PRE-OSTEOBLASTIC CELLS PROLIFERATIVE PROPERTY OF BIOACTIVE COMPOUNDS IN GERMINATED BROWN RICE: A PROMISING IN VITRO POTENTIALS IN OSTEOPOROSIS

[1]SANI ISMAILA MUHAMMAD, [2]MAZNAH ISMAIL

[1,2]Laboratory of Molecular Biomedicine, Institute of Bioscience
[2]Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine Usmanu Danfodiyo University Sokoto, PMB 2346 Nigeria
[3]Department of Nutrition and Dietetic, Faculty of Medicine and Health Sciences
E-mail: [1]snismaila99@gmail.com

Abstract:
Background: Application of brown rice in the area of biomedical research is increasing especially in the management of chronic metabolic diseases, butadequate effort has not been made to study the cellular toxicity and proliferative ability of its bioactives to properly ascertain its safety.
*Method***:** In this study, different *in vitro* assays were carried out to determine cell proliferation using Trypan blue exclusion assay (TBE) and Bromidine uracil assay(Brdu). Toxicity of the bioactives was measured by (MTT) and Neutral red assays (N.R) on MC3T3-E1 osteoblastic cells at varying concentrations. Alkaline phosphatase activity(ALP), collagen and protein synthesis of the cells were also quantified.
Results: A time-dependent, significant proliferative effect on the cells was observed at a concentration of 10µg/ml compared to other concentrations of 100 and 1000µg/ml for TBE and Brdu ($p<0.05$). No toxicity was recorded at a concentration between 0 and 1000µg/ml, using both MTT and N.R assays. An overall significant increase in alkaline phosphatase activity ($p=0.0194$) was observed. Collagen and protein synthesis increases significantly in ASG treated cells ($p<0.05$). Significant correlation was observed between Brdu and TBE results ($r^2= 0.842$, $p= 0.01$).
Conclusions: GBR-bioactives stimulates bone cell proliferation in-vitro possibly by increasing ALP synthesis which leads to new DNA formation, protein and collagen synthesis, and the bioactives has no toxic effect; further research is needed to ascertain the possibility of using these compounds in the management of osteoporosis.

Keywords: Cellular toxicity, cell proliferation, GBR- Bioactives, Osteoporosis

INTRODUCTION

The search for natural compounds from plants and their bioactive compounds in the management of chronic diseases have increased over the years. A number of researches have been conducted using food stuffs and their bioactive for prophylaxis and management of diseases related to oxidative stress such as Diabetes, arthritis, Osteoporosis, Cancer and cardiovascular diseases. Germinated brown rice is brown rice that undergoes the process of germination over time. The concept is to increase the concentration of the bioactive compounds within the brown rice. It involves the socking and stressing the brown rice seed until it sporulated. It was first invented by KayaharaandTukahara in Japan.(Kayahara&Tukahara, 2000) Previous studies on the use of germinated brown rice (GBR) have shown a promising trend in both laboratory and clinical trials in its possible application in the management of oxidative stress related diseases.(Hagiwara, Seki, &Ariga, 2004; Hayakawa et al., 2009;Latifah et al., 2010; Miura et al., 2006; G. Zhang et al., 2010)Proper detection or quantification of substances and compounds in a given biological sample cannot be achieved accurately by the application of a specific or single method. Transform infrared spectrophotometry (FTIR) is a spectroscopy which utilizes interferogram that received frequencies of a given sample simultaneously, it uses polychromatic radiation to measure the excitation of molecular bonds whose relative absorbance provide an index of the abundance of various functional groups.(Griffith & De Haseth, 1986)Toxicity screening is an essential step in the determination of safety and efficacy of any bioactive compound intended for either therapeutic or nutraceutical application.

Although previous study on GBR examines the acute and sub-chronic safety of GBR in mice,(Hutadilok-Towatana et al.)studies are limited on the screening of toxicity/viability of compounds in germinated brown rice using various in-vitro assays. MC3T3E-1 osteoblastic cell lines are widely used for testing the proliferative effect of compounds with osteoblastic activity *in vitro.*(Aoshima et al., 2003; E. M. Choi & Koo, 2003; E. M. Choi, Suh, Kim, Choue, & Koo, 2001; Quarles, Yohay, Lever, Caton, &Wenstrup, 1992;Seo, Cho, Kim, Shin, &Kwun, 2010; Xiang, Xu, Su, Hu, & Yan, 2011) Considering the need for an assessment of toxicity and osteoblastic activity of germinated brown rice and its bioactive compounds in-vitro, this study was designed to determine the safety and osteoblastogenesis ability of phenolic, ASG, γ-aminobutyric acid and γ-oryzanol from germinated brown rice using MC3T3-E1 osteoblastic cells as the model.

MATERIALS AND METHODS

Cells

MC3T3-E1 osteoblastic cell line subclone 4 from a mouse calvariae (*Musmusculus)* origin with a catalogue number (ATCC No. CRL-2593) was obtained from the American Culture Collection Manassas, VA 20108 USA(ATCC).Cells were thawed and transferred into a 25-cm^2 cell culture flask containing 5mls of α-MEM supplemented with 10% bovine serum and 1% antibiotics (penicillin streptomycin solution (10,000 units of penicillin and 10 mg of streptomycin in 0.9% NaCl) . Media was change every 2-3 days. Sub-culture was done after reaching 70% confluency at 72 hours in 75-cm^2 cell culture flasks containing 10mls of the media in a humidified atmosphere of 95% air and 5% CO_2 at 37ºC.

Reagents and chemicals

α-MEM (Minimum Essential Media) with ribonucleoside and deoxyribonucleoside, 2mM L-glutamine and 1mM sodium pyruvate, Penicillin, streptomycin and foetal bovine serum were purchased from Gibco Laboratories USA. Trypsin-EDTA, trypan blue dye, neutral red, MTT, 4-nitro-phenol phosphate, glycine and $ZnCl_2$ were obtained from Sigma (SIGMA-Aldrich Chemie, USA, GmbH, Germany). Brdu cell proliferation Assay kit (Cat.No QIA58) was obtained from Calbiochem EMD Chemicals Inc., affiliated to Merck KGaA, Darmstadt, Germany.All solvent were of analytical grade and were purchased from merckDarmstadt, Germany.

Germinated brown rice (phenolics), GABA, γ-oryzanol and Acylatedsterylglucosides (ASG)

Germination of brown rice, extraction and extraction of GBR-phenolic compounds were performed using 80% methanol as reported earlier.(Sani et al., 2012) GABA was extracted using 70% ethanol and quantified using HPLC-DAD (Agilent, Santa Clara, CA) as reported.(Rozan et al., 2000), while oryzanol was extracted using n-hexane and analyzed applying the method described earlier.(Azlan et al., 2008) .ASG was extracted using a combination of methanol and chloroform and analyzed by GCMS as reported.(Usuki et al., 2007)

Trypan blue exclusion (TBE) assay

TBE assays were used to determine cell proliferation. Cells in the log phase of growth were seeded in 6-well plates (2 mL of cell solution /well) at an initial concentration of $2x10^4$ cells/mL and allowed to attach for 24 hours, reaching about 50% confluency before treatment with various concentrations(10, 100 and 1000µg/ml) of GBR crude extract, ASG, GABA and oryzanol. Untreated wells were used as control. Each assay was run in triplicate. After 24, 48, and 72 hours of incubation at 37^0C of 3% CO_2, the cells were scraped gently using a cell scraper (30 cm), starting with the control wells, then wells containing the lowest concentration of the test compounds. The scraped cells were then transferred into a 15-ml falcon tube and centrifuged at 1000 rpm for 10 minutes at 4ºC. The media was discarded and the cells washed with 1 ml of phosphate buffered saline (PBS). Cells were centrifuged and the supernatant discarded, before adding another 1ml of PBS prior to vortexing. Then, 20 µl of the cell was dispensed into Neuber's chamber and 20µl of trypan blue stain added. The number of viable and non-viable cells were counted using an inverted microscope at x40 magnification, and the percentage viability using the formula:

% Viable cells = [1- (number of blue cells ÷ number of total cells) x100]

Brdu Proliferation Assay

The assay was carried out according to manufactures instructions. Briefly, Osteoblastic MC3T3-E1 cells (100 µl) were seeded on 96 well plate at a concentration of $1x10^4$ cells/ml, and later treated with various concentrations (10, 100, 1000µg/ml) of GBR and it bioactives for 24, 48, and 72hrs with some wells serving as two controls; one level as blank, containing only media, and the other containing cells but not label with Brdu. A working solution of Brdu was prepared by diluting it in a fresh media at 1: 2000.Then 20 µl of this working solution was pipetted to each treated well to be labeled and incubated for about 24hrs. Content were removed and 200 µl of fixative/denaturing solution was added to each well and incubated for 30minutes at room temperature, the content was removed by inverting over sink and tapping on paper towel. Anti-Brdu100 µl, was later added to each well and incubate for 1 hour at room temperature and the plate was washed three times with washing buffer. Then 100 µl of HRP conjugate was added and incubated for 30minutes at room temperature and the wells were washed three times. They were flooded with dH_2O and the content was removed, and 100 µl of substrate solution was added and incubated in the dark at room temperature for 15minutes a stop solution was added and the plate was read at 450 nm using spectrophotometer.

MTT cytotoxicity/viability assay

This assay was carried out following the same protocols describedearlier.(Mosmann, 1983)Cells were seeded in 96-well plates and incubated for 24 hours at 37ºC in 5% CO_2. At about 80% confluency, the cells were then treated with various concentrations (0, 15.63, 31.25, 62.5, 125, 250, 500 and 1000µg/ml) of the test compounds. The MTT was prepared by dissolving the powder in PBS and 10 µl of the prepared solution was added to each well. The plates were then incubated at 37ºC for 4 hours. The media was discarded and replaced with DMSO, before being mechanically shaken until the purple

formazan crystals were dissolved. Absorbance was determined using a spectrophotometer at 570 nm.

Neutral red (NR) uptake viability assay

This was determined according to a reported protocol first described by Borenfreund and Puerner (1985).(Borenfreund&Puerner, 1985) A stock solution of the neutral red dye was prepared by diluting 0.33 g of the dye in 100 ml of distilled water. It was then filtered using millipore filtering (0.2 mm pore size) and stored in the dark at room temperature until use. The required volume of the NR was prepared in complete α-MEM media at a final concentration of 50µg/ml. Cells were seeded in 96-well plates by pipetting 125 µl of the cell suspension into appropriate wells using a multichannel pipette. After 24 hours of incubation at 37ºC in a humidified atmosphere of 5% CO_2, the media was discarded and the cells washed with PBS. Using the multichannel pipette, the wellswere treated with various concentrations of the test compounds in triplicate, leaving 12 wells as negative control and 6 wells as blank. The plates were then incubated for another 48 hours at 37ºC (5% CO_2). The media was then discarded and the plate rinsed three times with PBS (200 µl each well), before being washed. Medium containing neutral red (50 µg/ml) was added, 200 µl to each well. After 3 hours of culture, the media was removed and the plate rinsed twice with PBS (200 µl each well). The desorbing solution (100 µl) was then added to each well, containing 1% glacier acetic acid, 50% ethanol and 49% H_2O. This was shaken for 15 minutes with a micromixer in the dark. The absorbance of the colored solution was measured using a microplate reader at 540 nm.

Alkaline phosphatase activity

The alkaline phosphatase activity was determined following the procedure described by the manufacturer of the substrate with some amendments (Sigma Product Number **N 4645**). In brief, cells were seeded in 96-well plates and incubated for 24 hours in a humidified atmosphere. They were then treated with the various test compounds and incubated for another 48 hours. p-nitrophenyl phosphate (pNPP) was used as the substrate for the assay. It was prepared by dissolving pNPP in 0.1 M glycine buffer containing 1 mM $MgCl_2$ and 1 mM $ZnCl_2$, pH 10.4, at a final concentration of 1mg/ml. Two hundred microlitres of the substrate were added to each well and incubated in the dark for about 30 minutes. This substrate produces a soluble end product that is yellow in colour, which was read spectrophotometrically at 405 nm.

Protein and Collagen synthesis

Protein was measured using the Bio-rad kit, which is a modified Bradford method. Bovine serum albumin was used as the standard at a concentration of 1mg/ml, seven dilutions of the standard were prepared. One hundred and sixty microliters of each standard and sample were pipette into a 96-well plate and 40 µl of the dye were added to each well. Samples and the reagent were mixed thoroughly using a multichannel pipette and then incubated at room temperature for about 30 minutes. Absorbance was measured using a spectrophotometer at 595 nm.

To determine the collage synthesized by the cells, the method used by (Seo et al., 2010) was adopted with some modifications. The absorbance of both the samples and the collagen standards was measured in a 96-well plate. A serial dilution of the collagen standard was made using collagen type I, in a concentration ranging from 0 to 400µg/ml. One hundred microlitres of the sample and standard were added to each well and incubated at 37ºC in a 5%-CO_2 atmosphere for 16 hours. The samples were then transferred to a dry oven for 24 hours and washed with 200 µl of distilled water. The wells were stained for an hour at 37 ºC using 100 µl of 0.1% (w/v) sirius red in saturated picric acid. Plates were washed twice using 200 µl of 10 mMHCl solution and 0.1 N NaOH at room temperature to dissolve the adsorbed collagen. The solution was mixed several times and the absorbance was measured spectrophotometrically at 540 nm.

