The potential developmental programming effect of oral curcumin administered during suckling on the bone health and plasma total osteocalcin in male and female Sprague Dawley rats fed a high-fructose diet post weaning

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Short title: Neonatal curcumin and bone health in adolescence

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Abstract

We investigated the effect of oral curcumin, on bone health of rats fed a high-fructose diet. Suckling pups (males= 65, females=63) were gavaged with 0.5% DMSO, curcumin (500mg.kg^{-1}), fructose (20%, w/v) or a combination of curcumin and fructose daily from postnatal days 6 to 21. The rats were weaned onto normal rat feed and half of the rats in each group had either plain tap water or fructose (20%, w/v) to drink for six weeks. Blood was assayed for plasma total osteocalcin. Morphometry and radiographic bone density assessments were made on the femora and tibiae. The lengths, masses and Seedor indices of the bones were similar ($p>0.05$, ANOVA) across the groups. Males that received curcumin with or without fructose during suckling and weaned onto a high-fructose diet had lower ($p\leq0.05$, ANOVA) osteocalcin concentration versus the other males. In females, curcumin alone or with fructose during suckling and subsequent feeding with a high-fructose diet resulted in lower ($p\leq0.05$, ANOVA) osteocalcin concentration versus rats administered the vehicle control during suckling followed by a high-fructose diet later. Neonatal curcumin-induced decrease in plasma total osteocalcin concentration may predispose to adverse consequences on glucose metabolism and bone health.

Keywords: Bone health, Metabolic syndrome, Childhood obesity, Plasma osteocalcin levels

Introduction

The prevalence of metabolic syndrome and its resultant deleterious effects are increasing globally (Falkner and Cossrow, 2014). In children and adolescents, the metabolic syndrome epidemic is driven by a rising prevalence of childhood obesity (Ludwig, 2018; Powell, 2018). The increase in childhood obesity has been linked to an increase in the consumption of high fructose-containing foods and physical inactivity (Fulkerson, 2018; Kral, 2018). Childhood obesity is a multisystem disorder that results in severe complications in most systems of the body, including the skeletal system (Han et al., 2010).

The skeletal system provides the structural framework for the body and plays an essential role in the regulation of glucose metabolism in the body by virtue of being an essential insulin target tissue (Tangseefa et al., 2018). Bone metabolism or remodelling continually occurs throughout an individual's life and involves tightly regulated processes of formation of new bone tissue and resorption of old tissues (Sánchez-Duffhues et al., 2015). Osteoblasts originate from the bone marrow mesenchyme and undergo continuous differentiation over childhood/adolescence to become bone matrix tissues as well as having an adipogenic function (Ganguly et al., 2017).

Osteocalcin is a bone-derived hormone that is exclusively secreted by osteoblasts and then released into the general circulation (Kanazawa, 2015). Osteocalcin undergoes carboxylation of three of its glutamyl residues which then enhances its binding to hydroxyapatite in the bone matrix (Yeap et al., 2015). There is a second form of osteocalcin which is undercarboxylated. This undercarboxylated form of osteocalcin plays an active role in glucose metabolism by serving as a link between bone metabolism and glucose metabolism (Kanazawa, 2015). Undercarboxylated osteocalcin facilitates insulin secretion directly by inducing the proliferation of the pancreatic β-cells and indirectly through its stimulation of

69 the secretion of glucagon-like peptide 1 from the intestines (Tangseefa et al., 2018).
70 Osteocalcin also stimulates the secretion of adiponectin from adipocytes, further enhancing
71 insulin sensitivity in the body (Kanazawa, 2015). Through a feedforward mechanism, both
72 insulin and adiponectin stimulate the expression of osteocalcin in the osteoblasts as shown in
73 figure 1 (Kanazawa, 2015). The osteoblasts have insulin receptors which control osteoblast
74 development and subsequent secretion of osteocalcin (Fulzele et al., 2010). Therefore, a
75 reduction in the blood osteocalcin concentration may disrupt the insulin signalling
76 mechanism possibly leading to the development of insulin resistance (Saleem et al., 2010).

77 Though bone remodelling occurs throughout life (Sánchez-Duffhues et al., 2015), most of the
78 bone mass increases in humans usually occur during adolescence and the peak bone mass is
79 achieved during this developmental stage (Boot et al., 2010). Events that interfere with these
80 bone mass increases result in suboptimal peak bone masses at adolescence and then
81 subsequently increase the risks of developing bone diseases, including fractures in old age
82 (Rizzoli et al., 2010; Lee, 2013). There is evidence that programming for long-term bone
83 health occurs during the early critical periods of developmental plasticity, a phenomenon
84 referred to as developmental programming (Devlin et al., 2013; Vickers, 2014). This
85 phenomenon whereby stressor events like nutritional perturbations in the critical periods of
86 development alter gene expression and modify the individual's physiology, can influence the
87 attainment of peak bone mass and therefore predispose to bone diseases in later life (Firth et
88 al., 2017). The period of developmental plasticity extends from pre-conception to infancy
89 (Firth et al., 2017) and during this critical window, epigenetic changes such as DNA
90 methylation, histone modification and micro RNA production are usually induced or
91 repressed by dietary and environmental factors (Park et al., 2017). Interventions in the
92 suckling period in rats have been shown to either program or reprogram for metabolic health

93 in later life. For example, neonatal administration of leptin in Wistar rats prevented the bone
94 suppressive effects of maternal undernutrition in adult offspring (Firth et al., 2017).

95 The administration of a high-fructose diet to rats is an established model for inducing
96 metabolic syndrome (Tappy and Mittendorfer, 2012; Mamikutty et al., 2014). Because of the
97 important role the skeletal system plays in metabolism, the metabolic changes induced by
98 fructose may affect bone health (Bass et al., 2013). Interestingly, the effects of fructose on
99 bone health have been rather conflicting with some studies reporting beneficial effects while
100 others reported negative effects. For instance, feeding a high-fructose diet for 12 weeks was
101 reported to lead to the formation of stronger bones with a better microarchitecture when
102 compared to glucose in a rat model (Bass et al., 2013). Jatkar et al. (2017) also reported no
103 negative effects on the bones of male BALB/cByJ (BALB) mice following 15 weeks of
104 consuming a high-fructose diet. However, fructose has been shown to lead to the generation
105 of reactive oxygen species in the body leading to oxidative stress that then causes an
106 imbalance in the bone remodelling process by increasing bone resorption through stimulation
107 of osteoclast activity (Yarrow et al., 2016).

