JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

# 90-Day Oral Toxicity Study of a Grape Seed Extract (IH636) in Rats

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To assess the safety of grape seed extract with less than 5.5% catechin monomers (IH636), 4 groups of male and female Sprague−Dawley rats were provided grape seed extract in the diet at levels of 0 (control), 0.5, 1.0, or 2.0% for a period of 90 days. All animals survived the duration of the study, and no significant changes in clinical signs, hematological parameters, organ weights, ophthalmology evaluations, or histopathological findings were observed. A significant increase in food consumption was observed in male and female rats provided the grape seed extract diets compared to that of the control rats, especially in male rats consuming 2.0% grape seed extract. This effect was not accompanied by increases in body weight gains. Grape seed extract appeared to increase the insoluble fraction of the diet. Male rats in the high-dose group exhibited decreased serum iron levels and decreased serum iron/total iron binding capacity ratio compared to those of the controls, although all values were within historical ranges for Sprague−Dawley rats. In conclusion, administration of the grape seed extract IH636 to male and female Sprague−Dawley rats in the feed at levels of 0.5, 1.0, or 2.0% for 90 days did not induce any significant toxicological effects.

**KEYWORDS: Grape seed extract; *Vitis vinifera*; flavonoid; proanthocyanidin; polyphenols; oral toxicity; rats**

## INTRODUCTION

Grape seed extract is a natural extract from the seeds of *Vitis vinifera*. A multitude of flavonoids are contained in grape seed extract. The most abundant of these are the proanthocyanidins, which are oligomers of monomeric flavan-3-ol units linked by carbon−carbon bonds (*1*−*3*). The major flavan-3-ols identified in grape seed extract are (+)-catechin, (−)-epicatechin, and (−)-epicatechin-3-*O*-gallate (*3*, *4*) (**Figure 1**). The most basic oligomeric proanthocyanidins are composed of flavan-3-ols units linked together from the C4 of one unit to either the C6 or C8 of the adjacent unit to form the B-type dimers and C-type trimers (*1*, *5*, *6*) **(Figure 2**). The further addition of flavan-3-ol units results in the formation of larger proanthocyanidin oligomers and polymers.

Flavonoids and flavan-3-ols are partially metabolized to lactones and phenolic acids by the intestinal microflora (*7*, *8*). These flavonoid and flavan-3-ol metabolites are absorbed through the intestinal lumen and are further metabolized by methylation, oxidation, or glucuronic conjugation. Flavonoids and their metabolites are eliminated mainly through urinary and fecal excretion and, to a certain extent, via respired carbon dioxide (*9*−*12*).

In addition to being present in the seeds of grapes, proanthocyanidins occur naturally in black and green teas, chocolate, coffee, cacao, red wine, and many fruits (*7*). A vast amount of literature has been published that provides evidence that these flavonoids possess antioxidant properties, and free radical scavenging and chelation abilities (*6*, *13*−*25*). Flavonoids have been reported to exert antiinflammatory actions and to modulate immune function (*26*, *27*). By reducing the permeability and fragility of capillaries, they also have a protective effect against vascular disorders (*28*). Flavonoids exert a cholesterol-lowering effect by enhancing reverse cholesterol transport and bile acid excretion, and by decreasing the intestinal absorption of dietary cholesterol (*29*−*31*). The results of epidemiological studies indicate an inverse relationship between cancer and the consumption of flavonoid-containing foods, especially fruits and green tea (*32*−*35*). The anti-carcinogenic properties of flavonoids and proanthocyanidins in particular are associated with cytotoxicity to cancer cells (*36*, *37*) and their ability to enhance the activity of enzymes that detoxify carcinogenic hydrocarbons by oxidation (*7*, *35*). Additional epidemiological studies on flavonoid consumption indicate an inverse relationship between dietary intake of flavonoids and coronary heart disease and stroke (*38*−*41*). By acting as free radical scavengers, proan-

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10.1021/jf011066w CCC: $22.00 
Published on Web 03/01/2002

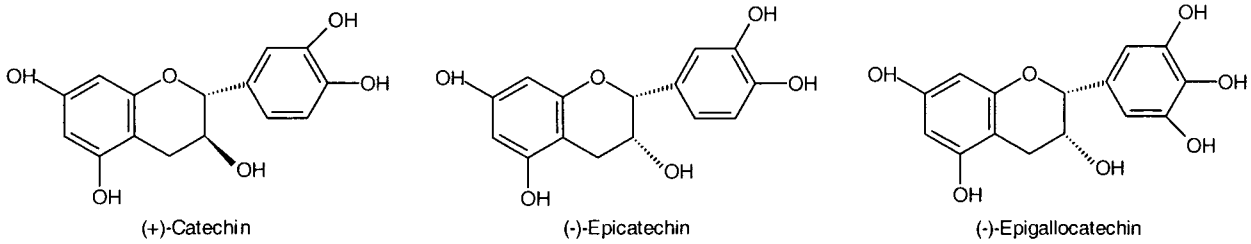


**Figure 1.** Structures of the major flavan-3-ols identified in grape seed extract.

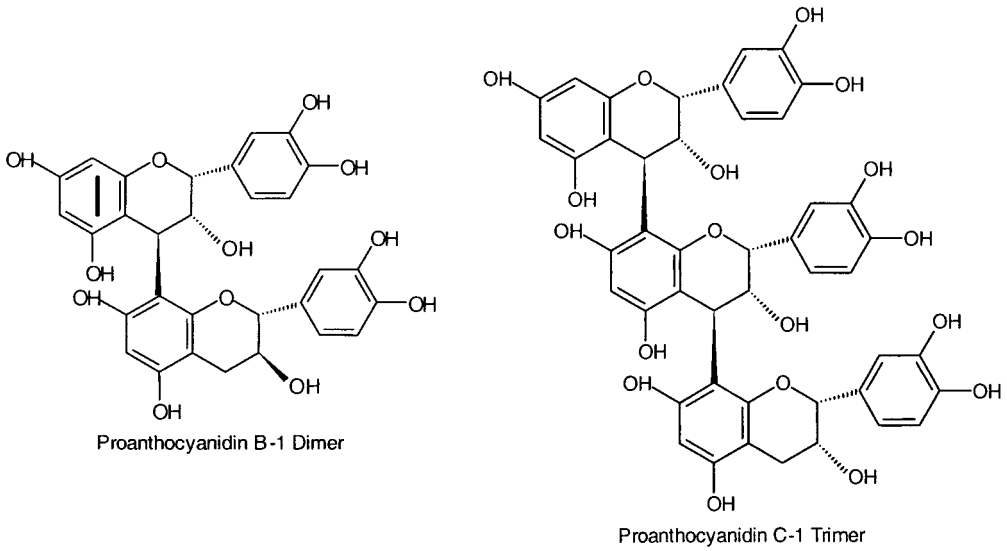


**Figure 2.** Structures of proanthocyanidin oligomers. The oligomeric proanthocyanidins are composed of flavan-3-ols units linked together from the C4 of one unit to either the C6 or C8 of the adjacent unit.

thocyanidins inhibit lipid peroxidation (*22, 28, 42*−*45*), a free-radical chain reaction that can produce cytotoxicity, disrupt lipid-containing membranes, and initiate low-density lipoprotein oxidation (*46*−*49*), a contributing factor to the development of atherosclerosis (*50, 51*). Flavonoids decrease the risk of cardiovascular disease by inhibiting platelet aggregation and thrombosis (*52*−*57*), and by exerting a sparing effect on other antioxidants, such as vitamins E and C (*52, 58*). By reducing oxidative stress, proanthocyanidins from grape seed exert a cardioprotective effect against ischemia reperfusion injury (*59*) and also protect gastric mucosal (*60*) and glial cells (*61*) from oxidative-stress induced injury.

Because of the increasing interest in flavonoids as dietary supplements (taken in caplet form with a typical daily dose between 50 and 150 mg) and a growing understanding of their potential health benefits, the safety of these substances must be established. The objective of the present study was to assess the oral toxicity of a water-extracted grape seed extract with less than 5.5% catechin monomers following administration to rats via dosed feed for a period of 90 days. At the highest concentration of 2.0 w/w % of grape seed extract with less than 5.5% catechin monomers (IH636) in the chow, the rats were consuming the extract at approximately 2 g/kg body weight/day, or 10−20 times the average human intake of plant-derived proanthocyanidins.

