 minutes. Subsequently, with rapid and immediate mixing, 0.5 ml Folin & Ciocalteu's Phenol reagent is added to all tubes. After 30 minutes of color development at room temperature, the absorbances of LDL and standard against blank are read at 750 nm within one hour. LDL concentration is calculated from the standard curve created by using different concentrations of protein standard.

References

Peterson, G. L. 1977. A simplification of the protein assay method of Lowry et al. which is more generally applicable. Anal. Bioch. 83:346.

Bensadoun, A. and Weinstein, D. 1976. Assay of proteins in the presence of interfering materials. Anal. Bioch. 70:241.

Lowry, O.H., Rosebrough, N.J., Lewis, F.A., and Randall, R. 1951. Protein measurement with the Folin Phenol reagent. J. Biol. Chem. 193:265.

APPENDIX N

Total Hemoglobin-Sigma 525A (St. Louis, MO) (Method)

Principle

This assay bases on a colorimetric cyanmethemoglobin method where total hemoglobin at alkaline pH is rapidly converted to the cyanoderivative. Drabkin's solution containing alkaline ferricyanide and cyanide reacts with all forms of hemoglobin. The absorbance of the cyanoderivative is dertermined at 540 nm.

Reagents

Drabkin's reagent

BRI-J-35 solution

Hemoglobin standard

Procedure

Erythrocytes are diluted 2.5-fold with water. To sample and blank tubes is added 5 ml Drabkin's reagent. Twenty microliters of diluted erythrocytes are added to tube with Drabkin's solution. The mixture is incubated at least 15 minutes at room temperature. The absorbance of sample against blank is read at 540 nm. The concentration of hemoglobin is calculated according to the standard cure created by using different concentrations of cyanhemoglobin standard.

Reference

Drabkin, D.L. and Austin, J.H. 1935. Spectrophotometric studies. II. Preparations from washed blood cells; nitric oxide hemoglobin and sulfhemoglobin. J. Biol. Chem. 112:51.

APPENDIX O

Medical Health History Questionnaire (Subject Questionnaire)

VIRGINIA POLYTECHNIC INSTITUTE AND STATE UNIVERSITY

Health History Questionnaire

Subject #: _____

Title of Project: The Beneficial Effects of Genestein Soy Protein in Humans

Investigators: Raga M. Bakhit, Ph.D and Oliver Chen

Please complete the following questionnaire as accurately as possible.

Name: _____ Age: _____

Date of Birth: _____19_____

Address:

_____

_____

_____

Phone: (540) _____(work)

Phone: (540) _____(home)

1. Do you have any known allergies? Y N

   If yes, please explain: (food, medical, other)

_____

_____

_____

_____

2. Do you currently take any medications? Y N

   If yes, please explain:

_____

_____

_____

_____

3. Do you currently exercise? Y N

   If yes, please explain: (what? how often?)

_____

_____

_____

_____

4. Please circle all that apply to you?

High Blood Pressure	Asthma	Smoking/Tobacco	
Skipped Heart Beats	Chest Discomfort	Fast Heart Rate	Diabetes
Heart Murmur	Short of Breath	Dizziness/Fainting	Joint Soreness

   Others

   If circled, please explain:

_____

_____

_____

_____

_____

5. Is there any reason not mentioned above that would limit your ability to perform high-intensity exercise?

Y N

If yes, please explain:

_____

_____

_____

_____

6. Has a physician ever told you to refrain from activity or exercise for an extended period of time?

Y N

If yes, please explain:

_____

_____

_____

_____

7. Do you have any difficulty with getting your blood taken?

Y N

If yes, please explain:

_____

_____

_____

Signature _____

Date _____

Thank you.

APPENDIX P

Volunter Information (Subject Questionnaire)

Instruction:

1. Please answer the following questions.
2. Please list a phone number where you can be reached.
3. Please send the completed form to me by attachment through e-mail.