108 Management of poor metabolic and bone health usually requires chronic or lifelong
109 medications which in addition to poor patient compliance, are also associated with side
110 effects (Inamdar and Kulkarni, 2017). Natural products such as plant polyphenols which are
111 perceived to be safe and readily available are currently being extensively researched for their
112 benefits in treating chronic conditions (Tsuda, 2012; Bahmani et al., 2014). Some of these
113 natural products have been used during suckling in rats as mentioned earlier, to program for
114 long term protection against some metabolic diseases in later life (Lembede et al., 2018;
115 Nyakudya et al., 2018).

Curcumin (diferuloylmethane) is a polyphenolic biologically active component of turmeric (Chainani-Wu, 2003; Sahebkar, 2013). Curcumin has demonstrated a wide array of biological activities in both *in vitro* and *in vivo* studies. Some of the documented biological activities of curcumin include: anti-obesity (Shao et al., 2012), anti-diabetic (Panahi et al., 2017), anti-oxidant (Ali et al., 2018) and anti-inflammatory (Elham et al., 2018). Additionally, curcumin has demonstrated beneficial effects in promoting good bone health by increasing bone strength and microarchitecture in ovariectomised postmenopausal rat model (Putnam et al., 2007; French et al., 2008).

Considering the previously established beneficial activity of curcumin against the components of metabolic syndrome and its ability to promote good bone health, we hypothesised that in rats, curcumin administration during suckling would program for improved bone health later in adolescence in the rats fed a high fructose diet post weaning. We show that there are no discernible changes to the bone health parameters, but the plasma total osteocalcin concentrations are decreased.

Materials and methods

Ethical clearance

The study protocols were approved by the Animal Ethics Screening Committee of the University of the Witwatersrand, Johannesburg (AESC 2016/04/18/B). The study complied with international ethical guidelines and standards for the use of laboratory animals.

Study location

The study was conducted in the Central Animal Services (CAS) unit and the Endocrinology and Metabolism Laboratory, School of Physiology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, Republic of South Africa.

Study animals: housing and care

One hundred and twenty-eight male and female Sprague Dawley pups which were six days old and delivered by fourteen dams sourced from the Central Animal Services Unit of the University of the Witwatersrand, Johannesburg were used in the study. The pups used were only those from dams with a litter size of between 8-12 pups to prevent litter size-effect on feeding. During the suckling phase, the pups nursed freely with their dams in perspex cages which were lined with wood shavings and shredded paper for environmental enrichment. The dams had *ad libitum* access to pelleted commercially sourced normal rat chow (Epol®, Johannesburg, South Africa) and plain tap water to drink. The ambient temperature in the animal room was maintained at 26 ± 2ºC with adequate ventilation and a 12-hour light cycle (lights switched on at 7.00am and off at 7.00pm).

At weaning (postnatal day 21), the rat weanlings were placed in individual cages under similar environmental conditions as the pre-weaning stage. The dams were returned to stock.

Chemicals and fructose used in the study

For this study, dimethyl sulfoxide (DMSO; Sigma-Aldrich, Missouri, USA), curcumin (Sigma-Aldrich, Missouri, USA) and fructose (Nature's choice, Randvaal, South Africa) were used. The DMSO was used as the vehicle in which the other treatments were dissolved.

Study design

The study was carried out in two interventional phases. The first phase of the study was conducted in the pre-weaning period; from post-natal day six to 21. The aim of the neonatal interventions was to induce programming of bone health. The second phase of the interventions extended from postnatal day 21 and continued for six weeks until postnatal day 63 (late adolescence) in order to determine whether the neonatal interventions had any effect on the response of the rats to a postweaning high fructose diet.

The pups (n=128: males = 65, females = 63) were allocated in a split-litter pattern to four treatment groups replicated by sex in the first phase of the study. The treatment groups were as follows:

Group 1 (control): DMSO (0.5%) in distilled water. DMSO was the vehicle used to dissolve curcumin and fructose.

Group 2: curcumin, 500mg.kg^{-1} body mass.

Group 3: fructose (20% w/v).

Group 4: curcumin 500mg.kg^{-1} + fructose (20%, w/v).

All the treatments were administered at 10ml.kg^{-1} body mass daily (between 8.30am and 10.00am) using an orogastric tube.

In the second phase of the study, the rats from each of the initial four groups were further sub-divided into two subgroups. While the two subgroups were weaned onto normal rat chow, one of the subgroups had plain tap water and the other had fructose (20%, w/v/) as their drinking fluid. The treatments in phase two were administered for six weeks until postnatal day 63. A schematic diagram of the study design is presented below (figure 2).

184 **Body mass measurements**

185 The pups were weighed daily using a digital weighing scale (Snowrex Electronic Scale,
186 Clover Scales, Johannesburg, South Africa) during the first phase of the study to adjust their
187 treatments to ensure constant dosing. In the second phase, the rats were weighed twice
188 weekly to monitor their growth and general health. The dams were also weighed twice
189 weekly as part of routine health monitoring and were returned to stock at weaning.

190 **Terminal procedures**

191 On postnatal day 63, the body masses of the rats were determined using a weighing scale
192 (Snowrex Electronic Scale, Clover Scales, Johannesburg, South Africa). The rats were
193 subsequently terminated by anaesthetic overdose using intraperitoneal sodium pentobarbitone
194 (150mg.kg^{-1} body mass, Euthapent; Kyron laboratories, South Africa). Blood was taken via
195 cardiac puncture and transferred into heparinised blood collecting tubes (Becton Dickinson
196 Vacutainer Systems Europe, Meylan Cedex, France). The blood was subsequently processed
197 in a centrifuge (Hermle Z 230A, B Hermle AG, Germany) at 4000 x g at 4ºC for 15 minutes
198 and the plasma was collected and stored in microtubes (Eppendorf, Hamburg, Germany) at
199 -80ºC (ESCO, Lexicon® ULT Freezer, Hatboro, PA, USA) for osteocalcin assay. The
200 carcasses of the rats were stored in a freezer until dissection of the long bones.

201

202 **Determination of bone morphometric parameters**

203 The carcasses were removed from the freezer and allowed to thaw overnight at 4°C. The left
204 hind limbs were carefully detached from the acetabulum and the femora and tibiae were
205 defleshed with a scalpel and scissors. The two bones were disarticulated from each other and
206 dried in an oven (Salvis) at 50°C for 5 days till constant masses were achieved. The masses
207 of the bones were determined using an electronic weighing scale (Precisa 310M, Instrulab,

Johannesburg, South Africa). The lengths of the bones were then determined using a digital Vernier caliper (Major Tech (Pty) Ltd., KTV 150 digital caliper, Elandsfontein, South Africa).