## MATERIALS AND METHODS

**Animals.** One hundred and sixty male and female Sprague−Dawley rats (80 rats/sex) were obtained from Charles River Laboratories (Raleigh, NC). The animals were quarantined and acclimatized for 7 days prior to the initiation of treatment. During this acclimatization period, the animals were fed Purina Certified Rodent Chow 5002 (pellets). Animals were assessed for viability, and serological evaluation of bacterial and viral infections was performed on 3 rats/sex.

The age of the animals at study initiation was approximately 9 to 10 weeks. The weights of the animals were 218−280 g and 175−215 g for male and female rats, respectively. General procedures for animal care and housing were in accordance with DHHS Publication No. (NIH) 86-23 (Revised, 1985) and the U.S. Department of Agriculture through the Animal Welfare Act (7 USC 2131) 1985, and Animal Welfare Standards incorporated in 9 CFR Part 3, 1991. Animals were housed under conventional conditions in suspended polycarbonate cages in groups of 2 to 3 animals/cage with Sani-Chips hardwood bedding (P. J. Murphy Forest Products, Montville, NJ). Temperature and relative humidity of the animal rooms were maintained at 62−79 °F and 23−56%, respectively. Room temperature varied only 3 °C from the accepted temperature range of 65−79 °C. This variation occurred twice during the study and for less than 4 h on each occasion. Four days before study initiation, the animals were randomly assigned to treatment groups using a computerized body-weight stratification procedure.

**Diets and Test Materials.** Animals were fed Ralston Purina Rodent Chow pre-ground to meal form, ad libitum. Drinking water was also provided to the animals ad libitum. Grape seed extract with less than 5.5% catechin monomers (IH636) was obtained from Dry Creek Nutrition, Inc. Five lots of IH636 were blended to form a composite batch. The composite batch was blended with the rodent chow to provide test diets containing levels of 0 (control), 0.5, 1.0, and 2.0% of IH636 (Average dose levels are presented in **Table 1**). A sixth lot was used separately at the end of the study. All lots complied with the current chemical and microbiological specifications for the product. The standard rodent chow without the test material was provided to the control group.

All diets were frozen at −21 to −19 °C upon receipt. The diets were refrigerated at 4° to 6 °C, protected from light after opening, and were maintained at room temperature (18−25 °C) 24 h prior to feeding to the animals. The presence of grape seed extract in the chow was demonstrated using the classical Folin−Ciocalteu (*62*) and Porter (*63*)

**Table 1.** Experimental Dose Groups

| group | GSE dose level (% w/w)[b] | (mg/kg body weight/day)[a] male | female |
|---|---|---|---|
| control[c] | 0 | 0 | 0 |
| low-dose group | 0.5 | 348 ± 67.6 | 469 ± 47.9 |
| mid-dose group | 1.0 | 642 ± 102.3 | 883 ± 93.7 |
| high-dose group | 2.0 | 1,586 ± 193.8 | 1,928 ± 171.6 |

[a] Values are means ± SD ($n = 20$ rats). [b] Weight of dry solid (g)/100 g of feed mixture. [c] Control animals received only Ralston Purina Rodent Chow pre-ground to meal form.

visible spectroscopy methodologies for determining the presence of polyphenols and proanthocyanidins, respectively. Samples of 0 (control), 0.5, 1.0, and 2.0% grape seed extract in chow were first extracted with methanol then subjected to the analyses. Greater absorbance at 760 nm (Folin−Ciocalteu) and 550 nm (Porter) for the samples with added grape seed extract than the control was indicative of the addition of grape seed extract to the chow.

Attainment of the target concentrations and content uniformity of the grape seed extract in the different test diets were determined by absorbance spectroscopy at 280 nm of methanol extracts of the chow. Standard regression analyses were performed. Target concentration attainment and content uniformity were defined as sample concentrations being within ± two methodology standard deviations of the mean concentration at the three levels of IH636 in the different test diets.

The stability of IH636 in the rodent chow was determined by absorbance spectroscopy at 280 nm and comparison to a "fingerprint" HPLC chromatogram. Standard regression analyses were performed on the UV spectroscopy. Stability was defined as sample concentrations being within ± two methodology standard deviations of the mean concentration at the three levels of IH636 in the rodent chow and no new unidentified chromatographic peaks at greater than 0.01 area percent appearing in the "fingerprint" chromatogram. For the "fingerprint" HPLC analysis, methanol extracts of the chow samples were dried. The dried samples were reconstituted with 30% ethanol/70% water to make 1000 ppm solutions. Aliquots of 25 $\mu$L of the 1000 ppm solution were injected onto a Zorbax SB-C18 4.6 × 150 mm, 5-$\mu$m column maintained at 30 °C. The injected material was eluted at 0.5 mL/min with a mobile phase gradient from 2.5% acetic acid/97.5% water to 2.5% acetic acid/17.5% water/80% acetonitrile in 85 min. The eluting material was monitored at 280 nm.

**Grape Seed Extraction.** Grape seed isolates were prepared by batch extraction with 100% water from dried grape seeds at up to 82 °C for up to 40 min. These isolates were purified by ultrafiltration and chromatography according to the process of Nafisi-Movaghar et al. (*64*) to produce IH636.

**Chemical Analyses.** Loss on drying (LOD) and ash were determined on neat IH636 by AOAC methods 925.09 and 923.03, respectively. IH636 is a complicated mixture of chemical classes. It was separated into ethyl acetate soluble and aqueous soluble fractions to facilitate chemical analysis by the method of Oszmianski and Sapis (*65*). The amino acid content of IH636 was then determined by hydrolysis of an aliquot of the aqueous soluble fraction by following the methodology of Battaglia et al. (*66*). The oligomeric polyphenols (OPC) content of IH636 was determined from the ethyl acetate soluble fraction by the methods of Vonk et al. (*67*) and Sun et al. (*68*). The monomeric proanthocyanidins in IH636 were quantified from the ethyl acetate soluble fraction according to the method of Fuleki and daSilva (*69*). The phytosterol content was determined by the method of Indyk (*70*), after a saponification and extraction of an aliquot of the ethyl acetate soluble fraction. The fatty acid content was quantified from the ethyl acetate soluble fraction by the method of Mehta et al. (*71*). The polysaccharide content of IH636 was determined from the aqueous soluble fraction by the method of Lopez-Barajas et al. (*72*).

**Experimental Design.** Testing was performed by SRI International (Menlo Park, CA) in compliance with Good Laboratory Practice (GLP) regulations established by the U. S. Food and Drug Administration (FDA) under Part 58 of Title 21 of the Code of Federal Regulations (CFR), Good Laboratory Practice for Nonclinical Laboratory Studies. The diets were provided to 4 groups of rats (20 rats/sex/group) containing IH636 at a level of 0 (controls), 0.5, 1.0, or 2.0% for a period of 90 days. The viability of the animals was checked twice daily Monday through Friday, and once daily on Saturdays, Sundays, and holidays. Clinical observations were performed once daily on study days 1 through 15, and weekly thereafter. One animal underwent an additional observation after sustaining a leg injury on day 32. Body weights were recorded on day 1 of the study, once weekly thereafter, and immediately before necropsy. Animals were fed the powdered diet in specially designed feed-troughs, which minimize spillage and contamination from feces by allowing only the head into the feeder. Food consumption was measured and recorded twice weekly by cage (2 or 3 animals per cage), by recording the difference in feed weight. An average apparent daily feed consumption was calculated for each rat. Food spillage was not measured because it was minimal and the use of Sani-Chip bedding (standard for GLP toxicology studies) makes such monitoring impractical. An ophthalmologic examination was performed on all animals prior to study initiation and again during the final week before necropsy (day 85).

Blood samples for clinical pathology evaluation were obtained from the animals on days 91 to 94 via the retro-orbital sinus under $CO_2/O_2$ anesthesia. Hematology and serum chemistry analyses were performed for all animals, and serum iron analysis was performed for the control and high-dose groups. Blood samples were analyzed for the following hematological parameters: red blood cell (RBC) count, hematocrit (HCT), hemoglobin (HGB), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCC), mean corpuscular hemoglobin (MCH), white blood cell (WBC) count, WBC differential counts [including absolute banded neutrophils (ANB), segmented neutrophils (ANS), lymphocyte (ALY), monocyte (AMO), eosinophil (AEO), and basophil (ABA)], platelet count (PLC), and reticulocyte count (RET). Serum samples were analyzed for the following clinical chemistry parameters: alanine aminotransferase (ALT), albumin (ALB), alkaline phosphatase (ALP), aspartate aminotransferase (AST), total bilirubin (TBI), blood urea nitrogen (BUN), calcium (CAL), chloride (CHL), cholesterol (CHO), creatinine (CRE), globulin (GLO), glucose (GLU), phosphorus (PHO), potassium (POT), total protein (TPR), sodium (SOD), iron (IRO), total iron binding capacity (TIBC), and iron/total iron binding capacity (ITC).