Statistical analysis

Samples were analyzed in triplicate, and data are expressed as mean ± standard deviation (SD) for three readings, Differences between the groups were evaluated by one-way ANOVA while the mean comparison was done by Turkey crammer LST using the JMP Statistical software version 10.0. Significance was considered at $p<0.05$.

RESULTS

Concentrations of the different bioactives in GBR

After extraction, our GBR-Phenolic content (80% Methanolic extract) was found to be 20.5±0.012mg/100g GBR, ASG 0.42- 0.51g/100g GBR, ASG 4.7mg/100g GBR,while our GABA content is 36mg/100g GBR as earlier reported.(Imam & Ismail, 2013; Sani et al., 2012)

Effects of GBR and its bioactive compounds on osteoblastic cell proliferation

Treatment of the cells with a concentration of 10µg/ml for 72hrs gave the highest proliferation rate in all the four compound tested using the trypan blue exclusion assay, with ASG having the highest (65 $x10^5$) cells/ml (Figure 2b) compared to other treatments with time ($p<0.05$). GBR- Phenolics treated cells ($57x10^5$) cells/ml figure 2a, cells treated with Oryzanol gives a proliferation rate of ($58\ x10^5$) cells/ml (Figure 2c), while those treated with GABA gave a proliferation rate of ($60\ x10^5$) cells/ml compared to other treatment concentrations with time as shown in(Figure 2d).

Bromodeoxyuridine (5-bromo-2'-deoxyuridine, BrdU) proliferation Assay

GBR and its bioactive compounds increase cell proliferation in a time- and dose-dependent manner. After treatment with 10, 100 and 1000µg/ml for 24, 48 and 72 hours, cells treated with ASG 10µg/ml exhibits the highest rate of proliferation. All the other compounds stimulates cell proliferation using the lowest concentration ($p<0.05$) as shown in (Table 2)

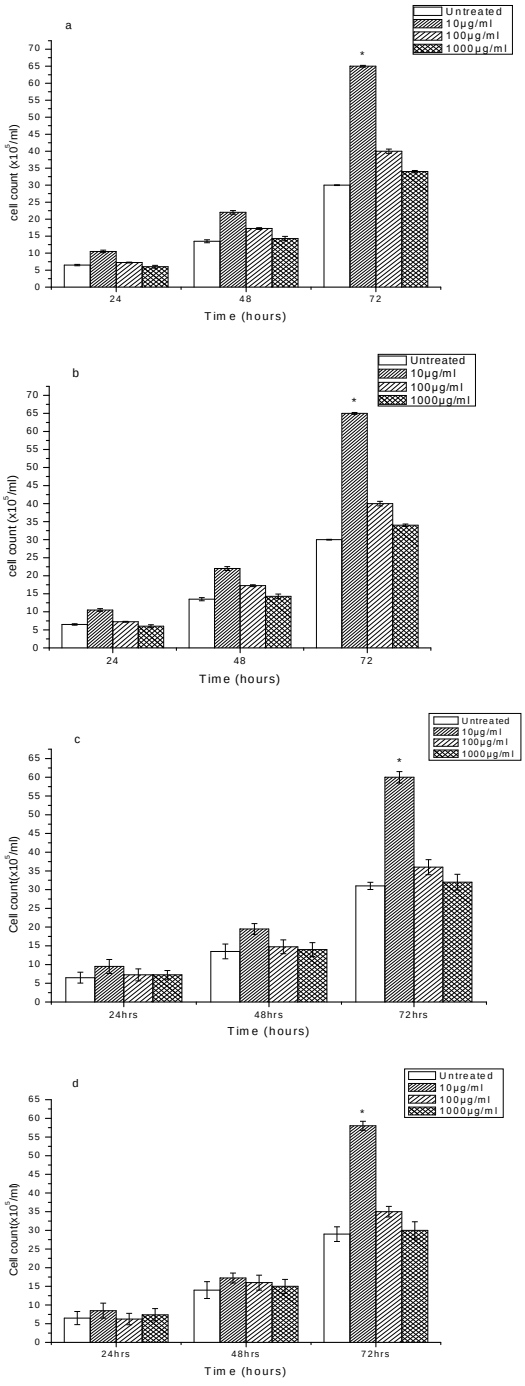


Figure2.Trypan blue exclusion (TBE) proliferation assays using three different concentrations (10, 100, and 1000µg/ml) of: (a) GBR Phenolic compounds; (b) ASG; (c) oryzanol; and (d) GABA at 24, 48 and 72 hours. ASG showed the strongest proliferative effect on the cells compared to the other compounds.

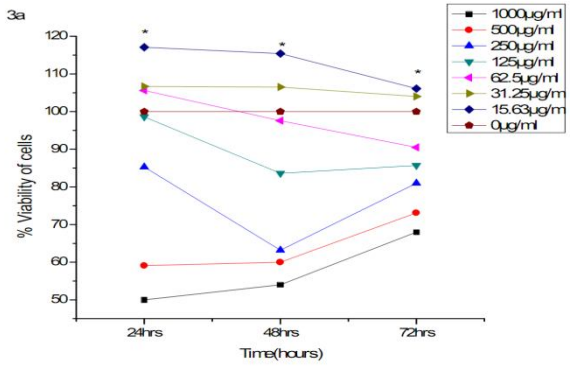
Table2: Mean ± SD Optical absorbance on the proliferation of MC3T3-E1cells in vitro after treatment with different concentrations with time of G B R- Phenolic compounds, ASG, Oryzanol and GABA

Treatment groups (µg/ml)	Incubation Time (hours) 24hrs	48hrs	72hrs
ASG 10	1.71±0.27^{a}	2.50 ±0.02^{a}	2.67±0.08^{a}
ASG100	1.39±.0.03^{d}	1.81 ±0.03^{c}	1.90±0.04^{c}
ASG1000	0.99±0.02^{f}	0.54 ±0.03^{e}	0.56±0.02^{e}
GABA10	1.59±0.02^{c}	2.11 ±0.09^{b}	2.47±0.01^{b}
GABA100	1.29±0.02^{e}	1.62±0.06^{d}	1.70±0.02^{d}
GABA1000	0.92±0.01^{f}	0.50±0.02^{e,f}	0.58±0.02^{e,f}
GBR10	1.62±0.03^{b,c}	2.38 ±0.02^{a}	2.59±0.06^{a,b}
GBR100	1.31±0.01^{d,e}	1.70 ±0.02^{c,d}	1.88±0.03^{d}
GBR1000	0.12±0.01^{g}	0.44±0.04^{e,f}	0.49±0.01^{e,f}
ORZ10	1.69±0.01^{a,b}	2.37 ±0.05^{a}	2.59±0.07^{a,b}
ORZ100	1.38±0.02^{d,e}	1.59 ±0.01^{c,d}	1.66±0.06^{c}
ORZ1000	0.94±0.10^{f}	0.52 ±0.04^{e}	0.62±0.01^{e}
Untreated	0.11±0.01^{g}	0.31 ±0.01^{f}	0.40±0.01^{f}

levels with different alphabets are significantly different ($p<0.05$)

Cytotoxicity study of GBR and its bioactive compounds on MC3T3-E1 osteoblastic cells

ASG-treated cells displayed greater viability with time compared to those treated the other three compounds. The MTT viability assays showed that cells treated with GBR had a viability rate of 117.1, 115.4 and 106.1% after 24, 48 and 72 hrs of treatment, respectively, at a low dose of 15.63 µg/ml. ASG-treated cells exhibited the highest viability rate of 135% after 24 hrs at a concentration of 62.5µg/ml, while GABA-treated cells displayed a rate of 116.9% after 48 hrs of treatment at 31.25 µg/ml. Cells administered gamma-oryzanol also displayed a high viability of 114.6% after 48 hrs of treatment at a concentration of 15.63 µg/ml. Administration of all four compounds maintained a high cell viability rate after treatment at the highest concentration of 1000 µg/ml for 72 hrs: GBR, 68%; ASG, 87%; GABA, 60%; and oryzanol, 77%.(figure 3a,b,c and d)



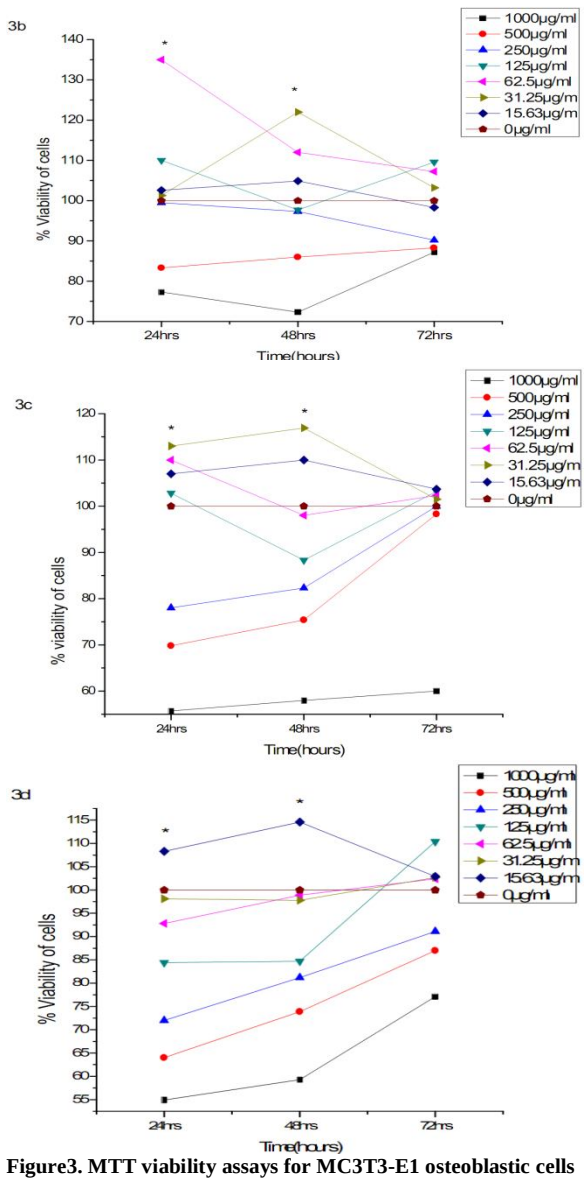


Figure3. MTT viability assays for MC3T3-E1 osteoblastic cells treated with different concentrations of: (a) GBR phenolic compounds; (b) ASG; (c) GABA; and (d) oryzanol

Treatment with GBR- Phenolic compounds at different concentration with time showed a viability rate of 117.1, 115.4 and 106.1% after 24, 48 and 72 hrs of incubation respectively, at a low dose of 15.63 µg/ml.ASG-treated cells exhibited the highest viability rate of 135% after 24 hrs of incubation at a concentration of 62.5µg/ml and 122% after 48hours respectively. GABA-treated cells displayed a percentage viability rate of 112% and 116.9% after 24 and 48hrs of incubation at 31.25 µg/ml. While cells treated with gamma-oryzanol also displayed a high viability of 108.3 and 114.6% after 24 and 48 hrs of incubation at a concentration of 15.63 µg/ml

Neutral red assays

In this assay, ASG-treated cells demonstrated the highest viability in terms of resistance to lysosomal damage, compared to those administered the other compounds ($p<0.05$).

Cells treated with ASG at a concentration of 250µg/ml gave the highest percentage in term of lysosomal function of about 170%. Lysosomal function was maintained even at higher concentrations of 500 and 1000 µg/ml with a percentage of 165 and 162 respectively. GBR-phenolic gave a percentage lysosomal function of 142 and 113% at 500 and 1000 µg/ml, while Oryzanol and GABA both gave a percentage lysosomal function of (156% and 138%) and (110 and 104%) at 500 and 1000 µg/ml respectively(Figure 4).

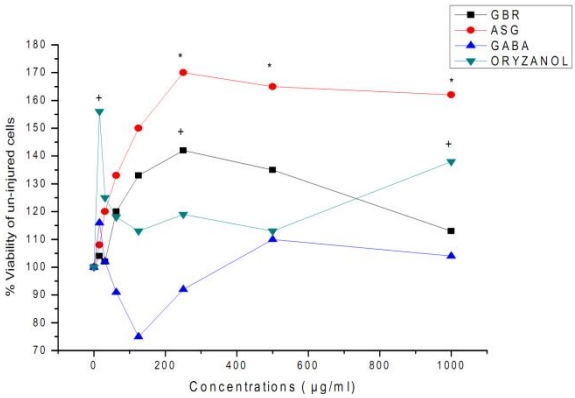


Figure4. Neutral red viability assays for MC3T3-E1 cells treated with GBR phenolic compounds, ASG, GABA and oryzanol after 72 hours of incubation.

levels with different symbols are significantly different ($p<0.05$)

No significant difference between levels with the same symbols ($p>0.05$)

Alkaline phosphatase activity.