The relative densities of the bones were computed using the Seedor index (Seedor et al., 1991) as follows:

Seedor index = mass/length ($mg.mm^{-1}$).

The higher the Seedor index, the greater the density of the bone (Monteagudo et al., 1997).

Radiographic assessment of the relative bone densities

Radiographs of representative samples of tibiae and femora from each treatment group were taken using a Shimadzu Mux 200 X-ray machine (Shimadzu Corporation, Kyoto, Japan). The bones were placed on a Fujifilm FCR 24×30 cm IP Cassette type CC at a 100 cm distance from the radiation light source. Settings of 2.8 MAS and 58 kV were used for exposure. The radiographs were digitised using a Fujifilm FCR PRIMA and viewed using MeDiViewer (version 1.3.2, JDK 1.3.1_08, Schaf Systemtechnik GmbH, Falkenstrabe 22, 91580 Petersaurach).

Determination of plasma osteocalcin concentration

Total plasma osteocalcin concentration was determined using a commercial rat-specific sandwich enzyme-linked immunosorbent assay kit (Elabscience®, Rat OC/BGP (Osteocalcin) ELISA kit, Houston, TX, USA). The ELISA protocol was followed according to the manufacturer's instructions. The plasma osteocalcin concentrations ($ng.\ mL^{-1}$) were extrapolated from standard curve generated using known osteocalcin concentrations.

Statistical analysis

Data was analysed using GraphPad Prism version 7.0 statistical software (GraphPad Software Inc., San Diego, CA, USA) and expressed as mean ± standard error of mean (SEM). Data was analysed using a one-way analysis of variance (ANOVA), followed by a comparison of the means using the Bonferroni *post hoc* test. Statistical significance was assumed when $p \leq 0.05$.

Results

Body mass changes

The body mass changes at induction, weaning and termination of male and female Sprague Dawley rats that were administered with oral curcumin during suckling are presented in figures 3a and 3b, respectively. There were no differences ($p > 0.05$, ANOVA) in the mean body masses across the treatment groups at each of the three time points in both male and female rats. However, both male and female rats gained significant ($p < 0.0001$, ANOVA) body mass from induction through weaning and termination.

Indices of femora and tibiae health

Tables 1a and 1b show the effect of the interventions on the lengths, masses and Seedor indices of femora and tibiae of male and female rats respectively. There were no differences ($p > 0.05$, ANOVA) across the treatment groups in the lengths, masses and Seedor indices of the femora and tibiae of the male and female rats. The representative radiographic images of the femora and tibiae of male and female Sprague Dawley rats that were administered with curcumin during suckling and subsequently fed a high-fructose diet post weaning are

presented as figures 4a and 4b. There were no discernible differences in the density of both the femora and tibiae of male and female rats across the treatment groups.

Plasma osteocalcin concentration changes

The effect of a high-fructose diet on the plasma concentration of osteocalcin in male and female Sprague Dawley rats that were orally administered with curcumin during suckling are presented in figures 5a and 5b. Male rats that were administered with curcumin alone and those administered with a combination of curcumin and fructose during suckling and subsequently fed a high-fructose diet had significantly lower ($p \leq 0.05$, ANOVA) total plasma osteocalcin concentration compared to that of rats from other treatment groups. In the female rats, administration of curcumin alone or combined curcumin and fructose during suckling and then subsequent feeding with a high-fructose diet post weaning resulted in significantly lower ($p \leq 0.05$, ANOVA) total plasma osteocalcin concentration compared to administration of DMSO during suckling and then feeding with a high-fructose diet post weaning.

Discussion

The major finding in this study was that administration of curcumin alone or in combination with fructose during suckling resulted in a reduced total plasma concentration of osteocalcin following a postweaning high-fructose diet in both male and female rats. The total plasma osteocalcin comprises of the carboxylated and undercarboxylated forms of osteocalcin, thus, the findings could be interpreted in two different ways. Whereas, a low concentration of carboxylated osteocalcin implies a low bone turnover, reduced bone loss and good bone health; a low concentration of undercarboxylated osteocalcin may imply an impairment of glucose metabolism probably because of the postweaning high fructose diet. It is important to

mention here that both the fasting blood glucose and plasma insulin concentration were similar across treatment groups in both male and female rats (data not shown but presented in supplemental tables 1 and 2).

A high-fructose diet is known to induce features of metabolic syndrome including hyperglycaemia and insulin resistance (Stanhope and Havel, 2010; Tappy and Mittendorfer, 2012). The low levels of osteocalcin that were noted only in the high fructose groups that had previously been administered with curcumin during suckling may imply that curcumin predisposed the rats to the adverse effects of the post weaning high fructose diet. Osteocalcin is an important non-collagenous protein biochemical marker of bone turnover that is found in abundance in the bone matrix (Nikel et al., 2018). Most of the osteocalcin exists in the carboxylated form and is involved in bone remodelling (Namai et al., 2018). Undercarboxylated osteocalcin is involved in glucose metabolism regulation and its deficiency leads to the development of insulin resistance (Saleem et al., 2010; Namai et al., 2018). Previous studies on the effects of neonatal administration of fructose in rats focused on its programming effects on metabolism (Huynh et al., 2008; Ghezzi et al., 2012) and not on long-term bone health. However, it has been previously shown that a 25% fructose (w/v) administered to 12-week old male Wistar rats did not induce any significant changes in the plasma osteocalcin concentration (Dai et al., 2017). It is therefore not surprising that we did not observe significant effects in the groups that had only fructose without curcumin when compared to the control in both sexes.

Contrary to our findings, curcumin was found to induce osteoblast differentiation by increasing the expression of genes that induce osteoblast differentiation, such as runt-related transcription factor 2 (Runx2) and osteocalcin in an *in vitro* study, (Son et al., 2018). The beneficial effects of curcumin on bone health were further shown in another study involving spinal cord injury subjects with low bone density where it prevented further bone loss and

303 improved the bone density (Hatefi et al., 2018). It is possible that curcumin only lowers
304 osteocalcin levels when a high fructose diet disrupts the positive feedback loop that exist
305 between osteocalcin and insulin concentrations (Kanazawa, 2015). Therefore, the pre-
306 weaning administration of curcumin may have epigenetically programmed the rats for lower
307 osteocalcin concentration later in life.