At the end of the study, during days 91−94, all animals were killed with approximately 100 mg of sodium phenobarbital/kg body weight administered intraperitoneally. All animals were subjected to gross necropsy, which included an external examination of all body orifices and surfaces, and an examination of all cranial, thoracic, and abdominal organs. Gross pathology findings were recorded. Samples of the following tissues were removed and fixed in phosphate-buffered 10% formalin: adrenal glands, aorta, urinary bladder, bone and marrow (from sternum), brain, cecum, cervix, colon, duodenum, esophagus, epididymides, eyes, femur, gross lesions (including tissue mass and abnormal regional lymph nodes, if identified), heart, ileum (including Peyer's patches), jejunum, kidneys, lungs and bronchi, liver, lymph nodes (mesenteric), mammary gland (to include nipple and surrounding tissue), ovaries, pancreas, pituitary, prostate, rectum, salivary gland, sciatic nerve, skeletal muscle, skin (abdominal; taken with mammary gland), spinal cord (mid-thoracic), spleen, stomach, testes, thymus, thyroid and parathyroids, tongue, trachea, uterus, and vagina. Organ weights were recorded for all animals for the following (paired organs were weighed together): prostate gland and seminal vesicles, adrenal glands, brain, heart, kidneys, liver, ovaries, spleen, testes, uterus, and thymus. No target organs were identified by gross pathological examination in animals of the high-dose group, and histopathological examination was therefore performed on animals in the control and high-dose groups only.

**Statistical Analysis.** Data on body weights, food consumption, clinical pathology, absolute organ weight, and organ-weight ratios were evaluated by one-way ANOVA, followed by Dunnett's test to compare the mean of each dose group with that of the control group. The probability level used to determine statistical significance was $P < 0.05$.

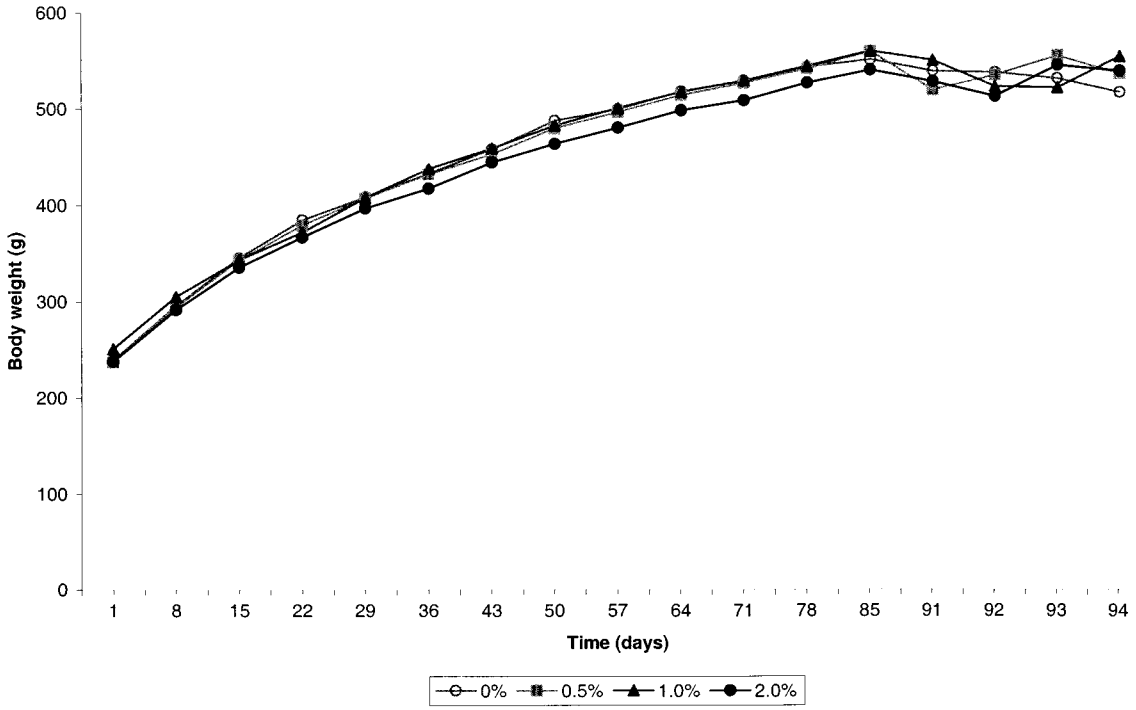


**Figure 3.** Body weights (g) for male Sprague–Dawley rats.

**Table 2.** Chemical Composition of Grape Seed Extract

| chemical class | % of grape seed extract |
|---|---|
| oligomeric polyphenols | 76.3 |
| polysaccharides | 10.8 |
| water | 5.7 |
| fatty acids | 2.8 |
| monomeric proanthocyanidins | 2.8 |
| amino acids | 2.1 |
| ash | 0.6 |
| phytosterols | 0.5 |
| total | 101.6 |

## RESULTS AND DISCUSSION

**Grape Seed Extract Characterization and Stability.** It was possible to complete a mass balance of IH636 with 100% accountability (**Table 2**). The nonpolyphenolic components in IH636 are typical compounds found in plant material. The target concentrations, content uniformity, and stability of the grape seed extract in the various chows were demonstrated to a 95% confidence level. No losses of IH636 were incurred due to instability under the storage conditions of −6 to −20 °C for 94 days. No new unidentified compounds were detected in the IH636 in rodent chow during the course of the stability study. All six batches of IH636 used in the study complied with the final product specifications with respect to the overall proanthocyanidin and monomer contents, heavy metal analysis, and microbial contamination.

**Clinical Observations.** No unscheduled deaths occurred during the study (**Table 3**). On day 32, one female rat in the 0.5% IH636 group sustained a leg injury during the closing of the cage, and 1 male rat in the control group exhibited opacity of the left eye commencing on day 64.

Beginning in week 5 of the study, sporadic alopecia was observed in both male and female rats in all groups (1−6 rats/sex/group) (**Table 3**). The alopecia in some male rats was accompanied by eschar formation (1−3 rats out of 20 males/group); however, there was no significant difference between the control group and IH636-treated groups. This effect is thought to be related to fighting between the male animals. It is a common occurrence with mid- to long-term housing of male rodents in group cages and is considered not to be compound-related. Ophthalmologic examination revealed no compound-related lesions in any of the animals. No other clinical observations were noted.

**Table 3.** Mortality and Clinical Observations for Male and Female Sprague–Dawley Rats Fed Diets Containing up to 2.0% GSE1 for 90 Days

| | dose group | | | |
|---|---|---|---|---|
| | 0 | 0.5% | 1.0% | 2.0% |
| | male | | | |
| found dead | 0 | 0 | 0 | 0 |
| moribund sacrifice | 0 | 0 | 0 | 0 |
| alopecia | 2 (29, 93)[a] | 6 (29, 93) | 4 (29, 94) | 3 (36, 94) |
| eschar formation | 2 (29, 85) | 3 (71, 93) | 3 (29, 94) | 2 (36, 78) |
| eye opacity | 1 (64, 94) | 0 | 0 | 0 |
| normal | 17 | 14 | 16 | 17 |
| | female | | | |
| found dead | 0 | 0 | 0 | 0 |
| moribund sacrifice | 0 | 0 | 0 | 0 |
| alopecia | 3 (29, 91) | 1 (50, 71) | 3 (64, 93) | 1 (43, 93) |
| eschar formation | 0 | 0 | 0 | 0 |
| limping/swelling[b] | 0 | 1 (32, 91) | 0 | 0 |
| normal | 17 | 18 | 17 | 19 |

[a] Numbers in parentheses ( ) are the first and last days of observation. [b] Animal no. 61 injured left front leg when technician was closing cage.