ALP concentration increases significantly with increase concentration. With an overall significance of p= (0.0194) when comparing between the treated groups at 72hrs after treatment. In ASG & GBR treated cells ($p<0.05$); ASG & ORZ (p=0.029); ASG& GABA (p=0.025). But no significant difference ($p> 0.05$) when comparing the level of ALP between A GBR&GABA, GBR&ORZ and also ORZ&GABA (figure 5)

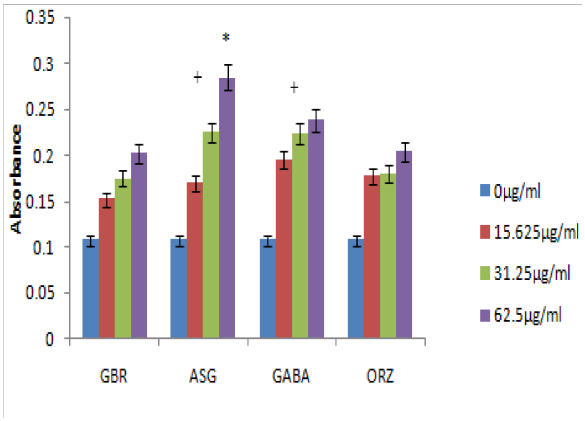


Figure 5.Alkaline phoshatase activity after 72 hours of incubating MC3T3-E1 osteoblastic cells with GBR and its bioactive compounds at different concentration

Alkaline phosphatase activity increased significantly ($p<0.05$) in groups treated with ASG .Treatment concentrations with the same symbol are not significant. ($p>0.05$)

One-way analysis of absorbance by compounds, analysis of variance (p= 0.0194*).

Protein and collagen synthesis

Protein synthesis significantly increased with increase concentration when comparing between ASG & GABA, ASG&ORZ ($p<0.05$). Group treated with ASG at a concentration of 15.62, 31.25 and 62.5µg/ml µg/ml gave the highest protein synthesis of about 110% at 72hrs.(Figure 6a). Collagen synthesis, in this assay, ASG at 500 µg/ml showed the highest significant rate of collagen synthesis ($p<0.05$) followed by oryzanol at the same concentration compared to the other treatment groups after 72hrs of incubation. (Figure 6)

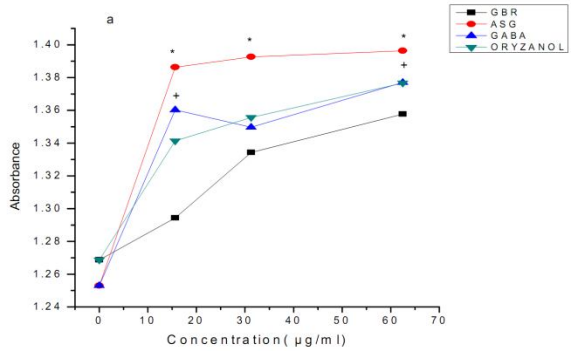


Figure 6a. Bio-rad protein synthesis assays in GBR and its bioactive compounds treated MC3T3-E1 cells at different concentrations with for after 72 hours of incubation.

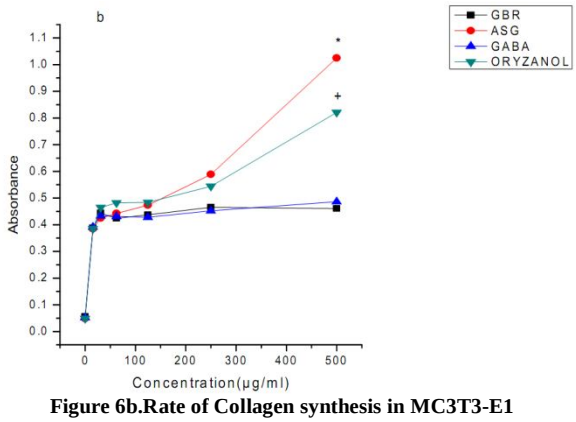


Figure 6b.Rate of Collagen synthesis in MC3T3-E1 Osteoblastic cells treated with different concentrations of GBR and its bioactive compounds

DISCUSSIONS

In this study,we found that, germinated brown rice and it bioactive compounds, especially ASG, stimulated osteoblastic proliferation and there was no observable toxic effect to cells even at higher concentrations. The high proliferative effect shown by ASG may be due to decreased in osteoclastic activities, given that ASG is known for its anti-inflammatory properties (Singh, 2005), whereby it reduces the synthesis of inflammatory cytokines and free radicals that leads to oxidative stress which also plays a key role in osteoporosis. Therefore decrease in reactive oxygen species (ROS) is the primary mechanism by which these compounds exert their osteoblastic effect(Muthusami et al., 2005) . Our results showed a significant increase in osteoblastic cell proliferation, even at the lowest concentrations of all the compounds tested (Figure 2 & Table2). Based on our TBE assay, ASG and other extracts from GBR shows a dose and time defendant increase on proliferation of MC3T3-E1 cells. To further substantiate our TBE results, Bromodeoxyuridine, which is a synthetic nucleoside that is an analogue to thiamidine, was used on the treated cells.Brdu is a proliferation assay that worked on the principles of ELISA. It involves the incorporation of Brdu into DNA of new proliferating cells. Increase in the optical density with time observed after incorporating Brdu into the treated cells shows an increase in DNA synthesis an increase uptake of the Brdu into DNA of the treated cells which demonstrates that new bone cells were formed by the addition of GBR and its bioactives to the cultured cells has the ability to synthesize DNA by activation of the precursors to nucleosides triphosphates, and by incorporation of activated nucleoside. From our TBE and Brdu results, at a concentration of 10µl/ml, germinated brown extracts have a greater cell proliferative effect on MC3T3-E1 cells. Our results from TBE assay have a strong correlation with that of Brdu proliferation assays in all the treated groups with GBR-Phenolics and ASG having r^2= 0.842, p = 0.01; and r^2= 0.714, p= 0.014.while GABA and Gamma-oryzanol having r^2= 0.851, p= 0.01 and r^2= 0.895, p= 0.01 respectively.

MTT and neutral red (NR) assays are used to determine cytotoxicity/cell viability. The former acts by staining the mitochondria of viable cells, while the latter stains the viable lysosomes. Although they act differently, they both gave the same percentages of viable cells over time in our treatments. Our MTT and NR assay results showed no toxicity to the cells either at the mitochondrial or lysosomallevelsandwere significant in terms of viability when the four different compounds were compared (Figure3a, b c & d and Figure 4). Cells treated with ASG at displayed the highest rate of viability, followed by those administered oryzanol, GABA, and then GBR extract, the cells being less viable at higher concentrations. The NR results also indicated that ASG-treated cells exhibited the highest viability compared to the rest of the treated cells. (Figure4). Our findings on enhanced osteoblastic activity following treatment were similar to those reported earlier by others.A pronounced increase in proliferation was reported when using Sanguisdrakonis resin in MC3T3-E1 cells. (W. Wang, Olson, Cheng, Guo, & Wang, 2012) Alkaline

phosphatase (ALP) activity is a prominent biochemical marker of bone formation. ALP levels increase markedly during bone cell proliferation due to DNA and protein synthesis. Cell differentiation leads to the formation of matured osteoblasts(Suh et al., 2007). Our results for ALP activity confirmed a significant increase in treated cells ($p<0.05$) with increasing concentrations in groups treated with GBR-phenolics and ASG (Figure 5). However, there was no significant increase in ALP levels with oryzanol or GABA treatment ($p>0.05$), although an overall significant elevation was observed when comparing the data among the four different treatments. Protein synthesis in the cells increases with time and concentration after treating the cells with GBR and it bioactive compounds (Figure 6a).

This indicates that new DNA was formed in the cells after treatment, which subsequently led to an increase in proliferation. Choi et al(E. M. Choi et al., 2001) reported an increase in ALP activity and protein synthesis in MC3T3-E1 cells administered soy bean extract, while increase in ALP activity in response to treatment with Red yeast rice and Sanguisdrakonis resin was reported by (Cho et al., 2010) and (W. Wang et al., 2012) respectively. Collagen is one of the major extracellular matrix components whose functions include the provision of mechanical strength in tissues and scaffolding for cell differentiation and growth(Aouacheria et al., 2004). Collagen levels also increased in cells after 72 hours of incubation with GBR and its bioactive compounds (Figure 6b). This is another indicator that germinated brown rice and its bioactive ingredients, particularly ASG, stimulate bone formation by the formation of new bone collagen which gives a mechanical support to new formed bone cells.

CONCLUSION

From the results obtained in this study, we found that GBR and it bioactive compounds stimulate the growth and proliferation of MC3T3-E1 osteoblastic cells by increasing cell proliferation, through the stimulation of protein and collagen synthesis, as well as alkaline phosphatase activity. Moreover, ASG exerted the strongest effects among all the bioactive ingredients studied, its mechanism probably due to the suppression of some inflammatory markers previously reported in osteoporosis and increases in osteoblastic activity. And the compound in GBR are non-toxic to bone cells at higher concentrations. Therefore GBR is safe to be used in the management of osteoporosis.

ACKNOWLEDGMENTS/DISCLOSURE

We wish to thank Padiberas National (BERNAS) ricecompany in Sri Tiram Jaya, Malaysia for funding this research.

REFERENCES

[1] Abukhadir, Seham Salem Ahmed, Mohamed, Norazlina, Makpol, Suzana, & Muhammad, Norliza. (2012). Effects of Palm Vitamin E on Bone-Formation-Related Gene Expression in Nicotine-Treated Rats. Evidence-Based Complementary and Alternative Medicine, 2012.

[2] Adeghate, E, & Ponery, AS. (2002). GABA in the endocrine pancreas: cellular localization and function in normal and diabetic rats. Tissue and Cell, 34(1), 1-6.

[3] Al Ali, Samer Hasan Hussein, Al-Qubaisi, Mothanna, Hussein, Mohd Zobir, Zainal, Zulkarnain, & Hakim, Muhammad Nazrul. (2011). Preparation of hippurate-zinc layered hydroxide nanohybrid and its synergistic effect with tamoxifen on HepG2 cell lines. International Journal of Nanomedicine, 6, 3099.

[4] Anderson, James W. (2003). Whole grains protect against atherosclerotic cardiovascular disease. Proceedings of the Nutrition Society, 62(01), 135-142.

[5] Aoshima, Yukihiro, Hasegawa, Yoshinori, Hasegawa, Sho, Nagasaka, Atsushi, Kimura, Tomofumi, Hashimoto, Satofumi, . . . Tsukagoshi, Norihiro. (2003). Isolation of GnafC, a polysaccharide constituent of Gnaphalium affine, and synergistic effects of GnafC and ascorbate on the phenotypic expression of osteoblastic MC3T3-E1 cells. Bioscience, biotechnology, and biochemistry, 67(10), 2068-2074.

[6] Aouacheria, Abdel, Cluzel, Caroline, Lethias, Claire, Gouy, Manolo, Garrone, Robert, & Exposito, Jean-Yves. (2004). Invertebrate data predict an early emergence of vertebrate fibrillar collagen clades and an anti-incest model. Journal of Biological Chemistry, 279(46), 47711-47719.

[7] Arden, NK, Baker, J, Hogg, C, Baan, K, & Spector, TD. (1996). The heritability of bone mineral density, ultrasound of the calcaneus and hip axis length: a study of postmenopausal twins. Journal of Bone and Mineral Research, 11(4), 530-534.

[8] Arjmandi, Bahram H, Birnbaum, Roger, Goyal, Noopur V, Getlinger, Mary J, Juma, Shanil, Alekel, Lee, . . . Kukreja, SC. (1998). Bone-sparing effect of soy protein in ovarian hormone-deficient rats is related to its isoflavone content. The American journal of clinical nutrition, 68(6), 1364S-1368S.

[9] Arjmandi, Bahram H, Getlinger, Mary J, Goyal, Noopur V, Alekel, Lee, Hasler, Clare M, Juma, Shanil, . . . Kukreja, Subhash C. (1998). Role of soy protein with normal or reduced isoflavone content in reversing bone loss induced by ovarian hormone deficiency in rats. The American journal of clinical nutrition, 68(6), 1358S-1363S.

[10] Arora, KS, Gupta, N, Singh, RA, Nagpal, S, & Arora, D. (2009). Role of free radicals in menopausal distress. J Clin Diagn Res, 3, 1900-1902.

[11] Arpels, John C. (1996). The female brain hypoestrogenic continuum from the premenstrual syndrome to menopause: A hypothesis and review of supporting data. Journal of reproductive medicine, 41(9), 633-639.

[12] Arshat, H, Tey, NP, & Ramli, N. (1989). A study on the age at menopause and menopausal symptoms among Malaysian women. Malaysian journal of reproductive health: a publication of the Reproductive Research Centre of the National Population and Family Development Board, Malaysia, 7(1), 1.

[13] Auf'mkolk, Bettina, Hauschka, Peter V, & Schwartz, Edith R. (1985). Characterization of human bone cells in culture. Calcified tissue international, 37(3), 228-235.