308 We did not find any differences in the bone lengths, masses and Seedor indices in both male
309 and female rats. This further suggests that it is probably the endocrine functions of the bones
310 that were affected by our interventions and not the bone density. Though postweaning
311 feeding with fructose has been reported in several studies not to exert any effect on the
312 morphometry of the femora and tibiae of rats (Ibrahim et al., 2017; Lembede et al., 2018);
313 Douard et al. (2013) had previously reported shorter femora in male Sprague Dawley rats that
314 were fed a 63% fructose diet for 4 weeks when compared to control rats due to decreased
315 calcium absorption and vitamin D deficiency caused by the high fructose diet. Fructose
316 metabolism in the body has been associated with generation of reactive oxygen species
317 leading to oxidative stress (Yarrow et al., 2016). This oxidative stress induces osteoblast
318 apoptosis which inhibits bone growth (Dai et al., 2017). In our present study, we did not
319 observe any obvious inhibition of bone growth as a result of either the administration of
320 curcumin or the high-fructose diet.

321 We did not observe any differences in the body masses of both male and female rats across
322 the treatment groups even though all the animals gained significant body mass through the
323 stages of the study. This suggests that both the neonatal and postweaning interventions did
324 not have any adverse effect on the growth of the rats. Although high fructose administration
325 has been shown to induce changes in body mass due to increased adiposity (Bocarsly et al.,
326 2010; Mamikutty et al., 2014), younger rats were shown to be resistant to this body mass
327 change due to their rapid growth and higher metabolic rate (Tillman et al., 2014) into which

they channel the consumed calories rather than into deposition of adipose tissue . Body mass changes due to fructose administration were found to become obvious only after postnatal day 100 in rats (Patel and Srinivasan, 2010). The rats in this study were terminated on postnatal day 63 and therefore this might explain the similarity in their body masses.

Though the use of Seedor index to estimate bone density is acceptable (Monteagudo et al., 1997), measuring bone mineral content and bone mineral density of bones with dual-energy x-ray absorptiometry (DXA) (VanGompel et al., 2017) would have been preferable. Bone breaking strength tests would have given more information on the density of the bones. We measured total osteocalcin concentration and not the different forms which may provide different results and suggest an alternative mechanism.

Conclusion

Oral administration of curcumin alone or with fructose during suckling reduced total plasma osteocalcin concentration following a postweaning high fructose diet. These findings suggest that while administration of curcumin during periods of developmental plasticity may worsen the disruption of glucose homeostasis induced by a high fructose diet, it probably reduces bone turnover. Thus, despite the potential role for curcumin in the prevention of fructose induced bone diseases, it should be used with caution in the neonatal age groups.

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Conflict of interest

The authors declare no conflict of interest.

Author contributions

KGI, EC and KHE contributed to the design of the work, the interpretation of the data and the revision of the manuscript. KGI, H.L.W and MTM contributed to the acquisition and interpretation of the data, and the drafting of the manuscript. All authors agree to be accountable for the entire content of the manuscript and approved the final version of the manuscript. All persons who qualify for authorship are listed as authors.

References

Ali BH, Suhail AS, Yousuf AS, Jamila AK, Shadia AB, Mohammed A, Priyadarsini M, Buthaina AD, Nadia AA, Heba NT, Javed Y, Abderrahim N, Mohammed AZA, Christina H, Nicole S (2018): Curcumin Ameliorates Kidney Function and Oxidative Stress in Experimental Chronic Kidney Disease. Basic Clin. Pharmacol. Toxicol. **122,** 65-73.

373 Bahmani M, Zargaran A, Rafieian-Kopaei M, Saki K (2014): Ethnobotanical study of
374 medicinal plants used in the management of diabetes mellitus in the Urmia, Northwest
375 Iran. Asian Pac. J. Trop. Biomed. **7,** S348-S354.

376 Bass EF, Baile CA, Lewis RD, Giraudo SQ (2013): Bone quality and strength are greater in
377 growing male rats fed fructose compared with glucose. Nutr. Res. **33,** 1063-1071.

378 Bocarsly ME, Powell ES, Avena NM, Hoebel BG (2010): High-fructose corn syrup causes
379 characteristics of obesity in rats: increased body weight, body fat and triglyceride
380 levels. Pharmacol. Biochem. Behav. **97,** 101-106.

381 Boot AM, De Ridder MA, Van Der Sluis IM, Van Slobbe I, Krenning EP, De Muinck
382 Keizer-Schrama SM (2010): Peak bone mineral density, lean body mass and fractures.
383 Bone **46,** 336-341.

384 Chainani-Wu N (2003) Safety and anti-inflammatory activity of curcumin: a component of
385 tumeric (*Curcuma longa*). J. Altern. Complement. Med. **9,** 161-168.

386 Dai P, Mao Y, Sun X, Li X, Muhammad I, Gu W, Zhang D, Zhou Y, Ni Z, Ma J (2017):
387 Attenuation of oxidative stress-induced osteoblast apoptosis by curcumin is associated
388 with preservation of mitochondrial functions and increased Akt-GSK3β signaling.
389 Cell. Physiol. Biochem. **41,** 661-677.

390 Devlin M, Grasemann C, Cloutie A, Louis L, Alm C, Palmert M, Bouxsein M (2013):
391 Maternal perinatal diet induces developmental programming of bone architecture. J.
392 Endocrinol. **217,** 69-81.

393 Douard V, Sabbagh Y, Lee J, Patel C, Kemp FW, Bogden JD, Lin S, Ferraris RP (2013):
394 Excessive fructose intake causes 1,25-(OH)(2)D(3)-dependent inhibition of intestinal
395 and renal calcium transport in growing rats. Am. J. Physiol. Endocrinol. Metab. **304,**
396 E1303-E1313.

397 Elham A, Abbas MA, Thomas J, Amirhossein S (2018): Therapeutic effects of curcumin in
398 inflammatory and immune- mediated diseases: A nature- made jack- of- all- trades?
399 J. Cell. Physiol. **233,** 830-848.

400 Falkner B, Cossrow ND (2014): Prevalence of metabolic syndrome and obesity-associated
401 hypertension in the racial ethnic minorities of the United States. Curr. Hypertens. Rep.
402 **16,** 1-8.

403 Firth EC, Gamble GD, Cornish J, Vickers MH (2017): Neonatal leptin treatment reverses the
404 bone-suppressive effects of maternal undernutrition in adult rat offspring. Sci. Rep. **7,**
405 7686, | DOI: https://doi.org/ 10.1038/s41598-017-07500-5.

406 French DL, Muir JM, Webber CE (2008): The ovariectomized, mature rat model of
407 postmenopausal osteoporosis: An assessment of the bone sparing effects of curcumin.
408 Phytomed. **15,** 1069-1078.

409 Fulkerson JA (2018): Fast food in the diet: Implications and solutions for families. Physiol.
410 Behav. **193,** 252-256.