Name: **Age:**

Phone number (home):

Phone number (office):

E-mail address:

How many hours do you exercise per week generally?

Do you smoke?

Do you take any vitamin supplement? If you do, how many tablets do you take a week?

Have you eaten any foods made of soy, such as soy burger, tofu, soy milk, and soy cheese? If you do, how often do you consume these foods?

Are you on any medication for any disease? If yes, please explain.

APPENDIX Q

Food Intake Form (Subject Handout, to be filled out and returned)

DAY _____ DATE _____ NAME _____

Time	Food/Beverage	Amount (cups, tbsp, oz., etc) Dimensions (5" banana)	Home/Out?

APPENDIX R

How to Ccomplete Food Intake Form (Subject Handout)

1. On the top of each form, write the date and day on intake record in the spaces provided.
2. Please write as clearly as possible because we will have a large number of these to analyze.
3. List all foods and beverages (including water and condiments) consumed during the 24-hour period.
4. Please describe food precisely. For example, “2% milk” vs. “milk” or whole wheat bread-Wonder brand’ vs. “bread”.
5. Please specify quantities consumed. If you are not certain on amounts, use measuring cups or spoons whenever possible.
6. Remember to list all additions to food and beverages, such as cream, sugar, butter, jelly, lemon, salad dressing, artificial sweeteners, catsup, etc. Do not forget ingredients added in food preparation such as vinegar, oils, and if fat and salt were used.
7. Describe how foods are prepared. For example: ½ cup frozen broccoli, steamed.
8. For beverages, you may use fluid ounces instead of cups, For example: 12 oz Pepsi.
9. For foods that do not fit in a cup or spoon, use dimensions. For example: 1 corn tortilla, 6 inches across; 1 piece of cheddar cheese 3”x2”x1”; 1 banana nut muffin 2”x1”.
10. For whole pieces of fruit or vegetables, you may use small, medium, or large. For example: one small Granny Smith apple.
11. List each food item, and the amount of each item, in mixed dishes. For example: 1 cup leaf lettuces, two ¼ “tomato slices, two 2” slices cucumber.
12. When including recipes, be sure to include how much the recipe yields in cups and your serving size. For example: Total recipe eight 1-cup servings. I had 1 cup.

AN EXAMPLE OF NOW TO FILL OUT YOUR FOOD INTAKE FORM

Time	Food/Beverage	Amount (C., tbsp, tsp, oz., etc) Dimensions (5" banana)	Home/ out?
7:00	Water	½ c	Home
7:15	Coffee	2 c	"
	Creamer-Carnation	2 tsp	"
	Frosted mini wheats	1 ½ c	"
	2% milk	2 c	"
8:30	Coffee-decaf brewed	1 c	School
	Creamer-Carnation	1 tsp	"
11:30	Bread-lite wheat-Nature Maid	2 slices	"
	Ham-92% fat free-Oscar Meyer	4 slices (4 oz)	"
	Mayo-Kraft Regular	1 tbsp	"
	Apple-red	1, 3-inch diameter	"
	Diet Pepsi	12 oz	"
3:00	Cookies-Chips Ahoy	6	"
	Iced tea-instant with lemon- Lipton	12 oz	"
6:15	McDonalds Quarter Pound w/Cheese	1	McDonalds
	French Fries	1 large order	"
	Vanilla Shakes	1 regular	"
	Side Salad	1	"
	Ranch Dressing	2 tbsp	"
8:00	Peach	1, 3-inch diameter	Home
	Cranapple Juice	10 oz	"

APPENDIX S

Common Weights and Measures (Subject Handout)

Fluids:
1 cup = 8 ounces
1 ounce = 2 tablespoons

Dry and fluid foods:
1 Cup = 16 tablespoons
1 tablespoon = 3 teaspoons

Please use standard measuring cup to measure the food you record.