[14] Azlan, Azrina, Ismail, Maznah, & Abdul Hamid, Azizah. (2008). Extraction and determination of oryzanol in rice bran of mixed herbarium UKMB: AZ 6807: MR 185, AZ 6808: MR 211, AZ6809: MR 29. ASEAN Food Journal, 15(1), 89-96.

[15] Azmi, Nur Hanisah, Ismail, Norsharina, Imam, Mustapha Umar, & Ismail, Maznah. (2013). Ethyl acetate extract of germinated brown rice attenuates hydrogen peroxide-induced oxidative stress in human SH-SY5Y neuroblastoma cells: role of anti-apoptotic, pro-survival and antioxidant genes. BMC complementary and alternative medicine, 13(1), 177.

[16] Babu, P Dinesh, Subhasree, RS, Bhakyaraj, R, & Vidhyalakshmi, R. (2009). Brown rice-beyond the color reviving a lost health food-a review. Magnesium, 187, 13.10.

[17] Barrett-Connor, Elizabeth, Mosca, Lori, Collins, Peter, Geiger, Mary Jane, Grady, Deborah, Kornitzer, Marcel, . . . Wenger, Nanette K. (2006). Effects of raloxifene on cardiovascular events and breast cancer in postmenopausal women. New England Journal of Medicine, 355(2), 125-137.

[18] Ben-Shachar, D, Laufer, D, Livne, E, & Silbermann, M. (1988). Picrotoxin, a gamma-aminobutyric acid-receptor antagonist, retards craniofacial development in the weaning rat: I. Effect on mandibular bone growth. Journal of craniofacial genetics and developmental biology, 8(4), 351.

[19] Bennetts, HW, Uuderwood, EJ, & Shier, FL. (1946). A specific breeding problem of sheep on subterranean clover pastures in Western Australia. Australian veterinary journal, 22(1), 2-12.

[20] Beral, Valerie, Banks, Emily, & Reeves, Gillian. (2002). Evidence from randomised trials on the long-term effects of hormone replacement therapy. Lancet, 360(9337), 942-944.

[21] Berendsen, Hemmie HG. (2000). The role of serotonin in hot flushes. Maturitas, 36(3), 155-164.

[22] Blake, Jennifer, & Bélisle, Serge. (2006). Menopause and Osteoporosis. JOGC-TORONTO-, 28(3), 185.

[23] Bohlhalter, Stephan, Mohler, Hanns, & Fritschy, Jean-Marc. (1994). Inhibitory neurotransmission in rat spinal cord: co-localization of glycine-and GABA< sub> A</sub>-receptors at GABAergic synaptic contacts demonstrated by triple immunofluorescence staining. Brain research, 642(1), 59-69.

[24] Borenfreund, Ellen, & Puerner, James A. (1985). A simple quantitative procedure using monolayer cultures for cytotoxicity assays (HTD/NR-90). Journal of Tissue Culture Methods, 9(1), 7-9.

[25] Britz, Steven J, Prasad, PVV, Moreau, Robert A, Allen, L Hartwell, Kremer, Diane F, & Boote, Kenneth J. (2007). Influence of growth temperature on the amounts of tocopherols, tocotrienols, and γ-oryzanol in brown rice. Journal of agricultural and food chemistry, 55(18), 7559-7565.

[26] Brown, ESTEBAN ORTEGA, Sundstrom, SUSAN A, Komm, BARRY S, Yi, Z, Teuscher, C, & Lyttle, CR. (1990). Progesterone regulation of estradiol-induced rat uterine secretory protein, complement C3. Biology of reproduction, 42(4), 713-719.

[27] Brzezinski, Amnon, Adlercreutz, Herman, Shaoul, Roger, Rosier, Ariel, Shmueli, Avigail, Tanos, Vasilus, & Schenker, Joseph G. (1997). Short-term effects of phytoestrogen-rich diet on postmenopausal women. Menopause, 4(2), 89-94.

[28] Brzezinski, Amnon, & Debi, Ayelet. (1999). Phytoestrogens: the "natural" selective estrogen receptor modulators? European Journal of Obstetrics & Gynecology and Reproductive Biology, 85(1), 47-51.

[29] Burge, Russel, Dawson-Hughes, Bess, Solomon, Daniel H, Wong, John B, King, Alison, & Tosteson, Anna. (2007). Incidence and Economic Burden of Osteoporosis-Related Fractures in the United States, 2005–2025. Journal of Bone and Mineral Research, 22(3), 465-475.

[30] Burger, Henry G. (2006). Physiology and endocrinology of the menopause. Medicine, 34(1), 27-30.

[31] Cao, Ying, Zhou, Zhichao, de Crombrugghe, Benoit, Nakashima, Kazuhisa, Guan, Hui, Duan, Xiaoping, . . . Kleinerman, Eugenie S. (2005). Osterix, a transcription factor for osteoblast differentiation, mediates antitumor activity in murine osteosarcoma. Cancer research, 65(4), 1124-1128.

[32] Caratti, Pietro, Ferraguto, Ludovico, & Riboldi, Chiara. (2006). Sustainable development data availability on the Internet.

[33] Carter, Lauren E, Kilroy, Gail, Gimble, Jeffrey M, & Floyd, Z Elizabeth. (2012). An improved method for isolation of RNA from bone. BMC biotechnology, 12(1), 5.

[34] Castelo-Branco, Camil, Cancelo, Maria Jesús, Villero, Jose, Nohales, Francisco, & Juliá, Maria Dolores. (2005). Management of post-menopausal vaginal atrophy and atrophic vaginitis. Maturitas, 52, 46-52.

[35] Cavagnini, F, Invitti, C, Pinto, M, Maraschini, C, Di Landro, A, Dubini, A, & Marelli, A. (1980). Effect of acute and repeated administration of gamma aminobutyric acid (GABA) on growth hormone and prolactin secretion in man. Acta Endocrinologica, 93(2), 149-154.

[36] Chan, Lawrence, & O'Malley, Bert W. (1976). Mechanism of action of the sex steroid hormones (first of three parts). The New England journal of medicine, 294(24), 1322.

[37] Chandrasekara, Anoma, & Shahidi, Fereidoon. (2010). Inhibitory activities of soluble and bound millet seed phenolics on free radicals and reactive oxygen species. Journal of agricultural and food chemistry, 59(1), 428-436.

[38] Charoenthaikij, Phantipha, Jangchud, Kamolwan, Jangchud, Anuvat, Prinyawiwatkul, Witoon, & Tungtrakul, Patcharee. (2010). Germination Conditions Affect Selected Quality of Composite Wheat-Germinated Brown Rice Flour and Bread Formulations. Journal of food science, 75(6), S312-S318.

[39] Chen, Yanyun, & Heiman, Mark L. (2001). Increased weight gain after ovariectomy is not a consequence of leptin resistance. American Journal of Physiology-Endocrinology And Metabolism, 280(2), E315-E322.

[40] Chlebowski, Rowan T, Wactawski-Wende, Jean, Ritenbaugh, Cheryl, Hubbell, F Allan, Ascensao, Joao, Rodabough, Rebecca J, . . . Chen, Chu. (2004). Estrogen plus progestin and colorectal cancer in postmenopausal women. New England Journal of Medicine, 350(10), 991-1004.

[41] Cho, Young-Eun, Alcantara, Ethel, Kumaran, Santhy, Son, Kun-Ho, Sohn, Ho-Yong, Lee, Jong-Hwa, . . . Kwun, In-Sook. (2010). Red yeast rice stimulates osteoblast proliferation and increases alkaline phosphatase activity in MC3T3-E1 cells. Nutrition Research, 30(7), 501-510.

[42] Choi, Eun Mi, & Koo, Sung Ja. (2003). Effects of soybean ethanol extract on the cell survival and oxidative stress in osteoblastic cells. Phytotherapy Research, 17(6), 627-632.

[43] Choi, Eun Mi, Suh, Kwang Sik, Kim, Young Seol, Choue, Ryo Won, & Koo, Sung Ja. (2001). Soybean ethanol extract increases the function of osteoblastic MC3T3-E1 cells. Phytochemistry, 56(7), 733-739.

[44] Choi, Kyung-Chul, Leung, Peter CK, & Jeung, Eui-Bae. (2005). Biology and physiology of Calbindin-D9k in female reproductive tissues: involvement of steroids and endocrine disruptors. Reproductive Biology and Endocrinology, 3(1), 66.

[45] Cicero, AFG, & Gaddi, A. (2001). Rice Bran Oil and γ-Oryzanol in the Treatment of Hyperlipoproteinaemias and Other Conditions. Phytotherapy Research, 15(4), 277-289.

[46] Compston, Juliet E. (2001). Sex steroids and bone. Physiological reviews, 81(1), 419-448.

[47] Contributors, Wikibooks. (2012). Human Physiology: Blacksleet River.

[48] Cooke, Paul S, Buchanan, David L, Lubahn, Dennis B, & Cunha, Gerald R. (1998). Mechanism of estrogen action: lessons from the estrogen receptor-α knockout mouse. Biology of reproduction, 59(3), 470-475.

[49] Copland, John A, Sheffield-Moore, Melinda, Koldzic-Zivanovic, Nina, Gentry, Sean, Lamprou, George, Tzortzatou-Stathopoulou, Fotini, . . . Vlahopoulos, Spiros A. (2009). Sex steroid receptors in skeletal differentiation and epithelial neoplasia: is tissue-specific intervention possible? Bioessays, 31(6), 629-641.

[50] Cos, Paul, De Bruyne, Tess, Apers, Sandra, Berghe, Dirk Vanden, Pieters, Luc, & Vlietinck, Arnold J. (2003). Phytoestrogens: recent developments. Planta Medica-Natural Products and Medicinal Plant Research, 69(7), 589-599.

[51] Crawford, Lesley A, Bown, Alan W, Breitkreuz, Kevin E, & Guinel, Frédérique C. (1994). The synthesis of [gamma]-aminobutyric acid in response to treatments reducing cytosolic pH. Plant Physiology, 104(3), 865-871.

[52] Cummings, SR. (1996). Treatable and untreatable risk factors for hip fracture. Bone, 18(3), S165-S167.

[53] Cummings, Steven R, & Melton, L Joseph. (2002). Epidemiology and outcomes of osteoporotic fractures. The Lancet, 359(9319), 1761-1767.

[54] Cunningham, Padraig, & Delany, Sarah Jane. (2007). k-Nearest neighbour classifiers. Multiple Classifier Systems, 1-17.

[55] de Aloysio, Domenico, Gambacciani, Marco, Meschia, Michele, Pansini, Francesco, Modena, Alberto Bacchi, Bolis, Pier Francesco, . . . Peruzzi, Elena. (1999). The effect of menopause on blood lipid and lipoprotein levels. Atherosclerosis, 147(1), 147-153.

[56] De Schryver, A, Huys, John, & Vakaet, Luc. (1992). Systemic treatment of early breast-cancer by hormonal, cytotoxic, or immune therapy: 133 randomized trials involving 31000 recurrences and 24000 deaths among 75000 women: 1. Lancet, 339(8784), 1-15.

[57] DE, Wimmer Teresa, Vanadin, Seifert-Klauss, ller Dirk, Mü, Tibor, Schuster, & Katharina, Goppel. (2011).