411 Fulzele K, Riddle RC, Digirolamo DJ, Cao X, Wan C, Chen D, Faugere M.-C, Aja S,
412 Hussain MA, Brüning JC, (2010): Insulin receptor signaling in osteoblasts regulates
413 postnatal bone acquisition and body composition. Cell **142,** 309-319.

414 Ganguly P, El-Jawhari JJ, Giannoudis PV, Burska AN, Ponchel F, Jone EA (2017): Age-
415 related changes in bone marrow mesenchymal stromal cells: a potential impact on
416 osteoporosis and osteoarthritis development. Cell Transplant. **26,** 1520-1529.

417 Ghezzi AC, Cambri LT, Botezelli JD, Ribeiro C, Dalia RA, Rostom De Mello, MA (2012):
418 Metabolic syndrome markers in wistar rats of different ages. Diabetol. Metab. Syndr.
419 **4,** 16; DOI: https://doi.org/10.1186/1758-5996-4-16.

420 Han JC, Lawlor DA, Kimm SYS (2010): Childhood obesity. The Lancet **375,** 1737-1748.

421 Hatefi M, Ahmadi MRH, Rahmani A, Dastjerd MM, Asadollahi K (2018): Effects of
422 Curcumin on Bone Loss and Biochemical Markers of Bone Turnover in Patients with
423 Spinal Cord Injury. World Neurosurg. **114,** e785-e791.

424 Huynh M, Luiken JJ, Coumans W, Bell RC (2008): Dietary fructose during the suckling
425 period increases body weight and fatty acid uptake into skeletal muscle in adult rats.
426 Obesity **16,** 1755-1762.

427 Ibrahim KG, Chivandi E, Mojiminiyi FBO, Erlwanger KH (2017): The response of male and
428 female rats to a high-fructose diet during adolescence following early administration
429 of *Hibiscus sabdariffa* aqueous calyx extracts. J. Dev. Orig. Health Dis. **8,** 628-637.

430 Inamdar SZ, Kulkarni RV (2017): Anti-Diabetics Drug Usage and Related Pharmaceutical
431 Care Issues among Geriatrics and Non-Geriatrics: A Comparative Assessment. Indian
432 J. Pharm. Pract. **10,** 161; DOI: https://doi.org/10.5530/ijopp.10.3.35.

433 Jatkar A, Kurland IJ, Judex S (2017): Diets High in Fat or Fructose Differentially Modulate
434 Bone Health and Lipid Metabolism. Calcified Tissue Int. **100,** 20-28.

435 Kanazawa I (2015): Osteocalcin as a hormone regulating glucose metabolism. World J.
436 Diabetes **6,** 1345-1354.

437 Kral TVE (2018): Behavioral phenotypes for childhood obesity: 2017 Alan N. Epstein
438 Research Award. Physiol. Behav. **192,** 206-209.

439 Lee K (2013): Sex-specific relationships between insulin resistance and bone mineral content
440 in Korean adolescents. J. Bone Miner Metab. **31,** 177-182.

441 Lembede BW, Erlwanger K, Nkomozepi P, Chivandi E (2018): Effect of neonatal orally
442 administered S-allyl cysteine in high-fructose diet fed Wistar rats. J. Dev. Orig.
443 Health Dis. **9,** 160-171.

444 Ludwig DS (2018) Epidemic childhood obesity: Not yet the end of the beginning. Pediatrics
445 e20174078, https://doi.org/10.1542/peds.2017-4078.

446 Mamikutty N, Thent ZC, Sapri SR, Sahruddin NN, Mohd Yusof MR, Haji Suhaimi F (2014):
447 The Establishment of Metabolic Syndrome Model by Induction of Fructose Drinking
448 Water in Male Wistar Rats. BioMed Res. Int. **2014,** 1-8.

449 Monteagudo M, Hernandez E, Seco C, Gonzalez-Riola J, Revilla M, Villa L, Rico H (1997):
450 Comparison of the bone robusticity index and bone weight/bone length index with the
451 results of bone densitometry and bone histomorphometry in experimental studies.
452 Cells Tissues Organs 1**60,** 195-199.

453 Namai F, Shigemori S, Sudo K, Sato T, Yamamoto Y, Nigar S, Ogita T, Shimosato T
454 (2018): Recombinant Mouse Osteocalcin Secreted by Lactococcus lactis Promotes
455 Glucagon-Like Peptide-1 Induction in STC-1 Cells. Curr. Microbiol. **75,** 92-98.

456 Nikel O, Poundarik AA, Bailey S, Vashishth D (2018): Structural role of osteocalcin and
457 osteopontin in energy dissipation in bone. J. Biomech. **80,** 45-52.

458 Nyakudya T, Mukwevho E, Nkomozepi P, Erlwanger K (2018): Neonatal intake of oleanolic
459 acid attenuates the subsequent development of high fructose diet-induced non-
460 alcoholic fatty liver disease in rats. J. Dev. Orig.Health Dis. **9,** 500-510.

461 Panahi Y, Khalili N, Sahebi E, Namazi S, Karimian MS, Majeed M, Sahebkar A (2017):
462 Antioxidant effects of curcuminoids in patients with type 2 diabetes mellitus: a
463 randomized controlled trial. Inflammopharmacology **25,** 25-31.

464 Park J.-H, Kim S.-H, Lee MS, Kim M.-S (2017): Epigenetic modification by dietary factors:
465 Implications in metabolic syndrome. Mol. Aspects Med. **54,** 58-70.

466 Patel MS, Srinivasan M (2010): Metabolic Programming Due to Alterations in Nutrition in
467 the Immediate Postnatal Period. J. Nutr. **140,** 658-661.

468 Powell D (2018): Governing the (un) healthy child-consumer in the age of the childhood
469 obesity crisis. Sport Educ. Soc. **23,** 297-310.

470 Putnam SE, Scutt AM, Bicknell K, Priestley CM, Williamson EM (2007): Natural products
471 as alternative treatments for metabolic bone disorders and for maintenance of bone
472 health. Phytother. Res. **21,** 99-112.

473 Rizzoli R, Bianchi ML, Garabédian M, Mckay HA, Moreno LA (2010): Maximizing bone
474 mineral mass gain during growth for the prevention of fractures in the adolescents and
475 the elderly. Bone **46,** 294-305.

476 Sahebkar A (2013): Why it is necessary to translate curcumin into clinical practice for the
477 prevention and treatment of metabolic syndrome? Biofactors **39,** 197-208.

478 Saleem U, Mosley Jr TH, Kullo IJ (2010): Serum osteocalcin is associated with measures of
479 insulin resistance, adipokine levels, and the presence of metabolic syndrome.
480 Arterioscler. Thromb. Vasc. Biol. **30,** 1474-1478.