Abbreviations:
Tablespoon = T or Tbsp
Teaspoon = t or tsp
Cup = C

Estimating Quantity of food:

1. For mixed dishes, please record approximate amounts of main ingredients eaten. For example, instead of "tuna casserole," list:
   2 oz. tuna fish, Bumble-bee, water-packed
   2 oz. cottage cheese, Breakshote's, 4% milk-fat
   1/2 C spiral noodles

2. For sandwiches, list ingredients separately. For example, a "turkey sandwich" is recorded as follows:
   2 slices bread, rye
   2 oz turkey
   1 leaf lettuce
   2 slices tomato
   1tsp. Mayonnaise, Weight-Watchers

3. All foods should be measured when ready for eating. Report the amount of meats, fruits and vegetables (etc) either raw or after cooking, depending on how they are served. For example, 3 cups of raw spinach may shrink to 3/4 cups cooked, so record 3/4 cups boiled spinach.

4. Please list whether the food was frozen, canned, or fresh, salted or unsalted.

5. Note the method of cooking: fried in peanut oil, boiled, baked, etc…

There is a ruler printed on this form to help you with portion sizes. This will help you measure foods like pizza, cakes, cookies, fruits… Please do not report weights unless you have a scale on which to weigh the food.

APPENDIX T

Activity Diary (Subject Handout, to be filled out and returned)

DAY _____ DATE _____ NAME _____

A. Sleep/Lying Down

B. Sitting Activities (little or not arm movement)
- B1 Eating
- B2 Reading/Writing
- B3 Watching television
- B4 Shaving
- B5 Driving
- B6 Typing/Computer work
- B7 Other

C. Light Activities (some arm movement while standing)
- C1 Office work
- C2 Standing/Slow walk
- C3 Housework
- C4 Personal Care (e.g. Dressing/Bathing)
- C5 Shopping
- C6 Light Gardening
- C7 Other

D. Moderate Activities (Moderate arm movement while standing or vigorous arm movement while sitting)
- D1 Walking (Vigorous pace)
- D2 Yardwork
- D3 Moderate Sports (e.g. Golf, Bowling)
- D4 Other

E. Hard Activities
- E1 Physical (e.g. Lifting/Carrying)
- E2 Vigorous Sports (e.g. Softball)
- E3 Aerobics
- E4 Dancing, Bicycling
- E5 Swimming
- E6 Snow Shoveling
- E7 Other

F. Very Hard Activities
- F1 Strenuous Physical Labor (e.g. Digging)
- F2 Exertive Sports (e.g. Running)
- F3 Single Tennis
- F4 Skiing
- F5 Basketball
- F6 Swimming Laps
- F7 Other

Write in the space provided the categorical value that corresponds best to the dominant activity of each 15-minute period.

Time	:00	:15	:30	:45
AM 5:00				
6:00				
7:00				
8:00				
9:00				
10:00				
11:00				
Noon 12:00				
1:00				
2:00				
3:00				
4:00				
5:00				
6:00				
7:00				
8:00				
9:00				
10:00				
11:00				
Midnite 12:00				
1:00				
2:00				
3:00				
4:00				

Adapted from: Bruce Elmore, An Evaluation of Five Physical Activity Assessment Methods in a Group of Women. (Ph.D. Dissertation, 1989)

APPENDIX U

Foods to Avoid (Subject Handout)