**Weight Gains.** All groups gained weight during the study period. On day 1 of the study, the mean body weight of the male rats in the 1.0% IH636 group was significantly higher than that in the control group; however, this was considered to be due to a randomization artifact (**Figure 3**). Slightly lower mean body weights were recorded for female rats in the 1.0 and 2.0% IH636 groups when compared to those of females in the control group (**Figure 4**). This effect was statistically significant only in the subgroup necropsied on day 91, and not in the groups necropsied on days 92 to 94. Because the difference in body weights was only slight, and given the fact that the effect was

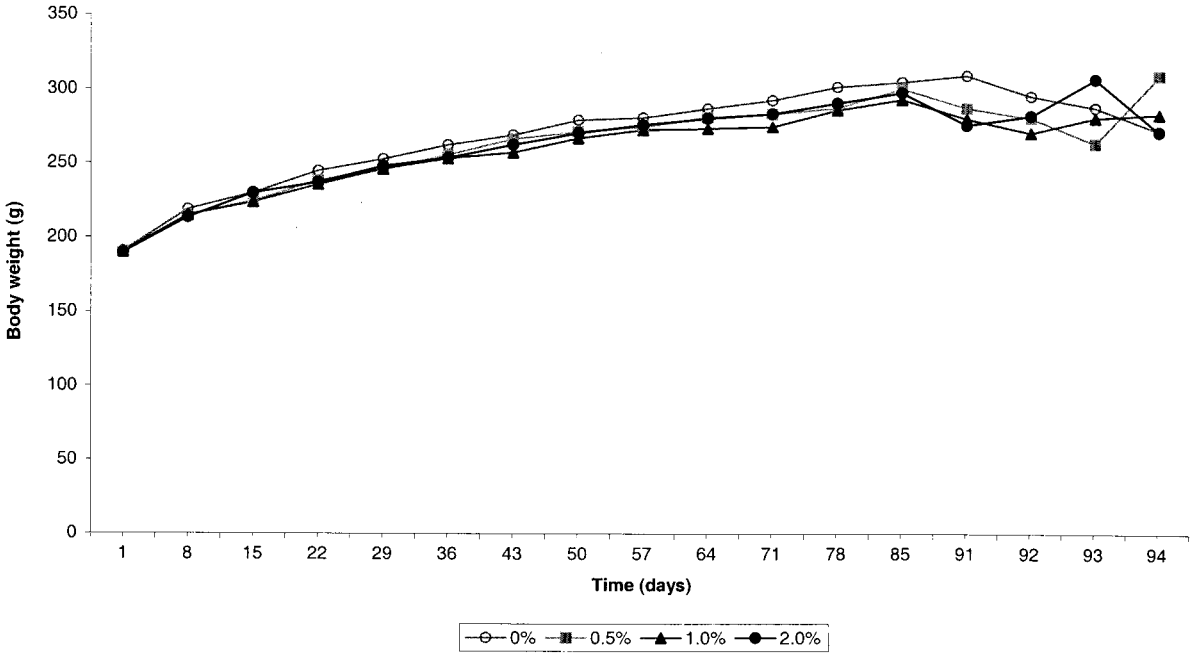


**Figure 4.** Body weights (g) for female Sprague–Dawley rats.

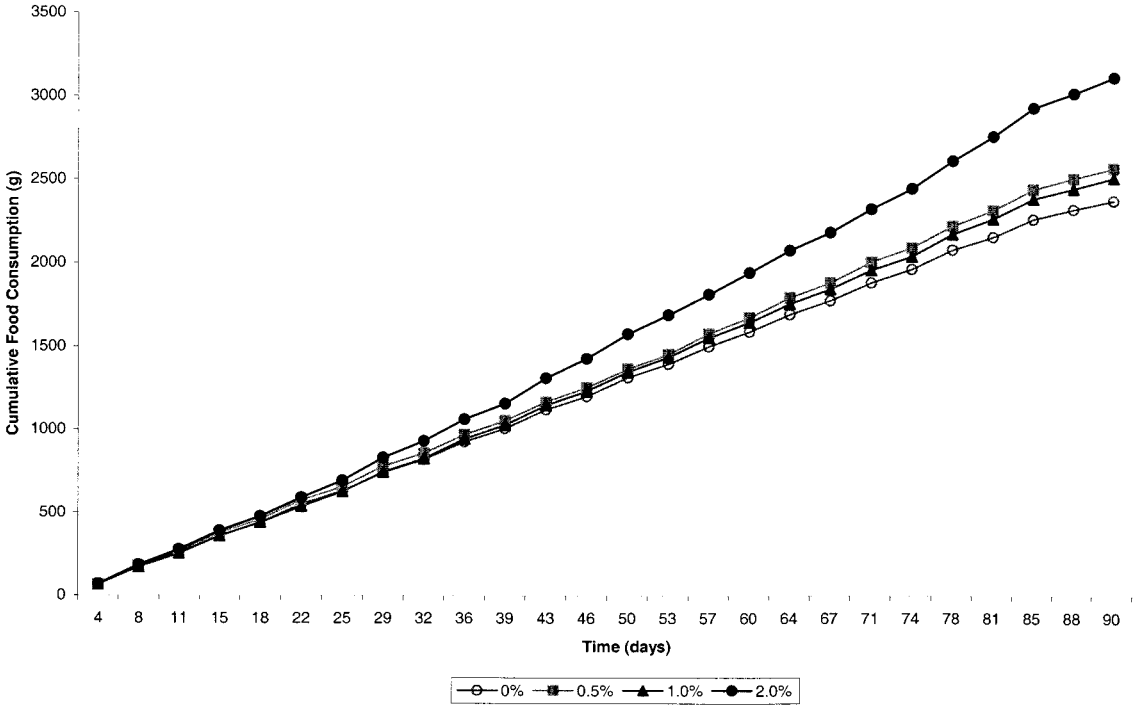


**Figure 5.** Cumulative food consumption (g) for male Sprague–Dawley rats.

statistically significant only in the day 91necropsy subgroup, this effect was considered to be of no biological significance.

**Food Consumption.** Food consumption was generally higher in male rat groups fed IH636-containing diets than that in the control group, possibly suggesting that rats preferred the flavor and texture of the blend over that of the conventional rodent chow (**Figure 5**). The estimated increased food consumption by male rats provided the 2.0% IH636 diets compared to that of males in the control group was statistically significant as early as the day 4−8 consumption measurement period, and remained higher than that of the control group throughout the duration of the study. The increase in apparent food consumption in male rats in the 0.5 and 1.0% IH636 groups also was statistically significant; however, these increases occurred in a more random pattern throughout the study. Sporadic, slight increases in food consumption also were observed for IH636-treated female rats compared to that of the control group, and these increases were statistically significant in only 9 out of 104 food consumption measurements (**Figure 6**). Increased food consumption by female rats provided the 2.0% IH636 diets compared to that of the control group was statistically significant during the final 2 weeks of the study period.

The increases in estimated food consumption by the male and female rats in the IH636 groups were not accompanied by increases in group body weight or in absolute organ weights. In addition, no significant differences were observed in the serum levels of albumin and globulin, or in total serum protein levels.