481 Sánchez-Duffhues G, Hiepen C, Knaus P, Ten Dijke P (2015): Bone morphogenetic protein
482 signaling in bone homeostasis. Bone **80,** 43-59.

483 Seedor JG, Quartuccio HA, Thompson DD (1991): The bisphosphonate alendronate (MK-
484 217) inhibits bone loss due to ovariectomy in rats. J. Bone Miner. Res. **6,** 339-346.

485 Shao W, Yu Z, Chiang Y, Yang Y, Chai T, Foltz W, Lu H, Fantus IG, Jin T (2012):
486 Curcumin prevents high fat diet induced insulin resistance and obesity via attenuating
487 lipogenesis in liver and inflammatory pathway in adipocytes. PloS One **7,** e28784;
488 DOI: https://doi.org/10.1371/journal.pone.0028784.

489 Son H.-E, Kim E.-J, Jang W.-G (2018): Curcumin induces osteoblast differentiation through
490 mild-endoplasmic reticulum stress-mediated such as BMP2 on osteoblast cells. Life
491 Sci. **193,** 34-39.

492 Stanhope KL, Havel PJ (2010): Fructose consumption: recent results and their potential
493 implications. Ann. N Y Acad. Sci. **1190,** 15-24.

494 Tangseefa P, Martin SK, Fitter S, Baldock PA, Proud G, Zannettino AC (2018):
495 Osteocalcin- dependent regulation of glucose metabolism and fertility: Skeletal

implications for the development of insulin resistance. J. Cell. Physiol. **233,** 3769-3783.

Tappy L, Mittendorfer B (2012): Fructose toxicity: is the science ready for public health actions? Curr. Opin. Clin. Nutr. Metab. Care **15,** 357-361.

Tillman EJ, Morgan DA, Rahmouni K, Swoap SJ (2014): Three months of high-fructose feeding fails to induce excessive weight gain or leptin resistance in mice. PloS one **9,** e107206; DOI: https://doi.org/10.1371/journal.pone.0107206.

Tsuda T (2012): Dietary anthocyanin- rich plants: biochemical basis and recent progress in health benefits studies. Mol. Nutr. Food Res. **56,** 159-170.

Vangompel ECW, Franks P, Robbins JA, Fenton JJ (2017): Incidence and predictors of repeat bone mineral densitometry: a longitudinal cohort study. J. Gen. Intern. Med. **32,** 1090-1096.

Vickers MH (2014): Developmental Programming and Transgenerational Transmission of Obesity. Ann. Nutr. Metab. **64,** 26-34.

Yarrow JF, Toklu HZ, Balaez A, Phillips EG, Otzel DM, Chen C, Wronski TJ, Aguirre JI, Sakarya Y, Tümer N (2016): Fructose consumption does not worsen bone deficits resulting from high-fat feeding in young male rats. Bone **85,** 99-106.

Yeap BB, Alfonso H, Chubb SP, Gauci R, Byrnes E, Beilby JP, Ebeling PR, Handelsman DJ, Allan CA, Grossmann M (2015): Higher serum undercarboxylated osteocalcin and other bone turnover markers are associated with reduced diabetes risk and lower estradiol concentrations in older men. J. Clin. Endocrinol. Metab. **100,** 63-71.

Figure 2: Schematic diagram of the study design

Newly born pups were obtained as described in the materials and methods. They were assigned to four treatment groups and treated with DMSO, curcumin (CC), fructose (FW), and a combination of curcumin and fructose (CCFW). NRC, normal rat chow; PTW, plain tap water; M, males; F, females.

Figures 3: Effect of a high-fructose diet on body mass changes from induction to termination of (a) male and (b) female Sprague Dawley rats that were administered curcumin orally during suckling

***= significantly different at $p<0.0001$. DMSO + TW= 10ml.kg^{-1} of a 0.5% dimethyl sulfoxide solution as neonates and plain tap water post-weaning, DMSO + FW= 10ml.kg^{-1} of a 0.5% dimethyl sulfoxide solution as neonates and fructose (20%, w/v) as drinking fluid post-weaning, CC + TW= Curcumin (500mg.kg^{-1} in 0.5% DMSO) as neonates and plain tap water post-weaning, CC + FW= Curcumin (500mg.kg^{-1} in 0.5% DMSO) as neonates and fructose (20%, w/v) as drinking fluid post-weaning, FW + TW= fructose (20%, w/v) as neonates and plain tap water post-weaning, FW + FW= fructose (20%, w/v) as neonates and fructose (20%, w/v) as drinking fluid post-weaning, CCFW + TW= curcumin (500mg.kg^{-1}) and fructose (20%, w/v) in 0.5% DMSO as neonates and plain tap water post-weaning,

539 CCFW + FW= curcumin (500mg.kg^{-1}) and fructose (20%, w/v) in 0.5% DMSO as neonates
540 and fructose (20%, w/v) post-weaning. Data expressed as mean ± SEM, n= 6-8 per group.

541

542 **Figures 4:** Representative radiographs showing the effect of a high-fructose diet on the of the
543 femora (top) and tibiae (bottom) of adolescent (a) male and (b) female Sprague Dawley rats
544 that were administered with curcumin during suckling

545 A= DMSO + TW= 10ml.kg^{-1} of a 0.5% dimethyl sulfoxide solution as neonates and plain tap
546 water post-weaning, B= DMSO + FW= 10ml.kg^{-1} of a 0.5% dimethyl sulfoxide solution as
547 neonates and fructose (20%, w/v) as drinking fluid post-weaning, C= CC + TW= Curcumin
548 (500mg.kg^{-1} in 0.5% DMSO) as neonates and plain tap water post-weaning, D= CC + FW=
549 Curcumin (500mg.kg^{-1} in 0.5% DMSO) as neonates and fructose (20%, w/v) as drinking fluid
550 post-weaning, E= FW + TW= fructose (20%, w/v) as neonates and plain tap water post-
551 weaning, F= FW + FW= fructose (20%, w/v) as neonates and fructose (20%, w/v) as drinking
552 fluid post-weaning, G= CCFW + TW= curcumin (500mg.kg^{-1}) and fructose (20%, w/v) in
553 0.5% DMSO as neonates and plain tap water post-weaning, H= CCFW + FW= curcumin
554 (500mg.kg^{-1}) and fructose (20%, w/v) in 0.5% DMSO as neonates and fructose (20%, w/v)
555 post-weaning. Representative images of n= 6-8 per group are shown.