MENOPAUSE AND OSTEOPOROSIS. Climacteric, 14(1), 57-97.
[58] Deecher, Darlene C. (2005). Physiology of thermoregulatory dysfunction and current approaches to the treatment of vasomotor symptoms. Expert opinion on investigational drugs, 14(4), 435-448.
[59] DEL RY, S. (2001). Increase in circulating levels of cardiac natriuretic peptides after hormone replacement therapy in postmenopausal women. Clinical Science, 101, 447-453.
[60] Deng, Hong-Wen, Chen, Wei-Min, Recker, Susan, Stegman, Mary Ruth, Li, Jin-Long, Davies, K Michael, . . . Recker, Robert R. (2000). Genetic determination of Colles' fracture and differential bone mass in women with and without Colles' fracture. Journal of Bone and Mineral Research, 15(7), 1243-1252.
[61] Drane, HM, Patterson, DSP, Roberts, BA, & Saba, N. (1980). Oestrogenic activity of soya-bean products. Food and Cosmetics Toxicology, 18(4), 425-427.
[62] Driskell, Judy A. (2009). Nutrition and Exercise Concerns of Middle Age: CRC Press.
[63] Duodu, KG, & Belton, PS. CHEMICAL COMPOSITION AND ANTIOXIDANT EFFECTS OF EXTRACTS FROM SORGHUM FLOUR AND BRAN.
[64] Eghbali-Fatourechi, Guitty, Khosla, Sundeep, Sanyal, Arunik, Boyle, William J, Lacey, David L, & Riggs, B Lawrence. (2003). Role of RANK ligand in mediating increased bone resorption in early postmenopausal women. Journal of Clinical Investigation, 111(8), 1221-1230.
[65] Enmark, Eva, Pelto-Huikko, Markku, Grandien, KAJ, Lagercrantz, Svetlana, Lagercrantz, Jacob, Fried, Gabriel, . . . Gustafsson, Jan-Åke. (1997). Human estrogen receptor β-gene structure, chromosomal localization, and expression pattern. Journal of Clinical Endocrinology & Metabolism, 82(12), 4258-4265.
[66] FISCHL, F. Definition AND Pathophysiology.
[67] Fisher, A, Srikusalanukul, W, Davis, M, & Smith, P. (2013). Cardiovascular diseases in older patients with osteoporotic hip fracture: prevalence, disturbances in mineral and bone metabolism, and bidirectional links. Clinical Interventions in Aging, 8, 239.
[68] Fraser, Ian S. (1997). New delivery systems for hormone replacement therapy. Maturitas, 27(1001), 17-17.
[69] Fraser, Ian S, & Mansour, Diana. (2006). Delivery systems for hormone replacement therapy.
[70] Freedman, Robert R. (2001). Physiology of hot flashes. American Journal of Human Biology, 13(4), 453-464.
[71] Freedman, Robert R. (2005). Pathophysiology and treatment of menopausal hot flashes. Paper presented at the Seminars in reproductive medicine.
[72] Freeman, Ellen W, Sammel, Mary D, Lin, Hui, Gracia, Clarisa R, Pien, Grace W, Nelson, Deborah B, & Sheng, Li. (2007). Symptoms associated with menopausal transition and reproductive hormones in midlife women. Obstetrics & Gynecology, 110(2, Part 1), 230-240.
[73] Fujimori, Sayumi, Hinoi, Eiichi, & Yoneda, Yukio. (2002). Functional GABA< sub> B</sub> receptors expressed in cultured calvarial osteoblasts. Biochemical and biophysical research communications, 293(5), 1445-1452.
[74] Furr, BJ. (1984). The pharmacology and clinical uses of tamoxifen. Pharmacol Therapeut, 25, 127-205.
[75] Gapstur, Susan M, Morrow, Monica, & Sellers, Thomas A. (1999). Hormone replacement therapy and risk of breast cancer with a favorable histology. JAMA: the journal of the American Medical Association, 281(22), 2091-2097.
[76] Gaumet, N, Seibel, MJ, Coxam, V, Davicco, MJ, Lebecque, P, & Barlet, JP. (1997). Influence of ovariectomy and estradiol treatment on calcium homeostasis during aging in rats. Archives of physiology and biochemistry, 105(5), 435-444.
[77] Genazzani, Andrea R, Stomati, Massimo, Bernardi, Francesca, Luisi, Stefano, Casarosa, Elena, Puccetti, Simone, . . . Luisi, Michele. (2004). Conjugated equine estrogens reverse the effects of aging on central and peripheral allopregnanolone and beta-endorphin levels in female rats. Fertility and sterility, 81, 757-766.
[78] Ghosh-Choudhury, Nandini, Harris, Marie A, Feng, Jian Q, Mundy, Gregory R, & Harris, Stephen E. (1994). Expression of the BMP 2 gene during bone cell differentiation. Critical Reviews™ in Eukaryotic Gene Expression, 4(2-3).
[79] Gooren, Louis JG, & Bunck, Mathijs CM. (2004). Androgen replacement therapy. Drugs, 64(17), 1861-1891.
[80] Gore, AC, Oung, T, & Woller, MJ. (2002). Age-Related Changes in Hypothalamic Gonadotropin-Releasing Hormone and N-Methyl-d-Aspartate Receptor Gene Expression, and their Regulation by Oestrogen, in the Female Rat. Journal of neuroendocrinology, 14(4), 300-309.
[81] Greendale, Gail A, & Judd, Howard L. (1993). The menopause: health implications and clinical management. Journal of the American Geriatrics Society, 41(4), 426.
[82] Greendale, Gail A, Lee, Nancy P, & Arriola, Edga R. (1999). The menopause. The Lancet, 353(9152), 571-580.
[83] Griffith, PR, & De Haseth, JA. (1986). Fourier transform infrared spectroscopy. Chem. Anal. Ser. Monogr. Anal. Chem. Appl, 83.
[84] Guerriero, Giulia. (2009). Vertebrate sex steroid receptors: evolution, ligands, and neurodistribution. Annals of the New York Academy of Sciences, 1163(1), 154-168.
[85] Guilliams, Thomas G. (2001). MENOPAUSE-A Natural Transition. The Standard, 4(1), 1-12.
[86] Gujral, H Singh, Sharma, P, Bajaj, R, & Solah, V. (2012). Effects of incorporating germinated brown rice on the antioxidant properties of wheat flour chapatti. Food Science and Technology International, 18(1), 47-54.
[87] Hafizah, Wan M, & Supriyanto, Eko. (2012). Automatic Generation of Region of Interest for Kidney Ultrasound Images Using Texture Analysis. International Journal of Biology and Biomedical Engineering, 6, 26-34.
[88] Hagiwara, Hiromi, Seki, Taiichiro, & Ariga, Toyohiko. (2004). The effect of pre-germinated brown rice intake on blood glucose and PAI-1 levels in streptozotocin-induced diabetic rats. Bioscience, biotechnology, and biochemistry, 68(2), 444-447.
[89] Harris, DM, Besselink, E, Henning, SM, Go, VLW, & Heber, D. (2005). Phytoestrogens induce differential estrogen receptor alpha-or beta-mediated responses in transfected breast cancer cells. Experimental biology and medicine, 230(8), 558-568.
[90] Harris, M, Nguyen, TV, Howard, GM, Kelly, PJ, & Eisman, JA. (1998). Genetic and environmental correlations between bone formation and bone mineral density: a twin study. Bone, 22(2), 141-145.
[91] Harrison, Eric, Adjei, Andrew, Ameho, Clement, Yamamoto, Shigeru, & Kono, Shinzo. (1998). The effect of soybean protein on bone loss in a rat model of postmenopausal osteoporosis. Journal of nutritional science and vitaminology, 44(2), 257.
[92] Harvie, Michelle, Howell, Anthony, Vierkant, Robert A, Kumar, Nagi, Cerhan, James R, Kelemen, Linda E, . . . Sellers, Thomas A. (2005). Association of gain and loss of weight before and after menopause with risk of postmenopausal breast cancer in the Iowa women's health study. Cancer Epidemiology Biomarkers & Prevention, 14(3), 656-661.
[93] Hayakawa, T, Suzuki, S, Kobayashi, S, Fukutomi, T, Ide, M, Ohno, T, . . . Nimura, T. (2009). Effect of pre-germinated brown rice on metabolism of glucose and lipid in patients with diabetes mellitus type 2. Journal of the Japanese Association of Rural Medicine, 58.
[94] Henderson, Victor W, Paganini-Hill, Annlia, Emanuel, Christina K, Dunn, Meleana E, & Buckwalter, J Galen. (1994). Estrogen replacement therapy in older women: comparisons between Alzheimer's disease cases and nondemented control subjects. Archives of Neurology, 51(9), 896.
[95] Herzberg, Mala, Foldes, J, Steinberg, R, & Menczel, J. (1990). Zinc excretion in osteoporotic women. Journal of Bone and Mineral Research, 5(3), 251-257.
[96] Herzberg, Mala, Lusky, Ayala, Blonder, Judith, & Frenkel, Yair. (1996). The effect of estrogen replacement therapy on zinc in serum and urine. Obstetrics & Gynecology, 87(6), 1035-1040.
[97] Hewitt, Sylvia Curtis, Deroo, Bonnie J, Hansen, Katherine, Collins, Jennifer, Grissom, Sherry, Afshari, Cynthia A, & Korach, Kenneth S. (2003). Estrogen receptor-dependent genomic responses in the uterus mirror the biphasic physiological response to estrogen. Molecular endocrinology, 17(10), 2070-2083.
[98] Hofbauer, Lorenz C, Khosla, Sundeep, Dunstan, Colin R, Lacey, David L, Spelsberg, Thomas C, & Riggs, B

Lawrence. (1999). Estrogen Stimulates Gene Expression and Protein Production of Osteoprotegerin in Human Osteoblastic Cells*. Endocrinology, 140(9), 4367-4370.

[99] Hu, Yiding, & Kupfer, David. (2002). Enantioselective metabolism of the endocrine disruptor pesticide methoxychlor by human cytochromes P450 (P450s): major differences in selective enantiomer formation by various P450 isoforms. Drug metabolism and disposition, 30(12), 1329-1336.

[100] Huang, Xuehui, Kurata, Nori, Wei, Xinghua, Wang, Zi-Xuan, Wang, Ahong, Zhao, Qiang, . . . Li, Wenjun. (2012). A map of rice genome variation reveals the origin of cultivated rice. Nature, 490(7421), 497-501.

[101] Hutadilok-Towatana, Nongporn, Wattanapiromsakul, Chatchai, & Thammarutwasik, Paiboon. Acute and subchronic toxicity evaluation of the hydroethanolic extract of germinated brown rice.

[102] Iafrati, Mark D, Karas, Richard H, Aronovitz, Mark, Kim, Sung, Sullivan, Theodore R, Lubahn, Dennis B, . . . Mendelsohn, Michael E. (1997). Estrogen inhibits the vascular injury response in estrogen receptor α-deficient mice. Nature medicine, 3(5), 545-548.

[103] Imam, Mustapha Umar, Azmi, Nur Hanisah, Bhanger, Muhammad Iqbal, Ismail, Norsharina, & Ismail, Maznah. (2012). Antidiabetic properties of germinated brown rice: a systematic review. Evidence-Based Complementary and Alternative Medicine, 2012.

[104] Imam, Mustapha Umar, & Ismail, Maznah. (2012). Effects of brown rice and white rice on expression of xenobiotic metabolism genes in type 2 diabetic rats. International journal of molecular sciences, 13(7), 8597-8608.

[105] Imam, Mustapha Umar, & Ismail, Maznah. (2013). Nutrigenomic effects of germinated brown rice and its bioactives on hepatic gluconeogenic genes in type 2 diabetic rats and HEPG2 cells. Molecular nutrition & food research.

[106] Imam, Mustapha Umar, Musa, Siti Nor Asma, Azmi, Nur Hanisah, & Ismail, Maznah. (2012). Effects of white rice, brown rice and germinated brown rice on antioxidant status of type 2 diabetic rats. International journal of molecular sciences, 13(10), 12952-12969.

[107] Ismail, Maznah, Al-Naqeeb, Ghanya, Mamat, WA, & Ahmad, Zalinah. (2010). Gamma-oryzanol rich fraction regulates the expression of antioxidant and oxidative stress related genes in stressed rat's liver. Nutr Metab, 7(23), 1-13.

[108] Ismail, Norsharina, Ismail, Maznah, Farhana Fathy, Siti, Asma Musa, Siti Nor, Umar Imam, Mustapha, Foo, Jhi Biau, & Iqbal, Shahid. (2012). Neuroprotective Effects of Germinated Brown Rice against Hydrogen Peroxide Induced Cell Death in Human SH-SY5Y Cells. International journal of molecular sciences, 13(8), 9692-9708.

[109] ITO, HIRONORI, TAKAHASHI, MASAKO, INOUE, SEIJI, KUSUMOTO, TOMOYUKI, YOSHIDA, TAKASHI, SAWAI, HIDEAKI, . . . YOSHIDA, NOBUTAKA. (2003). Menopause and osteoporosis. Medical Journal of Hiroshima City Hospital, 19(1), 17-19.

[110] Iwaki, Akira, Jingushi, Seiya, Oda, Yoshinao, Izumi, Toshihiro, Shida, Jun-Ichi, Tsuneyoshi, Masazumi, & Sugioka, Yoichi. (1997). Localization and quantification of proliferating cells during rat fracture repair: detection of proliferating cell nuclear antigen by immunohistochemistry. Journal of Bone and Mineral Research, 12(1), 96-102.

[111] Jacobs, DR, Meyer, Katie A, Kushi, Lawrence H, & Folsom, Aaron R. (1998). Whole-grain intake may reduce the risk of ischemic heart disease death in postmenopausal women: the Iowa Women's Health Study. The American journal of clinical nutrition, 68(2), 248-257.

[112] Jagger, CJ, Lean, JM, Davies, JT, & Chambers, TJ. (2005). Tumor necrosis factor-α mediates osteopenia caused by depletion of antioxidants. Endocrinology, 146(1), 113-118.

[113] Jakobs, C, Jaeken, J, & Gibson, KM. (1993). Inherited disorders of GABA metabolism. Journal of inherited metabolic disease, 16(4), 704-715.

[114] Jannoey, Panatda, Niamsup, Hataichanoke, Lumyong, Saisamorn, Suzuki, Toshisada, Katayama, Takeshi, & Chairote, Griangsak. (2010). Comparison of gamma-aminobutyric acid production in Thai rice grains. World Journal of Microbiology and Biotechnology, 26(2), 257-263.

[115] Jensen, J, Nilas, L, & Christiansen, C. (1990). Influence of menopause on serum lipids and lipoproteins. Maturitas, 12(4), 321-331.

[116] JUDD, HOWARD L, MELDRUM, DAVID R, DEFTOS, LEONARD J, & HENDERSON, BRIAN E. (1983). Estrogen replacement therapy: indications and complications. Annals of internal medicine, 98(2), 195-205.