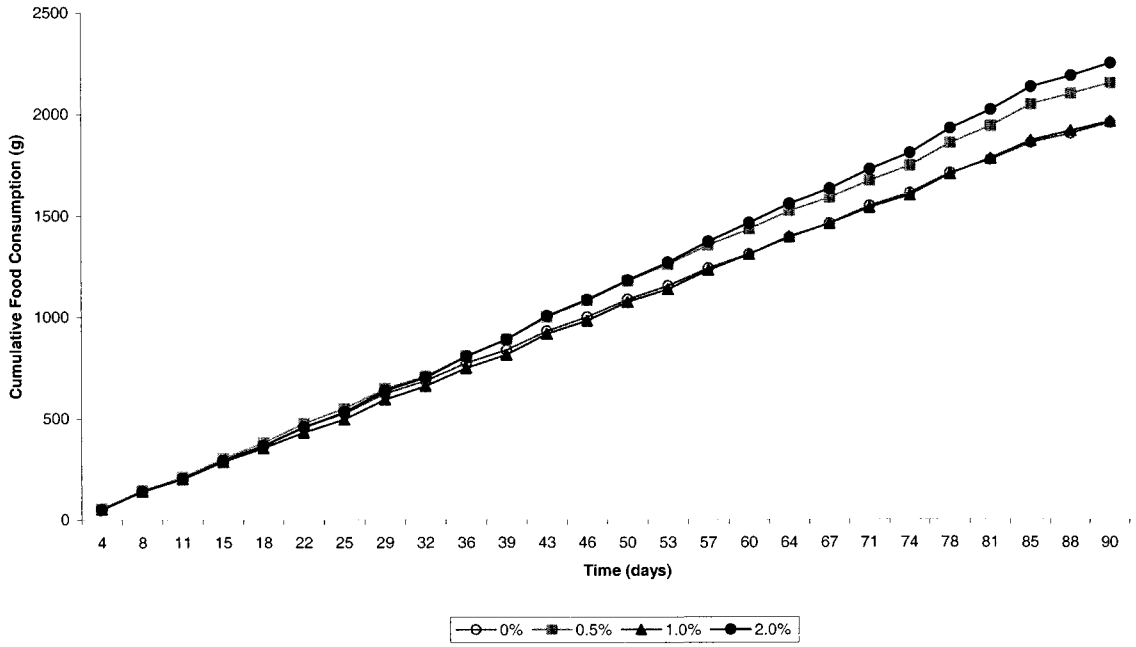


**Figure 6.** Cumulative food consumption (g) for female Sprague–Dawley rats.

**Table 4.** Effects of Proanthocyanidins (Condensed Tannins) on the Nutritive Value of Ingested Food

| positive effects | | negative effects | |
|---|---|---|---|
| observation | ref | observation | ref |
| extracellular antioxidant cytoprotective effects on the GI tract | Halliwell et al. (*84*) | prevents cellulolytic bacterial attachment | McAllister et al. (*85*) |
| low to moderate concentrations increase N retention by complexing protein at the pH of the lumen, releasing it in lower tomachs of ruminants | McNabb et al. (*86*) | impairs digestibility of amino acids (esp. glycine, proline, and histidine) and proteins, and increases fecal N excretion | pigs: Cousins et al. (*79*)<br>sheep: McNabb et al. (*87*)<br>humans: Bressani et al. (*88*) |
| prevents bloat in ruminants | McNabb et al. (*86*) | inhibits digestive enzymes trypsin and α-amylase in rats by formation of tannin-enzyme complexes | Griffiths, D. W. (*89*) |
| increases efficiency of urea recycled to the rumen | Waghorn et al. (*90*) | reduces food intake by lowering palatability due to astringency | Barry and Duncan (*91*) |
| better performance in high-producing dairy cows if moderate tannins in diet | Marten and Ehle (*92*) | inhibits electrolyte absorption in rat ileum | Silverstein et al. (*93*) |

Ever since Kühnau described plant flavonoids as "semi-essential" components of the human diet (*7*), the role, if any, of these dietary antioxidants in the maintenance of human health has been the focus of special attention. Their metabolism appears to be highly dependent upon their degree of polymerization (DP). Studies of chickens fed with $^{14}$C-labeled sorghum tannins (*73*) and sheep fed with $^{14}$C-labeled *Lotus pedunculatus* (*74*) indicated the proanthocyanidins were not absorbed. However, more recently, Déprez (*75*) compared the in vitro absorption of radiolabeled dimer, trimer, and oligomers (average degree of polymerization was 7) and the related (+)-catechin through a cell monolayer derived from the human intestinal cell line Caco-2. The monomer, dimer, and trimer were all absorbed to a similar extent, but the polymers were not and partially adhered to the cell surface. Further experiments by this group showed that the polymer was degraded by human colonic micro flora grown in vitro and anaerobically. In an elegant study using $^{14}$C-labeled proanthocyanidins from carob bean pods, Abia and Fry (*76*) have shown that 18 h after gavage 90−94% of the label was in the gut contents and/or feces. More than half of this originated in condensed tannins with higher DP becoming insoluble, mainly in the form of protein−tannin complexes.

Thus, there is a recent consensus that the monomeric, and possibly dimeric and trimeric, proanthocyanidins can be directly absorbed and that a small proportion of the higher DP compounds are degraded by colonic bacteria prior to absorption. However, the bulk of these condensed tannins form stable complexes with both dietary and endogenous proteins and fiber, resulting in increased fecal concentrations of these nutrients. It is this property that has allowed these compounds to be classified as "anti-nutrients" by some researchers. Just as there is some controversy over the metabolism of dietary flavanols, so there is evidence to support both beneficial and detrimental effects of these carbohydrates on the nutrition of animals. This has been well reviewed by Reed (*77*) and is summarized in **Table 4**. In short, the proanthocyanidins interact with proteins, especially proline to form insoluble complexes, as well as with other macromolecules and minerals in ingested food and those secreted by the gastrointestinal tract. This adds up to the occasional finding that high levels of procyanidins in feed results in increased consumption but no concomitant gain in body weight gain as observed in the present study in rats. Ruiz-Roso and co-workers (*78*) observed a similar phenomenon after feeding rats a diet containing 10% polyphenols (natural carob fiber) or 10% cellulose. This has also been seen in other omnivores such as pigs, after ingesting a diet with ∼3.4% condensed tannins (*79*).

Interestingly, a similar finding occurs when rats are fed a

**Table 5.** Mean Terminal Body Weights and Absolute Organ Weight (g) of Male and Female Sprague–Dawley Rats Fed Diets Containing up to 2.0% GSE1 for 90 Days

| | dose group | | | |
|---|---|---|---|---|
| | 0% | 0.5% | 1.0% | 2.0% |
| | male | | | |
| body weight (g) | 531 ± 47.3 | 536 ± 34.5 | 537 ± 43.3 | 531 ± 38.5 |
| adrenal glands (pair) (g) | 0.059 ± 0.0121 | 0.059 ± 0.0111 | 0.055 ± 0.0096 | 0.054 ± 0.0114 |
| brain (g) | 2.19 ± 0.108 | 2.25 ± 0.099 | 2.18 ± 0.092 | 2.21 ± 0.139 |
| heart (g) | 1.49 ± 0.169 | 1.46 ± 0.133 | 1.51 ± 0.151 | 1.48 ± 0.114 |
| kidneys (pair) (g) | 3.22 ± 0.373 | 3.23 ± 0.319 | 3.07 ± 0.264 | 3.15 ± 0.285 |
| liver (g) | 14.40 ± 2.346 | 14.43 ± 1.537 | 14.34 ± 1.712 | 14.60 ± 1.623 |
| prostate & seminal vesicles (g) | 3.79 ± 0.579 | 3.51 ± 0.509 | 3.54 ± 0.921 | 3.58 ± 0.724 |
| spleen (g) | 0.77 ± 0.103 | 0.95 ± 0.689 | 0.80 ± 0.111 | 0.76 ± 0.113 |
| testes (pair) (g) | 3.30 ± 0.316 | 3.34 ± 0.266 | 3.34 ± 0.251 | 3.31 ± 0.305 |
| thymus (g) | 0.430 ± 0.1195 | 0.439 ± 0.1247 | 0.421 ± 0.1008 | 0.437 ± 0.1420 |
| | female | | | |
| body weight (g) | 291 ± 21.6 | 285 ± 29.2 | 279 ± 24.5 | 284 ± 30.1 |
| adrenal glands (pair) (g) | 0.066 ± 0.0097 | 0.065 ± 0.0110 | 0.064 ± 0.0097 | 0.066 ± 0.0111 |
| brain (g) | 1.95 ± 0.105 | 1.90 ± 0.112 | 1.96 ± 0.104 | 1.95 ± 0.064 |
| heart (g) | 0.92 ± 0.087 | 0.88 ± 0.089 | 0.90 ± 0.070 | 0.88 ± 0.077 |
| kidneys (pair) (g) | 1.80 ± 0.152 | 1.77 ± 0.164 | 1.82 ± 0.194 | 1.78 ± 0.114 |
| liver (g) | 7.76 ± 1.039 | 7.82 ± 1.359 | 7.42 ± 0.751 | 7.36 ± 0.563 |
| ovaries (pair) (g) | 0.098 ± 0.0306 | 0.126 ± 0.1366 | 0.089 ± 0.0185 | 0.102 ± 0.0241 |
| spleen (g) | 0.49 ± 0.059 | 0.50 ± 0.064 | 0.50 ± 0.063 | 0.50 ± 0.047 |
| thymus (g) | 0.316 ± 0.0683 | 0.291 ± 0.0800 | 0.322 ± 0.1096 | 0.303 ± 0.0718 |
| uterus (g) | 0.623 ± 0.2051 | 0.677 ± 0.2267 | 0.652 ± 0.1329 | 0.677 ± 0.2106 |