Food Categories	Foods to Avoid	Acceptable Alternatives
Bread and Starches	Any baked product containing soy (soy flour, soy fiber, soy protein, soy concentrate, etc.) Soy noodles Soy chips	All baked products made without soy All other noodles (rice, semolina, regular wheat, corn, etc.) Potato, corn, vegetable chips
Vegetables	Edemame (edible green soybeans) Soy-containing: Spinach nuggets Broccoli nuggets Veggie patties Clover sprouts (read labels)	All other fresh and canned and frozen vegetables
Fruits	Please try to avoid Vitamins A- and C- supplemented juices (limit to < 2C/day)	All fruits, canned and fresh
Milk	Soy Milk/ soy powder Soy yogurts, puddings, and ice creams Soy cheeses Soy infant formulas	All other types of milk and milk's products (cow, goat, rice…)
Meats, beans, and Meat Substitutes	Tempeh Natto (fermented soy beans) Tofu (soybean curd) Soy Protein Isolate or concentrate Veggie burgers w/ soy Meatless chicken nuggets USDA commodity beef patties with TVP Meatless bacon Soy link-sausages Soy/meatless franks or hotdogs	(Vegetarians, compliment grains with nuts, cheeses) -Pumpkin seeds -Sunflower seeds -Almonds, Pecans, Walnuts

Food Categories	Foods to Avoid	Acceptable Alternatives
Meat and Meat substitutes continued	Legumes: -soybeans -kidney beans -navy beans -Pinto beans -Red beans -White beans -Broad beans	Tahini/sesame butter Almond butter Hazelnut butter

	-Chick peas -Pigeon peas -Split beans (lentils, split peas) -Mung beans -Peanuts **Peanut Butter** Soybean Butter	
Miscellaneous supplements	-Vitamins A, Beta-carotene, C, D, and E -Antioxidants supplements -Isoflavones -All Vit/Min supplements -Soy sauce -Soy paste and powder -most muscle/diet drinks,smoothies, shakes, etc. -most muscle/diet bars -Teas: Green tea Jasmine tea	

APPENDIX W

Compliance Survey (Subject Questionnaire)

Please honestly answer the following question (s). There is no penalty if you let us know how many tablets you did not take, if any. This is just to help us analyze your results more accurately.

Did you miss taking any tablet during the supplementation period?
Please circle the correct answer.

No Yes

If you answer is Yes, please fill in below the number of tablets you did not take?

_____ tablets

THANK YOU VERY MUCH FOR YOUR PARTICIPATION IN OUR STUDY.
WHITOUT YOU, WE COULD NOT HAVE FINISHED THIS EXPERIMENT.

APPENDIX V

VIRGINIA POLYTECHNIC INSTITUTE AND STATE UNIVERSITY

Informed Consent for Participants of Investigative Projects

Title of Project: Study to Test the Beneficial Effects of High Genistein Isoflavones on Humans

Investigators: Dr. Raga Bakhit, Oliver Chen

I. Purpose of the Research/Project:
I am being asked to participate in a research study to determine if soy protein has any positive effects on risk factors for heart disease, specifically LDL cholesterol and blood lipids. It will involve periodic exercise and blood draws to get adequate data. Healthy college aged people are being asked to participate in this research study.

II. Procedures: Prior to being included in the research study, I will complete a health history that will help determine if there may be reasons why I should not participate in this study. If the results of the health history indicate that I am an appropriate subject for this study, then I will be informed of when I should report to the Human Performance Laboratory at 230 War Memorial Hall for initial exercise testing.
Exercise sessions: I understand that I will perform 1 maximal exercise test on a cycle ergometer to determine the maximal amount of exercise that I can perform. This will be on the first day of the research study and I will need to report to the lab in the morning without eating or drinking 2 hours prior to arrival. During this test I will be connected by electrodes and cables to an electrocardiograph recorder which will enable the technician to monitor my heart rate. I will also be fitted with a breathing apparatus (mouthpiece and nose clip) which will measure my exhaled gases to properly measure oxygen consumption. This test will be on a cycle ergometer with the amount of effort gradually increased. As I understand it, the increase in effort will continue until I report to the technician that I am unable to continue or the pedal rate falls below 40 revolutions per minute. I will decide when I am unable to continue and the technician will stop the test when I so request.
The second and third exercise sessions will last 30 minutes. I will be connected to the electrocardiograph for heart rate but not the breathing apparatus. I will cycle on the ergometer for 30 minutes of a constant moderate intensity. It is important that I continue cycling for all 30 minutes but the session will stop at my request.
Blood will be taken before the first exercise session and immediately after the second and third exercise session. The total amount will be 28 ml each time and will be done by a Licensed Medical Technician.
During the study I understand that I am to consume bakery products that the investigators will provide. It is important that I follow instructions and eat the foods each day for 28 days.