[117] Karladee, D, & Suriyong, S. (2012). γ-Aminobutyric acid (GABA) content in different varieties of brown rice during germination. Science Asia, 38, 13-17.

[118] Kase, Nathan. (1973). Induction of ovulation with clomiphene citrate. Clinical obstetrics and gynecology, 16(3), 192-200.

[119] Kasugai, Shohei, Nagata, Toshihiko, & Sodek, Jaro. (1992). Temporal studies on the tissue compartmentalization of bone sialoprotein (BSP), osteopontin (OPN), and SPARC protein during bone formation in vitro. Journal of cellular physiology, 152(3), 467-477.

[120] Kayahara, Hiroshi, & Tukahara, K. (2000). Flavor. Paper presented at the Health and Nutritional Quality of Pre-Germinated Brown Rice. presented at 2000 International Chemical Congress of Pacific Basin Societies in Hawaii.

[121] Keenan, Michael J, Hegsted, Maren, Jones, Karen L, Delany, James P, Kime, James C, Melancon, Louis E, . . . Hong, Kelly D. (1997). Comparison of bone density measurement techniques: DXA and Archimedes' principle. Journal of Bone and Mineral Research, 12(11), 1903-1907.

[122] Kiing, Sie-Cheong, Yiu, Pang-Hung, Rajan, Amartalingam, & Wong, Sie-Chuong. (2009). Effect of Germination on γ-Oryzanol Content of Selected Sarawak Rice Cultivars. American Journal of Applied Sciences, 6(9), 1658-1661.

[123] Kim, SEOK JOONG, Han, DAESEOK, Moon, KWANG DEOG, & Rhee, JOON SHICK. (1995). Measurement of superoxide dismutase-like activity of natural antioxidants. Bioscience, biotechnology, and biochemistry, 59(5), 822.

[124] Komatsuzaki, Noriko, Tsukahara, Kikuichi, Toyoshima, Hidechika, Suzuki, Tadanao, Shimizu, Naoto, & Kimura, Toshinori. (2007). Effect of soaking and gaseous treatment on GABA content in germinated brown rice. Journal of Food Engineering, 78(2), 556-560.

[125] Komori, T, Yagi, H, Nomura, S, Yamaguchi, A, Sasaki, K, Deguchi, K, . . . Inada, M. (1997). Targeted Disruption of< i> Cbfa1</i> Results in a Complete Lack of Bone Formation owing to Maturational Arrest of Osteoblasts. Cell, 89(5), 755-764.

[126] Koshiyama, M, Konishi, I, Nanbu, K, Nanbu, Y, Mandai, M, Komatsu, T, . . . Fujii, S. (1995). Immunohistochemical localization of heat shock proteins HSP70 and HSP90 in the human endometrium: correlation with sex steroid receptors and Ki-67 antigen expression. Journal of Clinical Endocrinology & Metabolism, 80(4), 1106-1112.

[127] Kuiper, George GJM, Carlsson, BO, Grandien, KAJ, Enmark, Eva, Häggblad, Johan, Nilsson, Stefan, & Gustafsson, Jan-Åke. (1997). Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors α and β. Endocrinology, 138(3), 863-870.

[128] Kuiper, George GJM, Lemmen, Josephine G, Carlsson, BO, Corton, J Christopher, Safe, Stephen H, van der Saag, Paul T, . . . Gustafsson, Jan-Åke. (1998). Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor β. Endocrinology, 139(10), 4252-4263.

[129] Lai, Ming-Derg, Lee, LR, Cheng, KS, & Wing, LY. (2000). Expression of proliferating cell nuclear antigen in luminal epithelium during the growth and regression of rat uterus. Journal of endocrinology, 166(1), 87-93.

[130] Latifah, Saiful Yazan, Armania, Nurdin, Tze, Tan Hern, Azhar, Yaacob, Nordiana, Abdul Hadi, Norazalina, Saad, . . . Maznah, Ismail. (2010). Research Germinated brown rice (GBR) reduces the incidence of aberrant crypt foci with the involvement of β-catenin and COX-2 in azoxymethane-induced colon cancer in rats.

[131] Lean, Jenny M, Davies, Julie T, Fuller, Karen, Jagger, Christopher J, Kirstein, Barrie, Partington, Geoffrey A, . . . Chambers, Timothy J. (2003). A crucial role for thiol antioxidants in estrogen-deficiency bone loss. Journal of Clinical Investigation, 112(6), 915-923.

[132] Lean, Jenny M, Jagger, Chris J, Kirstein, Barrie, Fuller, Karen, & Chambers, Timothy J. (2005). Hydrogen peroxide is essential for estrogen-deficiency bone loss and osteoclast formation. Endocrinology, 146(2), 728-735.

[133] LEE, IAN R, DAWSON, STEPHEN A, WETHERALL, JOHN D, & HAHNEL, ROLAND. (1987). Sex hormone-binding globulin secretion by human hepatocarcinoma cells

is increased by both estrogens and androgens. Journal of Clinical Endocrinology & Metabolism, 64(4), 825-831.
[134] Lee, Jin Hwan. (2010). Changes in nutritional components throughout germination in paddy rice and brown rice. Journal of Food Science and Nutrition, 15(2), 113-119.
[135] Lerma-García, MJ, Herrero-Martínez, JM, Simó-Alfonso, EF, Mendonça, Carla RB, & Ramis-Ramos, G. (2009). Composition, industrial processing and applications of rice bran γ-oryzanol. Food Chemistry, 115(2), 389-404.
[136] Letchuman, GR, Wan Nazaimoon, WM, Wan Mohamad, WB, Chandran, LR, Tee, GH, Jamaiyah, H, . . . Ahmad Faudzi, Y. (2010). Prevalence of diabetes in the Malaysian national health morbidity survey III 2006. Med J Malaysia, 65(3), 180-186.
[137] Li, H, Cho, JY, Gao, TC, Choi, CR, Lee, KD, Cho, JE, . . . Ham, KS. (2008). Increment of physiologically active compounds in germinated brown rice treated with chitosan and its effect on obesity of rat fed a high fat diet. Journal of the Korean Society of Food Science and Nutrition, 37.
[138] Liu, Simin, Manson, JE, Stampfer, MJ, Hu, FB, Giovannucci, E, Colditz, GA, . . . Willett, WC. (2000). A prospective study of whole-grain intake and risk of type 2 diabetes mellitus in US women. American journal of public health, 90(9), 1409.
[139] Liyana-Pathirana, Chandrika M, & Shahidi, Fereidoon. (2006). Importance of insoluble-bound phenolics to antioxidant properties of wheat. Journal of agricultural and food chemistry, 54(4), 1256-1264.
[140] Lock, Margaret. (2002). Symptom reporting at menopause: a review of cross-cultural findings. Menopause International, 8(4), 132-136.
[141] MacGregor, FB, Abernethy, VE, Dahabra, S, Cobden, I, & Hayes, PC. (1989). Hepatotoxicity of herbal remedies. BMJ: British Medical Journal, 299(6708), 1156.
[142] Maggio, Dario, Barabani, Mauro, Pierandrei, Marco, Polidori, M Cristina, Catani, Marco, Mecocci, Patrizia, . . . Cherubini, Antonio. (2003). Marked decrease in plasma antioxidants in aged osteoporotic women: results of a cross-sectional study. Journal of Clinical Endocrinology & Metabolism, 88(4), 1523-1527.
[143] Mamiya, Takayoshi, Asanuma, Takamasa, Kise, Mitsuo, Ito, Yukihiko, Mizukuchi, Aya, Aoto, Hiromichi, & Ukai, Makoto. (2004). Effects of Pre-Germinated Brown Rice on. BETA.-Amyloid Protein-Induced Learning and Memory Deficits in Mice. Biological and Pharmaceutical Bulletin, 27(7), 1041-1045.
[144] Mandai, Masaki, Yamaguchi, Ken, Matsumura, Noriomi, Baba, Tsukasa, & Konishi, Ikuo. (2009). Ovarian cancer in endometriosis: molecular biology, pathology, and clinical management. International Journal of Clinical Oncology, 14(5), 383-391.
[145] Manyam, Bala V, Katz, Leonard, Hare, Theodore A, Kaniefski, Kathryn, & Tremblay, Robert D. (1981). Isoniazid-induced elevation of CSF GABA levels and effects on chorea in huntington's disease. Annals of neurology, 10(1), 35-37.
[146] Marie, Pierre J, Hott, Monique, Modrowski, Dominique, De Pollak, Cinderella, Guillemain, Joel, Deloffre, Pascale, & Tsouderos, Yannis. (2005). An uncoupling agent containing strontium prevents bone loss by depressing bone resorption and maintaining bone formation in estrogen-deficient rats. Journal of Bone and Mineral Research, 20(6), 1065-1074.
[147] Melton III, LJ. (1996). Epidemiology of hip fractures: implications of the exponential increase with age. Bone, 18(3), S121-S125.
[148] Metwally, AM, Habib, AM, & Khafagy, SM. (2009). Sterols and triterpene alcohols from rice bran oil. Planta medica, 25(01), 68-72.
[149] Mitlak, Bruce H, & Cohen, Fredric J. (1999). Selective estrogen receptor modulators. Drugs, 57(5), 653-663.
[150] Miura, Daiki, Ito, Yukihiko, Mizukuchi, Aya, Kise, Mitsuo, Aoto, Hiromichi, & Yagasaki, Kazumi. (2006). Hypocholesterolemic action of pre-germinated brown rice in hepatoma-bearing rats. Life sciences, 79(3), 259-264.
[151] Molina, Jeanmaire, Sikora, Martin, Garud, Nandita, Flowers, Jonathan M, Rubinstein, Samara, Reynolds, Andy, . . . Bustamante, Carlos D. (2011). Molecular evidence for a single evolutionary origin of domesticated rice. Proceedings of the National Academy of Sciences, 108(20), 8351-8356.
[152] Mook, Douglas G, Kenney, Nancy J, Roberts, Susan, Nussbaum, Alan I, & Rodier 3rd, WI. (1972). Ovarian-adrenal interactions in regulation of body weight by female rats. Journal of comparative and physiological psychology, 81(2), 198.
[153] Moonga, Baljit S, & Dempster, David W. (1995). Zinc is a potent inhibitor of osteoclastic bone resorption in vitro. Journal of Bone and Mineral Research, 10(3), 453-457.
[154] Mosmann, Tim. (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. Journal of immunological methods, 65(1), 55-63.
[155] Muhammad, Sani I, Ismail, Maznah, Mahmud, Rozi B, Salisu, Abubakar M, & Zakaria, Zuki A. (2013). Germinated brown rice and its bioactives modulate the activity of uterine cells in oophorectomised rats as evidenced by gross cytohistological and immunohistochemical changes. BMC complementary and alternative medicine, 13(1), 198.
[156] Musa, Asma SN, Umar, Imam Mustapha, & Ismail, Maznah. (2011). Physicochemical properties of germinated brown rice (Oryza sativa L.) starch. African Journal of Biotechnology, 10(33), 6281-6291.
[157] Muthusami, Sridhar, Ramachandran, Ilangovan, Muthusamy, Balaganesh, Vasudevan, Gopalakrishnan, Prabhu, Venkataraman, Subramaniam, Veni, . . . Narasimhan, Srinivasan. (2005). Ovariectomy induces oxidative stress and impairs bone antioxidant system in adult rats. Clinica Chimica Acta, 360(1), 81-86.
[158] Nabulsi, Azmi A, Folsom, Aaron R, White, Alice, Patsch, Wolfgang, Heiss, Gerardo, Wu, Kenneth K, & Szklo, Moyses. (1993). Association of hormone-replacement therapy with various cardiovascular risk factors in postmenopausal women. New England Journal of Medicine, 328(15), 1069-1075.
[159] Naciff, Jorge M, Jump, M Lynn, Torontali, Suzanne M, Carr, Gregory J, Tiesman, Jay P, Overmann, Gary J, & Daston, George P. (2002). Gene expression profile induced by 17α-ethynyl estradiol, bisphenol A, and genistein in the developing female reproductive system of the rat. Toxicological Sciences, 68(1), 184-199.
[160] Newby, P Kristen, Muller, Denis, Hallfrisch, Judith, Qiao, Ning, Andres, Reubin, & Tucker, Katherine L. (2003). Dietary patterns and changes in body mass index and waist circumference in adults. The American journal of clinical nutrition, 77(6), 1417-1425.
[161] Nguyen, Thi Hoa, Lee, Geun-Shik, Ji, Youn-Kyu, Choi, Kyung-Chul, Lee, Chang-Kyu, & Jeung, Eui-Bae. (2005). A calcium binding protein, calbindin-D9k, is mainly regulated by estrogen in the pituitary gland of rats during estrous cycle. Molecular brain research, 141(2), 166-173.
[162] Nys, Y, Baker, K, & Lawson, DEM. (1992). Estrogen and a calcium flux dependent factor modulate the calbindin gene expression in the uterus of laying hens. General and comparative endocrinology, 87(1), 87-94.
[163] Oh, Chan-Ho, & Oh, Suk-Heung. (2004). Effects of germinated brown rice extracts with enhanced levels of GABA on cancer cell proliferation and apoptosis. Journal of medicinal food, 7(1), 19-23.
[164] Omoto, Yoko, Kobayashi, Yasuhito, Nishida, Kazunori, Tsuchiya, Eiju, Eguchi, Hidetaka, Nakagawa, Ken, . . . Fujii, Yoshitaka. (2001). Expression, function, and clinical implications of the estrogen receptor β in human lung cancers. Biochemical and biophysical research communications, 285(2), 340-347.
[165] Oseni, Tawakalitu, Patel, Roshani, Pyle, Jennifer, & Jordan, V Craig. (2008). Selective estrogen receptor modulators and phytoestrogens. Planta medica, 74(13), 1656.
[166] Otto, Florian, Thornell, Anders P, Crompton, Tessa, Denzel, Angela, Gilmour, Kimberly C, Rosewell, Ian R, . . . Olsen, Bjorn R. (1997). < i> Cbfa1</i>, a Candidate Gene for Cleidocranial Dysplasia Syndrome, Is Essential for Osteoblast Differentiation and Bone Development. Cell, 89(5), 765-771.
[167] Owen, Maureen, & Friedenstein, AJ. (1988). Stromal stem cells: marrow-derived osteogenic precursors. Cell and molecular biology of vertebrate hard tissues, 136, 42-60.
[168] Owen, Thomas A, Aronow, Michael, Shalhoub, Victoria, Barone, Leesa M, Wilming, Laurens, Tassinari, Melissa S, . . . Stein, Gary S. (1990). Progressive development of the rat osteoblast phenotype in vitro: reciprocal relationships in

expression of genes associated with osteoblast proliferation and differentiation during formation of the bone extracellular matrix. Journal of cellular physiology, 143(3), 420-430.