**Table 6.** Terminal Histopathologic Observations for Male Sprague–Dawley Rats Fed Diets Containing 0 or 2.0% GSE1 for 90 Days

| | dose group[a] | | | |
|---|---|---|---|---|
| | 0% | | 2.0% | |
| | number of tissues examined | % of tissues containing lesions | number of tissues examined | % of tissues containing lesions |
| adrenal gland | 19 | | 18 | |
| accessory cortical nodule | 0 | 0.0 | 1 | 5.6 |
| epididymis | 19 | | 20 | |
| hypospermia | 0 | 0.0 | 1 | 5.0 |
| eye | 20 | | 20 | |
| cataract | 1 | 5.0 | 0 | 0.0 |
| retinal dysplasia | 1 | 5.0 | 0 | 0.0 |
| heart | 20 | | 20 | |
| degeneration, myofiber | 1 | 5.0 | 0 | 0.0 |
| hemorrhage | 0 | 0.0 | 3 | 15.0 |
| inflammation | 1 | 5.0 | 2 | 10.0 |
| kidney | 20 | | 20 | |
| casts, protein | 0 | 0.0 | 1 | 5.0 |
| nephropathy | 1 | 5.0 | 0 | 0.0 |
| cyst(s) | 1 | 5.0 | 0 | 0.0 |
| liver | 20 | | 20 | |
| fatty change | 1 | 5.0 | 1 | 5.0 |
| lung | 20 | | 19 | |
| inflammation | 0 | 0.0 | 1 | 5.3 |
| hemorrhage, fresh | 9 | 45.0 | 5 | 26.3 |
| lymph node, mesenteric | 20 | | 20 | |
| hemorrhage | 0 | 0.0 | 1 | 5.0 |
| mammary gland | 20 | | 20 | |
| no alveolar/ductal tissue present | 6 | 30.0 | 8 | 40.0 |
| pancreas | 20 | | 20 | |
| atrophy | 2 | 10.0 | 1 | 5.0 |
| fibrosis | 2 | 10.0 | 1 | 5.0 |
| hemorrhage | 1 | 5.0 | 1 | 5.0 |
| prostate gland | 20 | | 20 | |
| inflammation, chronic | 2 | 10.0 | 1 | 5.0 |
| prostate gland | 20 | | 20 | |
| inflammation | 3 | 15.0 | 2 | 10.0 |
| skeletal muscle (thigh) | 20 | | 20 | |
| leukocyte infiltration | 1 | 5.0 | 0 | 0.0 |
| testes | 20 | | 20 | |
| atrophy | 0 | 0.0 | 1 | 5.0 |
| thymus | 20 | | 20 | |
| hemorrhage, fresh | 5 | 25.0 | 0 | 0.0 |
| thyroid gland | 20 | | 20 | |
| cyst, ultimobranchial | 3 | 15.0 | 2 | 10.0 |

[a] Animals in the 0.5 and 1.0% dose groups were not evaluated histopathologically.

**Table 7.** Terminal Histopathologic Observations for Female Sprague–Dawley Rats Fed Diets Containing 0 or 2.0% GSE1 for 90 Days

| | dose group[a] | | | |
|---|---|---|---|---|
| | 0% | | 2.0% | |
| | number of tissues examined | % of tissues containing lesions | number of tissues examined | % of tissues containing lesions |
| eye | 20 | | 20 | |
| retinal dysplasia | 1 | 5.0 | 0 | 0.0 |
| hyaloid vessel remnant | 1 | 5.0 | 0 | 0.0 |
| hemorrhage | 1 | 5.0 | 0 | 0.0 |
| heart | 20 | | 20 | |
| fibrosis | 0 | 0.0 | 1 | 5.0 |
| hemorrhage | 3 | 15.0 | 4 | 20.0 |
| inflammation | 2 | 10.0 | 1 | 5.0 |
| kidney | 20 | | 20 | |
| calculus, microscopic observation | 3 | 15.0 | 6 | 30.0 |
| casts, protein | 0 | 0.0 | 1 | 5.0 |
| pelvic calculi | 0 | 0.0 | 1 | 5.0 |
| pelvis, dilation | 1 | 5.0 | 0 | 0.0 |
| liver | 20 | | 20 | |
| fatty change | 1 | 5.0 | 2 | 10.0 |
| lung | 20 | | 20 | |
| hemorrhage, fresh | 4 | 20.0 | 5 | 25.0 |
| pancreas | 20 | | 20 | |
| atrophy | 0 | 0.0 | 1 | 5.0 |
| thymus | 20 | | 20 | |
| hemorrhage, fresh | 7 | 35.0 | 1 | 5.0 |
| thyroid gland | 19 | | 20 | |
| cyst, ultimobranchial | 5 | 26.3 | 1 | 5.0 |

[a] Animals in the 0.5 and 1.0% dose groups were not evaluated histopathologically.

cellulose-enhanced diet. Freeman (*80*) reported increased consumption without weight gain during a 90-day study of microcrystalline cellulose (Avicel) administered at either 2.5% or 5.0% w/w in the diet. The author noted that the NOEL exceeded 50,000 mg/kg diet because there was no evidence of toxicity at the highest dose. In another study of a fibrous cellulose, Cellulon, given at 5% and 10% of the diet for 13 weeks, increased food consumption occurred in all the groups fed the fiber but there were no differences in body weight between fiber-fed and control groups. This was attributed to the altered nutritional value of the diet. Again, there was no evidence of treatment-related effects (*81*).

An alternative explanation for the apparent toxicity of a high-tannin diet may lie in the inhibition of post-digestive metabolism, or a systemic effect. In a study with rats, Mole et al. (*82*) found that the toxic effects of dietary condensed tannins were due to the impaired efficiency with which digested and absorbed nutrients were converted to new body substance and did not involve inhibition of food consumption or digestion. Butler and Rogler (*83*) also suggested possible systemic effects to include direct inhibition of a key metabolic pathway and/or the diversion of metabolism into detoxification of polyphenols or their degradation products.

**Necroscopy Observations.** Gross necropsy findings did not demonstrate any adverse effects in any organ. Organ weights were examined as a % of body weight and as a % of brain weight (**Table 5**). No statistically significant differences in organ weights were present in any of the male and female rats receiving the IH636 diets when compared to those of the control group. Histopathological evaluation of the tissues from rats in the high-dose and control groups revealed a number of lesions, the incidence of which was similar between the 2 groups (**Tables 6** and **7**). These types of lesions are commonly associated with Sprague−Dawley rats of this age and are considered to be related to spontaneous or iatrogenic causes. They were similar in severity in all groups and were graded as either minimal or mild and are therefore not considered compound-related.

**Clinical Chemistry.** Few significant differences in hematological and clinical chemistry values were observed between GSE-fed groups and the control group (**Tables 8** and **9**). Male rats in the 1.0 and 2.0% IH636 groups exhibited slight, statistically significant increases in serum sodium levels. Compared to the control group, slight, statistically significant decreases in blood urea nitrogen and creatine levels were observed in females provided the diet containing 2.0% IH636. These serum chemistry changes were not accompanied by histopathological findings and are considered not to be of toxicological significance.

Serum iron analysis was performed for the control and high-dose groups only (**Table 10**). The levels of serum iron (IRO) were significantly decreased (14−17% lower) in male rats in the 2.0% IH636 group compared to those in the control group. Consequently, the serum iron/total iron binding capacity (ITC) ratio was significantly reduced. No significant changes were observed for total iron binding capacity (TIBC). There were no significant differences in clinical chemistry values for serum iron, total iron binding capacity, and iron/total iron binding capacity between the females in the 2.0% IH636 group and females in the control group.

In the present rat study, the serum iron levels of male rats in the control and 2.0% IH636 groups were 175 and 151 $\mu$g/dL, respectively. Historically, the levels of serum iron in male Sprague−Dawley rats average 202 $\pm$ 49 $\mu$g/dL in rats 6 to 8 weeks of age and 152 $\pm$ 70 $\mu$g/dL in rats 19 to 21 weeks of age (*94*). Although the mean serum iron levels of the rats in the control and 2.0% IH636 groups varied statistically, the levels of both groups are within the range of these historical limits. No histological evidence supporting any physiological changes from a pronounced decrease in serum iron was observed. In addition, the results of the clinical hematology analyses showed no changes in male rats in the IH636-fed groups in hematological parameters that would be expected to be altered by a decrease in circulating iron, such as red blood cells, hemoglobin, or hematocrit (**Table 11**).