556

557 **Figures 5:** Effect of a high-fructose diet on the plasma concentration of total osteocalcin of
558 (a) male and (b) female Sprague Dawley rats that were administered curcumin during
559 suckling

560 a, b, c, d= significantly different at $p \leq 0.05$. DMSO + TW= 10ml.kg^{-1} of a 0.5% dimethyl
561 sulfoxide solution as neonates and plain tap water post-weaning, DMSO + FW= 10ml.kg^{-1} of
562 a 0.5% dimethyl sulfoxide solution as neonates and fructose (20%, w/v) as drinking fluid

post-weaning, CC + TW= Curcumin (500mg.kg^{-1} in 0.5% DMSO) as neonates and plain tap water post-weaning, CC + FW= Curcumin (500mg.kg^{-1} in 0.5% DMSO) as neonates and fructose (20%, w/v) as drinking fluid post-weaning, FW + TW= fructose (20%, w/v) as neonates and plain tap water post-weaning, FW + FW= fructose (20%, w/v) as neonates and fructose (20%, w/v) as drinking fluid post-weaning, CCFW + TW= curcumin (500mg.kg^{-1}) and fructose (20%, w/v) in 0.5% DMSO as neonates and plain tap water post-weaning, CCFW + FW= curcumin (500mg.kg^{-1}) and fructose (20%, w/v) in 0.5% DMSO as neonates and fructose (20%, w/v) post-weaning. Data expressed as mean ± SEM, n= 6-8 per group.

584

585 **Tables 1:** Effect of a high-fructose diet on the lengths, masses, Seedor and robusticity indices
586 of the bones of (a) male and (b)female Sprague Dawley rats that were fed curcumin during
587 suckling

588

589 DMSO + TW= 10ml.kg^{-1} of a 0.5% dimethyl sulfoxide solution as neonates and plain tap
590 water post-weaning, DMSO + FW= 10ml.kg^{-1} of a 0.5% dimethyl sulfoxide solution as
591 neonates and fructose (20%, w/v) as drinking fluid post-weaning, CC + TW= Curcumin
592 (500mg.kg^{-1} in 0.5% DMSO) as neonates and plain tap water post-weaning, CC + FW=
593 Curcumin (500mg.kg^{-1} in 0.5% DMSO) as neonates and fructose (20%, w/v) as drinking fluid
594 post-weaning, FW + TW= fructose (20%, w/v) as neonates and plain tap water post-weaning,
595 FW + FW= fructose (20%, w/v) as neonates and fructose (20%, w/v) as drinking fluid post-
596 weaning, CCFW + TW= curcumin (500mg.kg^{-1}) and fructose (20%, w/v) in 0.5% DMSO as
597 neonates and plain tap water post-weaning, CCFW + FW= curcumin (500mg.kg^{-1}) and
598 fructose (20%, w/v) in 0.5% DMSO as neonates and fructose (20%, w/v) post-weaning. Data
599 expressed as mean ± SEM, n= 6-8 per group.

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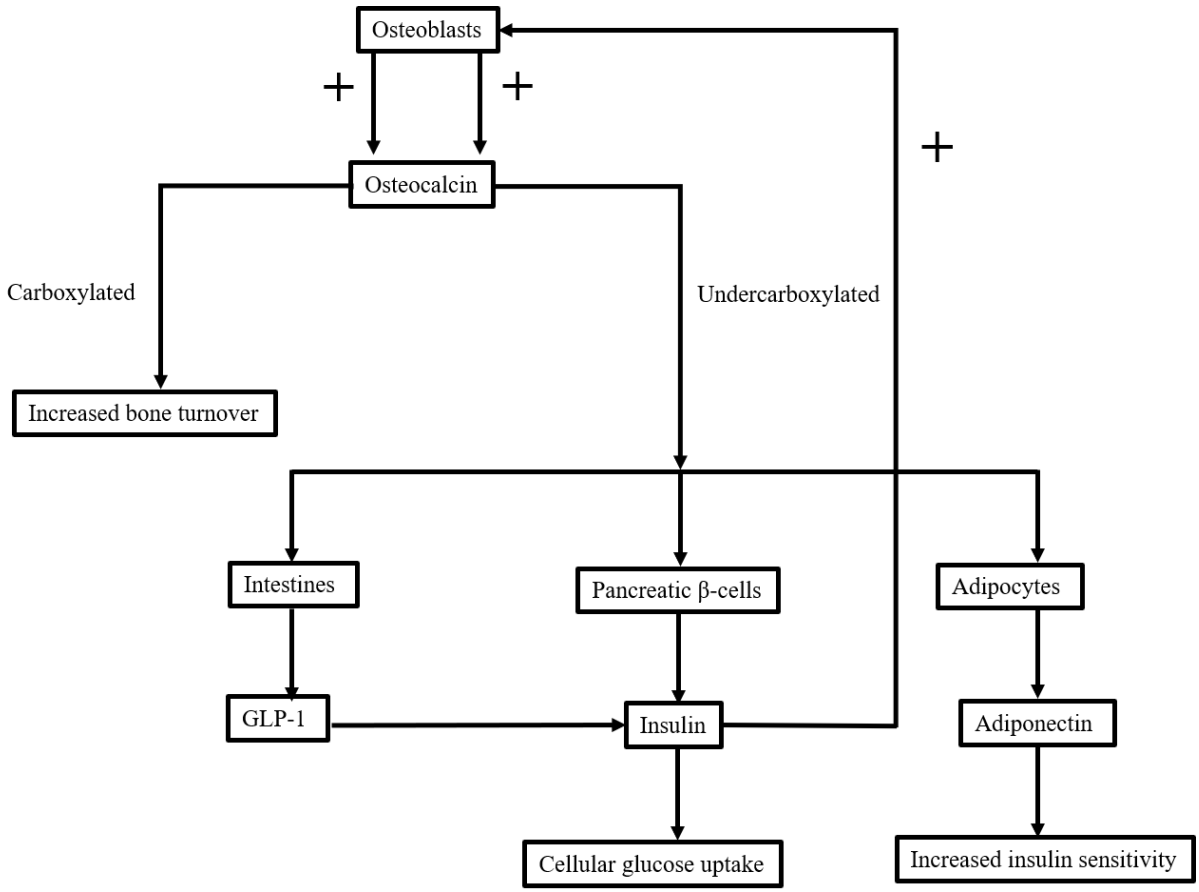

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608 **Figure 1:** schematic representation of the interactions between osteoblasts, pancreatic β cells