III. Risks: It is my understanding and I have been informed that there exists the possibility during exercise of adverse physiologic responses during the tests. I have been informed that these changes could include abnormal blood pressure, fainting, disorders of the heart beat, and in rare instances, heart attack, stroke, or death. Every effort will be made to minimize these risks by evaluation of the preliminary information relating to your health and by observations during testing. Other possible discomforts I may experience in this study include leg fatigue, muscle soreness, a dry mouth (from the mouthpiece), pain, bleeding and local bruising at the site the blood was taken. I understand that the registered nurse or licensed medical technician collecting the blood samples, and that the technicians who may be handling the samples, will be wearing gloves at all times. I also understand that a registered nurse or licensed medical technician, certified exercise specialist and other support personnel will be present during all exercise testing to minimize the risks during exercise. I also understand that there is also a working telephone in the exercise testing area that can be used to alert the emergency rescue squad on the campus of Virginia Tech. Their average response time in getting to the Laboratory for Health and Exercise Science is approximately 4 to 5 min.

IV. Benefits of the Project: My participation in this project will provide valuable information that will help clarify the benefits of soy protein for reducing risk factors for cardiovascular disease. In addition I will receive information about my physical fitness level and outcomes of the study, if desired.

V. Confidentiality/Anonymity: I understand that the results of this study will be kept strictly confidential. At no time will the researchers release my results of this study without your written consent. The information I provide will have my name removed and only a subject number (not the social security number) will identify me during analysis and written reports of this research.

VI. Compensation: I understand that there is $50 dollar compensation available for participating in this project.

VII. Freedom to Withdraw: I understand that I may withdraw from this study at any time. There will be no penalty or loss of benefits to which I am otherwise entitled.

VIII. Approval of Research: This project has been approved by the Institutional Review Board.

IX. Subjects Responsibilities: I know of no reason that I cannot participate in this study. I have the following responsibilities:

- Accurately report my medical history.
- Arrive at the laboratory at my scheduled for each exercise session.
- Arrive at the laboratory at least 2 hours after wating or drinking.
- Arrive to give blood after fasting 12 hours since the previous night.
- Eat bakery products following instructions daily.
- Report any unusual signs/symptoms during the study.

- **X. Subjects Permission:** I have read and understand the informed consent and conditions of this project. I have had all my questions answered. I hereby acknowledge the above and give my voluntary consent for participation in this project.

If I participate, I may withdraw at any time without penalty. I agree to abide by all the rules of this project.

_____ _____

Signature Date

Should I have any questions about this research or its conduct, I may contact:

Oliver Chen	961-0211
Investigator	Phone
Dr. Raga Bakhit	231-6784
Faculty Advisor	Phone
E. R. Stout	231-9359
Chairman, IRB Research Division	Phone

CURRICULM VITA

Chung-Yen (Oliver) Chen

Antioxidants Research Lab
Jean Mayer USDA Human Nutrition Research Center on Aging
Tufts University, Boston, MA 02111
E-mail: chchen2@vt.edu

EDUCATION

Ph.D. 1996-2001	Human Nutrition, Foods & Exercise, Virginia Tech at Blacksburg, VA, graduate program in Human Nutrition.
M.S. 1993-1995	Animal Science, Colorado State University at Ft. Collin, graduate program in Animal Nutrition.
B. S. 1987-1991	Animal Science, National Taiwan University at Taipei, Taiwan