Dietary iron absorption may be affected by the formation of insoluble iron complexes within the intestinal lumen (*95*) or by altered intestinal permeability (*96*). Various studies have been published that aimed to determine the effects of flavonoids on

**Table 8.** Terminal Hematological Values for Sprague–Dawley Rats Fed Diets Containing up to 2.0% GSE1 for 90 Days

| | dose group | | | |
|---|---|---|---|---|
| test(s) | untreated feed, 0% | 0.5% | 1.0% | 2.0% |
| WBC ($\times 10^3$/mm$^3$) | | | | |
| males | 12.8 ± 2.94 | 14.5 ± 5.14 | 13.1 ± 3.68 | 12.1 ± 3.57 |
| females | 6.4 ± 2.55 | 6.9 ± 2.53 | 6.7 ± 2.46 | 6.5 ± 2.80 |
| RBC ($\times 10^6$/mm$^3$) | | | | |
| males | 8.66 ± 0.435 | 8.56 ± 0.464 | 8.64 ± 0.536 | 8.50 ± 0.366 |
| females | 7.43 ± 0.394 | 7.45 ± 0.343 | 7.37 ± 0.439 | 7.41 ± 0.418 |
| HGB (g/dL) | | | | |
| males | 15.0 ± 0.81 | 15.0 ± 0.68 | 15.2 ± 0.57 | 15.1 ± 0.49 |
| females | 14.0 ± 0.67 | 13.9 ± 0.70 | 13.9 ± 0.83 | 14.0 ± 0.63 |
| HCT (%) | | | | |
| males | 44.4 ± 2.21 | 44.3 ± 1.98 | 44.7 ± 1.76 | 44.4 ± 1.61 |
| females | 41.1 ± 2.09 | 41.1 ± 1.71 | 41.1 ± 2.38 | 41.3 ± 1.87 |
| MCV (fl) | | | | |
| males | 51 ± 1.6 | 52 ± 1.4 | 52 ± 1.9 | 52 ± 2.0 |
| females | 55 ± 1.9 | 55 ± 1.5 | 56 ± 1.7 | 56 ± 1.9 |
| MCH (pg) | | | | |
| males | 17.3 ± 0.83 | 17.5 ± 0.46 | 17.6 ± 0.75 | 17.8 ± 0.70 |
| females | 18.8 ± 0.75 | 18.7 ± 0.61 | 18.9 ± 0.52 | 18.9 ± 0.62 |
| MCC (%) | | | | |
| males | 33.8 ± 1.19 | 33.8 ± 0.47 | 33.9 ± 0.56 | 34.1 ± 0.62 |
| females | 34.0 ± 0.75 | 33.9 ± 0.94 | 33.9 ± 0.46 | 33.9 ± 0.45 |
| PLC ($\times 10^3$/mm$^3$) | | | | |
| males | 1351 ± 295.9 | 1212 ± 220.7 | 1316 ± 193.0 | 1255 ± 213.5 |
| females | 1124 ± 113.1 | 1171 ± 141.3 | 1142 ± 220.5 | 1159 ± 144.4 |
| RET (% RBC) | | | | |
| males | 1.0 ± 0.35 | 1.0 ± 0.61 | 1.1 ± 0.61 | 1.1 ± 0.54 |
| females | 1.3 ± 0.45 | 1.3 ± 0.68 | 1.2 ± 0.74 | 1.2 ± 0.47 |
| ANS (/mm$^3$) | | | | |
| males | 1507 ± 705.3 | 2232 ± 3309.1 | 1769 ± 693.4 | 1686 ± 794.6 |
| females | 623 ± 230.0 | 528 ± 202.7 | 903 ± 932.3 | 770 ± 550.1 |
| ANB (/mm$^3$) | | | | |
| males | 0 | 0 | 0 | 0 |
| females | 0 | 0 | 0 | 0 |
| ALY (/mm$^3$) | | | | |
| males | 10770 ± 3053.8 | 11792 ± 5178.7 | 10847 ± 3580.1 | 10008 ± 3305.0 |
| females | 5556 ± 2470.8 | 6193 ± 2484.4 | 5561 ± 2367.4 | 5509 ± 2727.5 |
| AMO (/mm$^3$) | | | | |
| males | 414 ± 251.5 | 360 ± 223.6 | 373 ± 189.1 | 269 ± 151.7 |
| females | 177 ± 131.0 | 156 ± 109.3 | 164 ± 127.7 | 150 ± 85.0 |
| AEO (/mm$^3$) | | | | |
| males | 75 ± 98.8 | 76 ± 122.6 | 86 ± 81.1 | 111 ± 127.3 |
| females | 20 ± 43.5 | 13 ± 27.7 | 26 ± 35.4 | 31 ± 41.4 |
| ABA (/mm$^3$) | | | | |
| males | 0 | 0 | 0 | 0 |
| females | 0 | 0 | 0 | 0 |

**Table 9.** Clinical Chemistry Values for Sprague–Dawley Rats Fed Diets Containing up to 2.0% GSE1 for 90 Days

| | dose group | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | untreated feed, 0% | | 0.5% | | 1.0% | | 2.0% | |
| | male | female | male | female | male | female | male | female |
| TPR (g/dL) | 7.1 ± 0.44 | 7.6 ± 0.64 | 7.1 ± 0.37 | 7.8 ± 0.47 | 7.0 ± 0.45 | 7.7 ± 0.30 | 7.0 ± 0.35 | 7.5 ± 0.41 |
| ALB (g/dL) | 3.3 ± 0.17 | 3.9 ± 0.33 | 3.3 ± 0.14 | 3.9 ± 0.28 | 3.3 ± 0.19 | 3.9 ± 0.26 | 3.3 ± 0.15 | 3.8 ± 0.25 |
| GLO (g/dL) | 3.8 ± 0.32 | 3.8 ± 0.34 | 3.8 ± 0.25 | 3.9 ± 0.25 | 3.7 ± 0.31 | 3.8 ± 0.17 | 3.7 ± 0.28 | 3.7 ± 0.22 |
| ALP (IU/L) | 102 ± 19.1 | 48 ± 15.6 | 118 ± 147.8 | 52 ± 23.0 | 103 ± 18.6 | 52 ± 22.7 | 118 ± 36.5 | 44 ± 21.5 |
| BUN (mg/dL) | 17 ± 2.9 | 18 ± 2.9 | 16 ± 2.1 | 18 ± 4.3 | 18 ± 2.6 | 16 ± 4.1 | 16 ± 2.1 | 14 ± 2.2[b] |
| CRE (mg/dL) | 0.6 ± 0.07 | 0.7 ± 0.08 | 0.6 ± 0.15 | 0.7 ± 0.06 | 0.6 ± 0.06 | 0.7 ± 0.07 | 0.6 ± 0.03 | 0.6 ± 0.20[b] |
| AST (IU/L) | 93 ± 53.4 | 83 ± 55.9 | 170 ± 423.6 | 74 ± 10.8 | 72 ± 11.2 | 96 ± 83.3 | 72 ± 5.7 | 75 ± 12.6 |
| ALT (IU/L) | 51 ± 36.6 | 42 ± 24.8 | 169 ± 584.8 | 38 ± 7.8 | 40 ± 13.2 | 55 ± 64.7 | 41 ± 6.2 | 34 ± 13.7 |
| CHO (mg/dL) | 90 ± 26.0 | 94 ± 21.2 | 83 ± 25.9 | 91 ± 27.9 | 78 ± 16.9 | 98 ± 19.5 | 81 ± 16.4 | 90 ± 18.2 |
| TBI (mg/dL) | 0.1 ± 0.04 | 0.1 ± 0.05 | 0.3 ± 0.98 | 0.2 ± 0.05 | 0.1 ± 0.05 | 0.1 ± 0.05 | 0.1 ± 0.04 | 0.1 ± 0.05 |
| GLU (mg/dL) | 132 ± 32.3 | 123 ± 15.8 | 132 ± 37.9 | 122 ± 23.5 | 133 ± 35.0 | 114 ± 19.5 | 133 ± 20.1 | 127 ± 39.4 |
| CAL (mg/dL) | 10.7 ± 0.33 | 11.0 ± 0.47 | 10.5 ± 2.17 | 11.3 ± 0.37 | 11.1 ± 0.46 | 11.2 ± 0.39 | 11.1 ± 0.32 | 10.5 ± 2.41 |
| CHL (mEq/L) | 99 ± 1.8 | 99 ± 1.3 | 99 ± 1.6 | 98 ± 2.3 | 99 ± 1.6 | 98 ± 1.8 | 99 ± 1.1 | 99 ± 2.1 |
| PHO (mg/dL) | 7.7 ± 0.81 | 6.4 ± 1.04 | 7.8 ± 0.45 | 6.8 ± 0.96 | 8.2 ± 0.68 | 6.8 ± 1.14 | 8.1 ± 0.65 | 6.4 ± 0.81 |
| SOD (mEq/L) | 145 ± 1.1 | 144 ± 2.0 | 146 ± 1.5 | 144 ± 2.5 | 146 ± 1.0[a] | 144 ± 1.4 | 146 ± 0.6[a] | 145 ± 1.4 |
| POT (mEq/L) | 5.7 ± 0.59 | 5.4 ± 0.49 | 5.7 ± 0.52 | 5.6 ± 0.42 | 5.7 ± 0.46 | 5.4 ± 0.34 | 5.9 ± 0.39 | 5.4 ± 0.39 |