[169] Palacios, S, Henderson, VW, Siseles, N, Tan, D, & Villaseca, P. (2010). Age of menopause and impact of climacteric symptoms by geographical region. Climacteric, 13(5), 419-428.

[170] Panzer, Claudia, & Guay, André. (2009). Continuing Medical Education: Testosterone Replacement Therapy in Naturally and Surgically Menopausal Women (CME). The journal of sexual medicine, 6(1), 8-18.

[171] Papaconstantinou, Andriana D, Fisher, Benjamin R, Umbreit, Thomas H, Goering, Peter L, Lappas, Nicholas T, & Brown, Ken M. (2001). Effects of β-estradiol and bisphenol A on heat shock protein levels and localization in the mouse uterus are antagonized by the antiestrogen ICI 182,780. Toxicological Sciences, 63(2), 173-180.

[172] Papoutsi, Zoi, Kassi, Eva, Fokialakis, Nikolas, Mitakou, Sofia, Lambrinidis, George, Mikros, Emmanuel, & Moutsatsou, Paraskevi. (2007). Deoxybenzoins are novel potent selective estrogen receptor modulators. Steroids, 72(9), 693-704.

[173] Patel, M, & Naik, SN. (2004). Gamma-oryzanol from rice bran oil: a review. J Sci Ind Res India, 63, 569-578.

[174] Patisaul, Heather B, Whitten, Patricia L, & Young, Larry J. (1999). Regulation of estrogen receptor beta mRNA in the brain: opposite effects of 17β-estradiol and the phytoestrogen, coumestrol. Molecular brain research, 67(1), 165-171.

[175] Perret, Christine, L'Horset, Fabienne, & Thomasset, Monique. (1991). DNase I-hypersensitive sites are associated, in a tissue-specific manner, with expression of the calbindin-D9k-encoding gene. Gene, 108(2), 227-235.

[176] Pfeilschifter, Johannes, Köditz, Roland, Pfohl, Martin, & Schatz, Helmut. (2002). Changes in proinflammatory cytokine activity after menopause. Endocrine reviews, 23(1), 90-119.

[177] Philosophe, R, & Seibel, MM. (1991). Menopause and cardiovascular disease. NAACOG's clinical issues in perinatal and women's health nursing, 2(4), 441.

[178] Powers, MICHAEL E, Yarrow, JOSHUA F, McCoy, SEAN C, & Borst, STEPHEN E. (2008). Growth hormone isoform responses to GABA ingestion at rest and after exercise. Medicine and science in sports and exercise, 40(1), 104.

[179] Powles, Trevor J, Hickish, Tamas, Kanis, John A, Tidy, Alwynne, & Ashley, Sue. (1996). Effect of tamoxifen on bone mineral density measured by dual-energy x-ray absorptiometry in healthy premenopausal and postmenopausal women. Journal of Clinical Oncology, 14(1), 78-84.

[180] Quarles, L Darryl, Yohay, Daniel A, Lever, Laura W, Caton, Rashmi, & Wenstrup, Richard J. (1992). Distinct proliferative and differentiated stages of murine MC3T3-E1 cells in culture: An in vitro model of osteoblast development. Journal of Bone and Mineral Research, 7(6), 683-692.

[181] Raisz, Lawrence G. (2005). Pathogenesis of osteoporosis: concepts, conflicts, and prospects. Journal of Clinical Investigation, 115(12), 3318-3325.

[182] Raz, Raul. (2001). Hormone replacement therapy or prophylaxis in postmenopausal women with recurrent urinary tract infection. Journal of Infectious Diseases, 183(Supplement 1), S74-S76.

[183] Reiswig, JD, Frazer, GS, & Inpanbutr, N. (1995). Calbindin-D9k expression in the pregnant cow uterus and placenta. Histochemistry and cell biology, 104(2), 169-174.

[184] Renn, Joerg, Schaedel, Marianne, Volff, Jean-Nicolas, Goerlich, Roland, Schartl, Manfred, & Winkler, Christoph. (2006). Dynamic expression of< i> sparc</i> precedes formation of skeletal elements in the Medaka (< i> Oryzias latipes</i>). Gene, 372, 208-218.

[185] Riggs, B Lawrence, Khosla, Sundeep, & Melton, L Joseph. (2002). Sex steroids and the construction and conservation of the adult skeleton. Endocrine reviews, 23(3), 279-302.

[186] Rivera-Gonzalez, Ramon, Petersen, Donna N, Tkalcevic, George, Thompson, David D, & Brown, Thomas A. (1998). Estrogen-induced genes in the uterus of ovariectomized rats and their regulation by droloxifene and tamoxifen. The Journal of Steroid Biochemistry and Molecular Biology, 64(1-2), 13-24.

[187] Roohinejad, Shahin, Omidizadeh, Alireza, Mirhosseini, Hamed, Saari, Nazamid, Mustafa, Shuhaimi, Meor Hussin, Anis Shobirin, . . . Abd Manap, Mohd Yazid. (2011). Effect of pre-germination time on amino acid profile and gamma amino butyric acid (GABA) contents in different varieties of Malaysian brown rice. International Journal of Food Properties, 14(6), 1386-1399.

[188] Rosenkranz, K, Hinney, A, Ziegler, A, Hermann, H, Fichter, M, Mayer, H, . . . Hebebrand, J. (1998). Systematic mutation screening of the estrogen receptor beta gene in probands of different weight extremes: identification of several genetic variants. Journal of Clinical Endocrinology & Metabolism, 83(12), 4524-4524.

[189] Ross, Ronald K, Paganini-Hill, Annlia, Wan, Peggy C, & Pike, Malcolm C. (2000). Effect of hormone replacement therapy on breast cancer risk: estrogen versus estrogen plus progestin. Journal of the National Cancer Institute, 92(4), 328-332.

[190] Rossmanith, Winfried G, & Ruebberdt, Wiebke. (2009). What causes hot flushes? The neuroendocrine origin of vasomotor symptoms in the menopause. Gynecological Endocrinology, 25(5), 303-314.

[191] Roy, Edward J, & Wade, George N. (1977). Role of food intake in estradiol-induced body weight changes in female rats. Hormones and behavior, 8(3), 265-274.

[192] Rozan, Pascale, Kuo, Yu-Haey, & Lambein, Fernand. (2000). Free amino acids present in commercially available seedlings sold for human consumption. A potential hazard for consumers. Journal of agricultural and food chemistry, 48(3), 716-723.

[193] Rubin, Carl-Johan, Lindberg, Johan, Fitzsimmons, Carolyn, Savolainen, Peter, Jensen, Per, Lundeberg, Joakim, . . . Kindmark, Andreas. (2007). Differential gene expression in femoral bone from red junglefowl and domestic chicken, differing for bone phenotypic traits. BMC genomics, 8(1), 208.

[194] Sani, Ismaila Muhammad, Iqbal, Shahid, Chan, Kim Wei, & Ismail, Maznah. (2012). Effect of acid and base catalyzed hydrolysis on the yield of phenolics and antioxidant activity of extracts from Germinated Brown Rice (GBR). Molecules, 17(6), 7584-7594.

[195] Saunders, PT, Fisher, JS, Sharpe, RM, & Millar, MR. (1998). Expression of oestrogen receptor beta (ER beta) occurs in multiple cell types, including some germ cells, in the rat testis. Journal of endocrinology, 156(3), R13-R17.

[196] Sawaddiwong, Rutairat, Jongjareonrak, Akkasit, & Benjakul, Soottawat. (2008). Phenolic content and antioxidant activity of germinated brown rice as affected by germination temperature and extraction solvent. KMITL Science Journal, 8, 45-49.

[197] Scholz, Gunthard, Windeisen, Elisabeth, Liebner, Falk, Baucker, E, & Bues, Claus-Thomas. (2010). Wood anatomical features and chemical composition of Prosopis kuntzei from the Paraguayan Chaco. IAWA J, 31, 39-52.

[198] Schroeder, Andreas, Mueller, Odilo, Stocker, Susanne, Salowsky, Ruediger, Leiber, Michael, Gassmann, Marcus, . . . Ragg, Thomas. (2006). The RIN: an RNA integrity number for assigning integrity values to RNA measurements. BMC molecular biology, 7(1), 3.

[199] Seetharamaiah, GS, & Prabhakar, JV. (1986). Oryzanol content of Indian rice bran oil and its extraction from soap stock. Journal of food science and technology, 23(5), 270-273.

[200] Seo, Hyun-Ju, Cho, Young-Eun, Kim, Taewan, Shin, Hong-In, & Kwun, In-Sook. (2010). Zinc may increase bone formation through stimulating cell proliferation, alkaline phosphatase activity and collagen synthesis in osteoblastic MC3T3-E1 cells. Nutrition research and practice, 4(5), 356-361.

[201] Setchell, Kenneth DR, & Cassidy, Aedin. (1999). Dietary isoflavones: biological effects and relevance to human health. The journal of nutrition, 129(3), 758S-767S.

[202] Sgroi, Dennis C, Teng, Sarena, Robinson, Greg, LeVangie, Rebbecca, Hudson, James R, & Elkahloun, Abdel G. (1999). In vivo gene expression profile analysis of human breast cancer progression. Cancer research, 59(22), 5656-5661.

[203] Shughrue, Paul J, Lane, Malcolm V, & Merchenthaler, Istvan. (1997). Comparative distribution of estrogen receptor-α and-β mRNA in the rat central nervous system. Journal of Comparative Neurology, 388(4), 507-525.

[204] Shuster, Lynne T, Rhodes, Deborah J, Gostout, Bobbie S, Grossardt, Brandon R, & Rocca, Walter A. (2010). Premature menopause or early menopause: long-term health consequences. Maturitas, 65(2), 161-166.

[205] Simkin-Silverman, Laurey R, Wing, Rena R, Boraz, Miriam A, & Kuller, Lewis H. (2003). Lifestyle intervention can prevent weight gain during menopause: results from a 5-year randomized clinical trial. Annals of Behavioral Medicine, 26(3), 212-220.

[206] Simoncini, Tommaso, & Genazzani, Andrea R. (2003). Non-genomic actions of sex steroid hormones. European Journal of Endocrinology, 148(3), 281-292.

[207] Singh, Amrit Pal. (2005). Promising Phytochemicals from Indian Medicinal Plants. Ethnobotanical Leaflets, 2005(1), 18.

[208] Songtip, Parisut, Jangchud, Kamolwan, Jangchud, Anuvat, & Tungtrakul, Patcharee. (2012). Physicochemical property changes in germinated brown rice flour from different storage periods of paddy rice. International Journal of Food Science & Technology, 47(4), 682-688.

[209] Sooriyamoorthy, M, & Gower, DB. (1989). Hormonal influences on gingival tissue: relationship to periodontal disease. Journal of Clinical Periodontology, 16(4), 201-208.

[210] Stahl, Sarah, Chun, Tae-Yon, & Gray, Wesley G. (1998). Phytoestrogens act as estrogen agonists in an estrogen-responsive pituitary cell line. Toxicology and applied pharmacology, 152(1), 41-48.

[211] Stearns, Vered, Ullmer, Lynda, Lopez, Juan F, Smith, Yolanda, Isaacs, Claudine, & Hayes, Daniel F. (2002). Hot flushes. The Lancet, 360(9348), 1851-1861.

[212] Stein, Gary S, Lian, JANE B, & Owen, THOMAS A. (1990). Relationship of cell growth to the regulation of tissue-specific gene expression during osteoblast differentiation. The FASEB journal, 4(13), 3111-3123.