WORK EXPERIENCE

1999-2001	Graduate Teaching Assistant for the class, "Foods and Nutrition", in Department of Human Nutrition, Foods & Exercise at Virginia Tech.
1999-2000	Interpreter in three Chinese Food Managers Certification Courses offered by Virginia Tech and Virginia Cooperative Extension.
1999 summer	Mentor in the minority internship program at Virginia Tech, responsible for teaching students laboratory techniques and knowledge.
1999	Graduate Assistant specialized in computer applications in Department of Human Nutrition, Foods & Exercise at Virginia Tech.
1998 summer	Mentor in the minority internship program at Virginia Tech, responsible for teaching students laboratory techniques and knowledge.
1997-1998	President of Chinese Student Association at Virginia Tech.
1997 summer	Mentor for two undergraduate students at Virginia Tech, responsible for teaching laboratory skills and guiding them to conduct an animal study.
1991-1993	Corporal, Secretary of General in Army of Republic of China.
1988-1991	Chair of extracurricular activity of 1991 class in Animal Science at National Taiwan University.
1988	Coordinator of Sixtieth Anniversary Party of National Taiwan University.
1987-1991	Tutor of middle school students.

REASEARCH EXPERIENCE

2001	Attended the Molecular Biology and PCR Workshop held by New England Biolabs at Smith College.
1998	Research Assistant in Department of Biological Engineer at Virginia Tech. Worked in a project, "The application of useless soy hull in production of gelatin".
1997-1998	Research Assistant in Department of Psychology at Virginia Tech. Worked as an assistant in a project, "The relationship between Cancer incidence and family dietary habits".

1997 Research Assistant in Department of Human Nutrition, Foods & Exercise at Virginia Tech. Conducted a project, "The Effect of Acute Strenuous Exercise on the Activities of Antioxidant Enzymes and Plasma Genistein Concentration in Rats Fed with Genistein Supplemented Diet".

1989-1991 Research Assistant in Department of Animal Science at National Taiwan University. Worked as an assistant in a project, "The Development of Enzyme in Small Intestine and Pancreas by Different Feeds in Piglets".

1988-1989 Research Assistant in Department of Animal science at National Taiwan University. Worked as an assistant in a project, "The Effect of B Vitamins on the Growth of Duck Fetus".

LABORATORY AND COMPUTER SKILLS

Laboratory

Biochemical assays:

Antioxidant enzymes (superoxide dismutase, catalase, and glutathione peroxidase)

Glucose-6-phosphate dehydrogenase

Vitamin A, C, and E and uric acid determinations by HPLC

Plasma and liver isoflavone determination by HPLC

Homocysteine determination by HPLC

Plasma, tissue, and LDL malondialdehyde determinations (in vitro and in vivo) by HPLC or a spectrophotometer method

Glutathione determination by HPLC or a spectrophotometer method LDL separation by ultracentrifugation

Conjugated diene determination

C-reactive protein by immunoassay

Lipid profile determination

FRAP method determining total antioxidant activity

Molecular biology assays:

Extraction and purification of RNA and DNA

Northern, Southern, and Western blot

PCR, RT-PCR, and PCR subcloning

Construction and screening of genomic and cDNA libraries

Restriction enzyme digestion

Gel electrophoresis

Construction of recombinant DNA molecules

Cloning in plasmid and phage vectors

Bacterial transformation

Techniques in exercise research:

Maximum oxygen capacity (VO_2max) determination in human

Conducting sub-maximum exercise test on human

Rat exercise on treadmill

Animal handling:

Create feed formulas for experimental animals

Raise rat, pig, steer, and duck

Collect tissue sample from experimental animals such as skeletal muscle, liver, heart, and small intestine

Computer Dietary analysis program, Nutritionist V, Word, Excel, PowerPoint, Access, SAS, Jmpin, SPSS, Photoshop, and Web page making.