[a] Mean significantly different from 0% control ($P < 0.05$). [b] Mean significantly different from 0% control ($P < 0.01$).

iron absorption in laboratory animals and humans (*97*−*99*). Red wine, but not white wine, was reported to decrease non-heme iron absorption in humans, and, in the presence of food (a bread roll, high in phytate), the inhibitory effect on iron absorption was doubled (*97*). In human volunteers, tea consumption decreased iron absorption from solutions of ferric chloride and

**Table 10.** Terminal Serum Iron Chemistry Values for Male and Female Sprague–Dawley Rats Fed Diets Containing 0 or 2.0% GSE1 for 90 Days

| | IRO ($\mu$g/dL) | TIBC ($\mu$g/dL) | TTC (NA) |
|---|---|---|---|
| untreated feed, 0% | | | |
| male | 175 ± 32.6 | 591 ± 70.5 | 30 ± 5.5 |
| female | 344 ± 88.5 | 591 ± 123.3 | 60 ± 17.2 |
| 2.0% dose group | | | |
| male | 151 ± 22.0[a] | 610 ± 54.1 | 25 ± 37[a] |
| female | 332 ± 108.6 | 590 ± 75.2 | 56 ± 15.5 |

[a] Mean significantly different from 0% control ($P < 0.01$).

ferrous sulfate from bread, rice, and uncooked, but not cooked, hemoglobin (*98*, *99*). These inhibitory effects were overcome by other nutrient factors in the diet, such as ascorbic acid (*100*) and meat (*96*). In rats, although iron absorption was decreased with the administration of iron in a tea solution, substitution of drinking water with tea for a period of 3 days did not affect iron absorption (*96*).

Other published investigations have focused on the actions of the monomeric constituents of flavonoids, such as (+)-catechin, (−)-epicatechin, (+)-gallocatechin, and (−)-epigallocatechin. In a study designed to measure the inhibitory effect of different polyphenol structures on iron absorption in humans, researchers at the University of Goteburg provided a bread meal with a variety of phenolic acids, catechin, or tannic acid with glucose (*101*). Tannic acid, gallic acid, and chlorogenic acid caused decreases in iron absorption of 88, 50, and 30%, respectively, whereas catechin had no effect, indicating that the content of iron-binding galloyl groups may determine the inhibitory effect of flavonoids on dietary iron absorption. The weight % value for gallation of IH636 was identified using thiolysis followed by HPLC (results not shown). Thiolysis degrades the oligomers to their respective monomer flavan-3-ols as well as providing the proportion of galloylated units and the ratio of trihydroxylated to dihydroxylated units). Approximately 10% w/w of IH636 is gallated; therefore, according to the hypothesis put forth by Brune and co-workers (*101*), if iron complexation is dependent upon the presence of gallated flavonoids, only 10% of ingested IH636 could contribute to inhibition of dietary iron absorption.

Although several studies provide some evidence for an inhibition of dietary iron absorption by flavonoids through complexation in the intestinal lumen, no general consensus has been reached as to whether this effect has physiological consequences. In addition, the various nutrient factors in the diet that enhance or inhibit the absorption of iron are confounding factors in determining the effect on iron absorption of one particular dietary component. There is no evidence of iron deficiency in vegetarians, whose diet usually contains exaggerated quantities of fruit. They are able to obtain, on average, >90% of their daily iron intake (primarily as non-heme iron) from foods other than meat. In a study designed to evaluate the responsiveness of serum and fecal ferritin to differences in iron absorption, 21 women ate a nonvegetarian or an ovolactovegetarian diet for 2 separate 8-week periods (*102*). Although non-heme iron absorption in women consuming the ovolactovegetarian was less than 50% of the absorption in women consuming the meat diet, no effects were observed in hemoglobin, transferrin saturation, serum ferritin, or erythrocyte protoporphyrin, indicating a possible physiological adaptation to lowered iron absorption or intestinal responsiveness to iron bioavailability (*103*).

## CONCLUSIONS

Overall, the ingestion of IH636 at dietary levels of up to 2% was well tolerated by male and female Sprague−Dawley rats. Results from this study do not provide any evidence of toxicity at 2% IH636 in the diet as demonstrated by the findings in clinical observations, body weight and food consumption measurements, ophthalmoscopic examinations, hematology, serum chemistry, organ weights, or histopathology. Although slight increases in serum sodium levels in male rats in the 1.0 and 2.0% IH636 groups, and decreased blood urea nitrogen and creatine levels in females provided 2.0% IH636 were observed, the serum chemistry changes, in the absence of histopathological findings, were not considered to be of toxicological significance. Similarly, although decreased levels of serum iron and the serum iron/total iron binding capacity (14−17% lower) were observed in male rats in the 2.0% IH636, the serum iron levels were within the range of historical levels reported by Loeb and Quimby (1989). In conclusion, at 2.0% in the diet, IH636 produced no significant compound-related toxicity in Sprague−Dawley rats.

Of greater importance to the consuming public is the effect that ingesting normal levels of proanthocyanidins might have on the nutrient value of the diet. In man, there are no toxicological studies per se, but in a recent paper Dubnick and Omaye (*104*) have reviewed more than 150 reports of the effect of wine, grape, and tea antioxidant polyphenols on atherosclerosis and ischemic heart disease. In the majority of these studies, the doses of proanthocyanidins ingested were between 75 mg and 300 mg, or equivalent to those present in 1−3 glasses of wine or a few cups of tea; in other words, a small increase above normal consumption. Not only did the authors conclude that the available data indicate that wine and tea polyphenols possess biological activity that may modify risk factors associated with cardiovascular disease, but also that the rare side effects have been observed only after the ingestion of pharmacological doses. For example, some stomach discomfort was noted in subjects

**Table 11.** Summary of Results of the Serum Iron and Clinical Hematology Analyses of Male Sprague–Dawley Rats Fed Diets Containing up to 2.0% GSE1 for 90 Days

| | dose group | | | |
|---|---|---|---|---|
| test(s) | untreated feed, 0% | 0.5% GSE1 | 1.0% GSE1 | 2.0% GSE1 |
| TIBC ($\mu$g/dL) | 591 ± 70.5 | NA[a] | NA | 610 ± 54.1 |
| IRO ($\mu$g/dL) | 175 ± 32.6 | NA | NA | 151 ± 22.0[b] |
| ITC | 30 ± 5.5 | NA | NA | 25 ± 3.7[b] |
| WBC ($\times 10^3$/mm$^3$) | 12.8 ± 2.94 | 14.5 ± 5.14 | 13.1 ± 3.68 | 12.1 ± 3.57 |
| RBC ($\times 10^6$/mm$^3$) | 8.66 ± 0.435 | 8.56 ± 0.464 | 8.64 ± 0.536 | 8.50 ± 0.366 |
| HGB (g/dL) | 15.0 ± 0.81 | 15.0 ± 0.68 | 15.2 ± 0.57 | 15.1 ± 0.49 |
| HCT (%) | 44.4 ± 2.21 | 44.3 ± 1.98 | 44.7 ± 1.76 | 44.4 ± 1.61 |

[a] NA = Not Available. [b] Mean significantly different from 0% control ($P < 0.01$).

taking ~1 g/day epigallocatechin gallate supplements. This is approximately the dose in >10 cups of green tea/day (*105*).

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**Received for review August 9, 2001. Revised manuscript received October 30, 2001. Accepted November 1, 2001.**

JF011066W