[213] Suchowersky, Oksana, & Muthipeedika, Joy. (2005). A case of late-onset chorea. Nature Clinical Practice Neurology, 1(2), 113-116.

[214] Suh, Seok-Jong, Yun, Woo-Sik, Kim, Kap-Sung, Jin, Un-Ho, Kim, June-Ki, Kim, Myung-Sunny, . . . Kim, Cheorl-Ho. (2007). Stimulative effects of< i> Ulmus davidiana</i> Planch (Ulmaceae) on osteoblastic MC3T3-E1 cells. Journal of Ethnopharmacology, 109(3), 480-485.

[215] Sundstrom, Susan A, Komm, BS, Ponce-de-Leon, Helga, Yi, Zheng, Teuscher, Cory, & Lyttle, CR. (1989). Estrogen regulation of tissue-specific expression of complement C3. Journal of Biological Chemistry, 264(28), 16941-16947.

[216] Takahata, Yoshifumi, Takarada, Takeshi, Hinoi, Eiichi, Nakamura, Yukari, Fujita, Hiroyuki, & Yoneda, Yukio. (2011). Osteoblastic γ-aminobutyric acid, type B receptors negatively regulate osteoblastogenesis toward disturbance of osteoclastogenesis mediated by receptor activator of nuclear factor κB ligand in mouse bone. Journal of Biological Chemistry, 286(38), 32906-32917.

[217] Thakur, MK, & Paramanik, V. (2009). Role of steroid hormone coregulators in health and disease. Hormone Research in Paediatrics, 71(4), 194-200.

[218] Tham, Doris M, Gardner, Christopher D, & Haskell, William L. (1998). Potential health benefits of dietary phytoestrogens: a review of the clinical, epidemiological, and mechanistic evidence. Journal of Clinical Endocrinology & Metabolism, 83(7), 2223-2235.

[219] Tian, Su, Nakamura, Kozo, & Kayahara, Hiroshi. (2004). Analysis of phenolic compounds in white rice, brown rice, and germinated brown rice. Journal of agricultural and food chemistry, 52(15), 4808-4813.

[220] Tsay, Jaime, Yang, Zheiwei, Ross, F Patrick, Cunningham-Rundles, Susanna, Lin, Hong, Coleman, Rhima, . . . Giardina, Patricia J. (2010). Bone loss caused by iron overload in a murine model: importance of oxidative stress. Blood, 116(14), 2582-2589.

[221] Tsypin, Maxim, & Röder, Heinrich. On the Reliability of kNN Classification. Lecture Notes in Engineering and Computer Science, 2167.

[222] Tyler, Varro E. (1994). Herbs of choice: the therapeutic use of phytomedicinals: Pharmaceutical Products Press (imprint of Haworth Press, Inc.).

[223] Ueda, Takako, & Armstrong, D. (2009). Preventive effect of natural and synthetic antioxidants on lipid peroxidation in the mammalian eye. Ophthalmic research, 28(3), 184-192.

[224] Ushiyama, T, Ueyama, H, Inoue, K, Ohkubo, I, & Hukuda, S. (1999). Expression of genes for estrogen receptors α and β in human articular chondrocytes. Osteoarthritis and cartilage, 7(6), 560-566.

[225] Usuki, Seigo, Ariga, Toshio, Dasgupta, Somsankar, Kasama, Takeshi, Morikawa, Keiko, Nonaka, Shota, . . . Robert, K Yu. (2008). Structural analysis of novel bioactive acylated steryl glucosides in pre-germinated brown rice bran. Journal of lipid research, 49(10), 2188-2196.

[226] Usuki, Seigo, Ito, Yukihiko, Morikawa, Keiko, Kise, Mitsuo, Ariga, Toshio, Rivner, Michael, & Yu, Robert K. (2007). Effect of pre-germinated brown rice intake on diabetic neuropathy in streptozotocin-induced diabetic rats. Nutrition & metabolism, 4(1), 25.

[227] Van der Graaf, Y, de Kleijn, MJ J, & Schouw, YT van der. (1997). Menopause and cardiovascular disease. Journal of Psychosomatic Obstetrics & Gynecology, 18(2), 113-120.

[228] Van Nguyen, Nguu, & Ferrero, Aldo. (2006). Meeting the challenges of global rice production. Paddy and Water Environment, 4(1), 1-9.

[229] Veldhuis, Johannes D, Evans, William S, Shah, Nikhita, Story, E Shannon, Bray, Megan J, & Anderson, Stacey M. (1999). Proposed Mechanisms of Sex-Steroid—Hormone Neuromodulation of the Human GH—IGF-I Axis Sex-Steroid Interactions with Growth Hormone (pp. 93-121): Springer.

[230] Vincent, Ann, & Fitzpatrick, Lorraine A. (2000). Soy isoflavones: are they useful in menopause? Paper presented at the Mayo Clinic Proceedings.

[231] Vogel, Victor G, Costantino, Joseph P, Wickerham, D Lawrence, Cronin, Walter M, Cecchini, Reena S, Atkins, James N, . . . Wade, James L. (2010). Update of the national surgical adjuvant breast and bowel project study of tamoxifen and raloxifene (STAR) P-2 trial: preventing breast cancer. Cancer Prevention Research, 3(6), 696-706.

[232] Vogel, Victor G, Costantino, Joseph P, Wickerham, D Lawrence, Cronin, Walter M, Cecchini, Reena S, Atkins, James N, . . . Wade III, James L. (2006). Effects of tamoxifen vs raloxifene on the risk of developing invasive breast cancer and other disease outcomes. JAMA: the journal of the American Medical Association, 295(23), 2727-2741.

[233] Voss, Meike R, Stallone, John N, Li, Min, Cornelussen, Richard NM, Knuefermann, Pascal, & Knowlton, Anne A. (2003). Gender differences in the expression of heat shock proteins: the effect of estrogen. American Journal of Physiology-Heart and Circulatory Physiology, 285(2), H687-H692.

[234] Vural, Perin, Akgül, Cemil, & Canbaz, Mukaddes. (2005). Effects of menopause and tibolone on antioxidants in postmenopausal women. Annals of clinical biochemistry, 42(3), 220-223.

[235] Wang, Christina, & Swerdloff, Ronald S. (1997). Androgen replacement therapy. Annals of medicine, 29(5), 365-370.

[236] Wang, Elizabeth A, Rosen, Vicki, D'Alessandro, Josephine S, Bauduy, Marc, Cordes, Paul, Harada, Tomoko, . . . LaPan, Peter. (1990). Recombinant human bone morphogenetic protein induces bone formation. Proceedings of the National Academy of Sciences, 87(6), 2220-2224.

[237] Wang, Huei-ju, & Murphy, Patricia A. (1994). Isoflavone content in commercial soybean foods. Journal of agricultural and food chemistry, 42(8), 1666-1673.

[238] Wang, Weizhuo, Olson, Douglas, Cheng, Bin, Guo, Xiong, & Wang, Kunzheng. (2012). Sanguis Draconis resin stimulates osteoblast alkaline phosphatase activity and mineralization in MC3T3-E1 cells. Journal of Ethnopharmacology.

[239] Ward, Eric J, Parsons, Kim, Holmes, Elizabeth E, Balcomb III, Ken C, Ford, John KB, Altenburger, A, . . . Gunz, P. (2009). The role of menopause and reproductive senescence in a long-lived social mammal. Frontiers in zoology, 6(4).

[240] Warren, Michelle P, Shortle, Barbara, & Dominguez, Jennifer E. (2002). Use of alternative therapies in menopause. Best Practice & Research Clinical Obstetrics & Gynaecology, 16(3), 411-448.

[241] Watanabe, Masahito, Maemura, Kentaro, Kanbara, Kiyoto, Tamayama, Takumi, & Hayasaki, Hana. (2002). GABA and GABA receptors in the central nervous system and other organs. International review of cytology, 213, 1-47.

[242] Watts, Nelson B, Notelovitz, Morris, Chrystie Timmons, M, Allen Addison, W, Wiita, Brinda, & Downey, Laurence J.

(1995). Comparison of oral estrogens and estrogens plus androgen on bone mineral density, menopausal symptoms, and lipid-lipoprotein profiles in surgical menopause. Obstetrics & Gynecology, 85(4), 529-537.

[243] Weihua, Zhang, Saji, Shigehira, Mäkinen, Sirpa, Cheng, Guojun, Jensen, Elwood V, Warner, Margaret, & Gustafsson, Jan-Åke. (2000). Estrogen receptor (ER) β, a modulator of ERα in the uterus. Proceedings of the National Academy of Sciences, 97(11), 5936-5941.

[244] Williams, J Koudy, Honoré, Erika K, Washburn, Scott A, & Clarkson, Thomas B. (1994). Effects of hormone replacement therapy on reactivity of atherosclerotic coronary arteries in cynomolgus monkeys. Journal of the American College of Cardiology, 24(7), 1757-1761.

[245] Wing, Rena R, Matthews, Karen A, Kuller, Lewis H, Meilahn, Elaine N, & Plantinga, Pam L. (1991). Weight gain at the time of menopause. Archives of internal medicine, 151(1), 97.

[246] Witteveen-Lane, Caralee. (2008). Interactive Journey Through the Innerbody Web Site. Journal of Consumer Health on the Internet, 12(4), 361-371.

[247] Wong, Li Ping, & Nur Liyana, AH. (2007). A survey of knowledge and perceptions of menopause among young to middle aged women in federal territory, Kuala Lumpur, Malaysia. JUMMEC JUMMEC, 22.

[248] Wong, Ting, Guin, C, Bottiglieri, Teodoro, & Snead, O Carter. (2003). GABA, γ-hydroxybutyric acid, and neurological disease. Annals of neurology, 54(S6), S3-S12.

[249] Wu, Qingli, Wang, Mingfu, & Simon, James E. (2004). Analytical methods to determine phytoestrogenic compounds. Journal of Chromatography B, 812(1), 325-355.

[250] Wu, Xindong, Kumar, Vipin, Quinlan, J Ross, Ghosh, Joydeep, Yang, Qiang, Motoda, Hiroshi, . . . Philip, S Yu. (2008). Top 10 algorithms in data mining. Knowledge and Information Systems, 14(1), 1-37.

[251] Xiang, Mei-Xian, Xu, Zong, Su, Han-Wen, Hu, Jinyue, & Yan, Yun-Jun. (2011). Emodin-8-O-β-D-Glucoside from Polygonum Amplexicaule D. Don var. Sinense Forb. Promotes Proliferation and Differentiation of Osteoblastic MC3T3-E1 Cells. Molecules, 16(1), 728-737.

[252] Yahyaoui, R, Esteva, I, Haro-Mora, JJ, Almaraz, MC, Morcillo, S, Rojo-Martínez, G, . . . Hernando, V. (2008). Effect of long-term administration of cross-sex hormone therapy on serum and urinary uric acid in transsexual persons. Journal of Clinical Endocrinology & Metabolism, 93(6), 2230-2233.

[253] Yamaguchi, Masayoshi. (1998). Role of zinc in bone formation and bone resorption. The Journal of Trace Elements in Experimental Medicine, 11(2-3), 119-135.

[254] Yamaguchi, Masayoshi, Oishi, Hidetoshi, & Suketa, Yasunobu. (1987). Stimulatory effect of zinc on bone formation in tissue culture. Biochemical pharmacology, 36(22), 4007-4012.

[255] Yan, Chengxin, Sang, Nong, & Zhang, Tianxu. (2003). Local entropy-based transition region extraction and thresholding. Pattern Recognition Letters, 24(16), 2935-2941.

[256] Yin, Weiling, & Gore, Andrea C. (2006). Neuroendocrine control of reproductive aging: roles of GnRH neurons. Reproduction, 131(3), 403-414.

[257] Zhang, Chi, Tang, Wanjin, Li, Yang, Yang, Fan, Dowd, Diane R, & MacDonald, Paul N. (2011). Osteoblast-specific transcription factor Osterix increases vitamin D receptor gene expression in osteoblasts. PloS one, 6(10), e26504.

[258] Zhang, Geng, Malik, Vasanti S, Pan, An, Kumar, Shuba, Holmes, Michelle D, Spiegelman, Donna, . . . Hu, Frank B. (2010). Substituting brown rice for white rice to lower diabetes risk: a focus-group study in Chinese adults. Journal of the American Dietetic Association, 110(8), 1216-1221.

[259] Zhang, Rong, Lu, Hongzhi, Tian, Su, Yin, Jie, Chen, Qing, Ma, Li, . . . Niu, Yujie. (2010). Protective effects of pre-germinated brown rice diet on low levels of Pb-induced learning and memory deficits in developing rat. Chemico-biological interactions, 184(3), 484-491.

[260] Zhang, Weilie, Yamada, Masahisa, Gomeza, Jesus, Basile, Anthony S, & Wess, Jürgen. (2002). Multiple muscarinic acetylcholine receptor subtypes modulate striatal dopamine release, as studied with M1–M5 muscarinic receptor knock-out mice. The Journal of neuroscience, 22(15), 6347-6352.

★★★