HONOR, AWARD, AND FELLOWSHIP

2001	First Place Winner of the 2001 IFT Nutrition Division Graduate Paper Competition, Mark L. Bieber Award for Best Paper
2001	Outstanding Graduate Student Service Award
2001	Outstanding Teaching Assistant Nomination
2000	Third Place Winner of Institute of Food Technologists Nutrition Division Graduate Paper Competition
2000	Hepler Summer 2000 Research Fellowship from Department of Human Nutrition, Foods & Exercise at Virginia Tech
2000	Travel Fund Award from Virginia Tech
1999	James Moran Memorial Award from College of Human Resources and Education at Virginia Tech
1997-present	Kappa Omicron Nu Honor Society

THESIS AND DIESSERTATION

M.S. Thesis	The Effect of Dietary Crude Protein Amounts on Tissue α- Tocopherol Concentrations in Steers during Growth.
Ph.D. Dissertation	Soy Isoflavones Modulated Antioxidant Defense Systems and Decreased Lipid Peroxidation in Rats and Humans.

RESEARCH INTEREST

Conducting research on human subjects and animals, with particular emphasis in:

- Antioxidants and exercise on aging
- Potential roles of phytochemicals, especially genistein, in the process of carcinogenesis
- Mechanism of genistein action at the molecular level
- Soy protein effect on blood cholesterol in different population groups
- Soy isoflavones and their antioxidant effects on LDL and atherosclerosis

PUBLICATIONS

C. Chen. 2001. Safe and nutritious seafood in Virginia. Translated into Chinese. Virginia Cooperative Extension Publication 348-962.

A.A. Hertzler and **C. Chen**. 2001. Recipes and the Internet. Submitted to Journal of the American Dietetic Association.

C. Chen, G.I. Holtzman, and R.M. Bakhit. 2001. High-Genistin Isoflavone Supplementation Effect on Erythrocyte Antioxidant Enzymes and Tissue's TBARS in Acutely Exercised Rats. Submitted to Can. J. Physiol. Pharmacol.

ABSTRACTS AND PRESENTATIONS

C. Chen and R. M. Bakhit. 1998. The Effect of Acute Strenuous Exercise on the Activities of Antioxidant Enzymes and Plasma Genistein Concentration in Rats Fed with Genistein Supplemented Diet. FASEB J 17:4:A560.

C. Chen and R. M. Bakhit. 1999. The Effect of Genistein on Antioxidative Defense System and Plasma Lipid Profiles in Aged Rats. FASEB J13:5:A904.

R. M. Bakhit, V. L. Admas, and **C. Chen**. 1999. The Effect of Soy Protein Intake on Plasma Lipid Profiles and Plasma TBARS in Male College Subjects. FASEB J 13:5:A904.

C. Chen and R. M. Bakhit. 1999. The Effects of Genistein on the Activities of Antioxidant Enzymes in Strenuously Exercised Rats. Presented in Third International Symposium on the Role of Soy.

C. Chen and R. M. Bakhit. 2000. The Optimal Amount of Genistein Necessary for its Antioxidative and Cholesterol-Lowering Effects in Rats FASEB J 14:4:A519.

C. Chen and R. M. Bakhit. 2000. The Optimal Amount of Genistein Necessary for its Antioxidative Effect in Rats. Presented in 2000 Institute of Food Technologists Annual Meeting and FOOD EXPO.

S.C. Shehadeh, **C. Chen**, and R.M. Bakhit. 2000. Soy Protein and Isoflavone Effect on Lipid Oxidation and Blood Lipid Profiles in Humans Participating in Moderate Physical Activity. FASEB J 14:4:A519.

C. Chen and R.M. Bakhit. 2001. The Optimal Amount of Isoflavone Intake Necessary to Diminish Oxidative Stress Parameters in Rats. FASEB J. 15:4:A608.

C. Chen and R.M. Bakhit. 2001. Dietary Isoflavone Supplementation Diminished Oxidative Stress but did not Alter Plasma Lipid Profiles in Rats. Presented in 2001 Institute of Food Technologists Annual Meeting and FOOD EXPO.