609 and adipocytes

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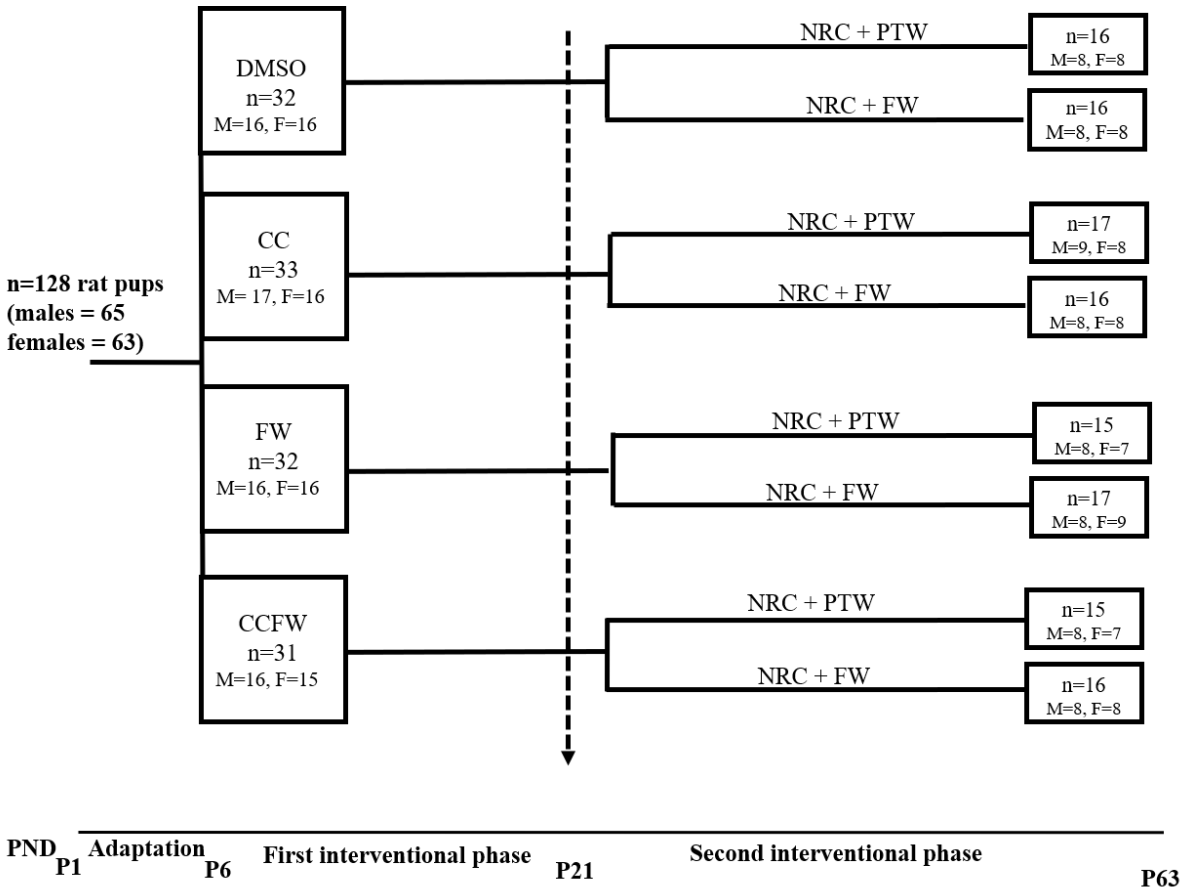

Figure 2: schematic diagram of the study design

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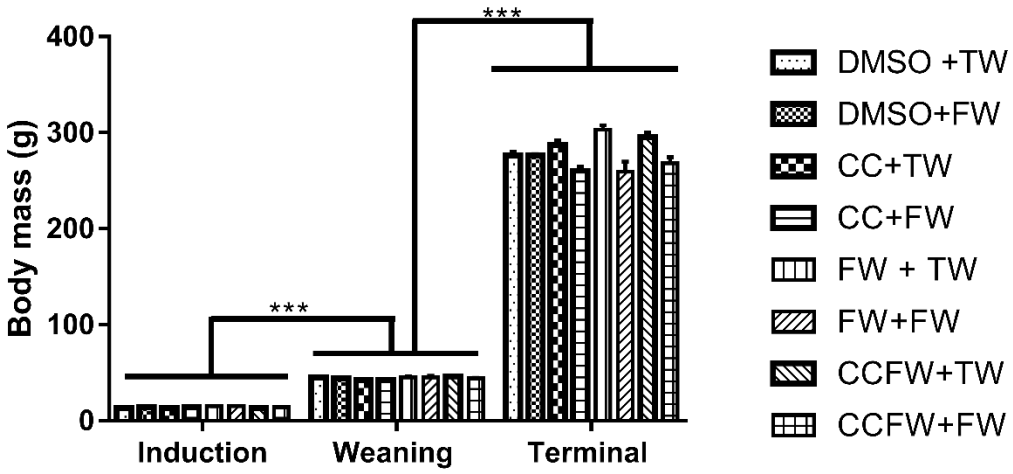
629 **A**



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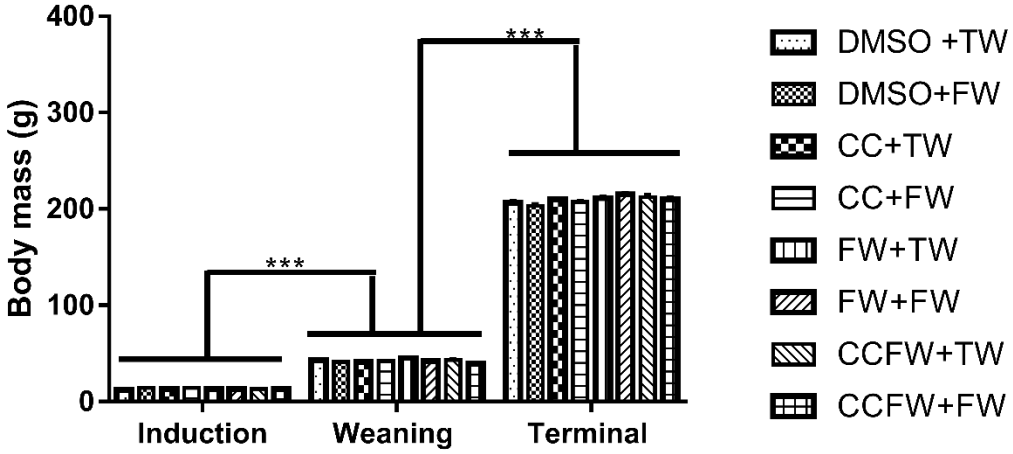
631 **B**



632

633 **Figure 3:** Effect of a high-fructose diet on body mass changes of male (A) and female (B)

634 Sprague Dawley rats that were administered oral curcumin during suckling

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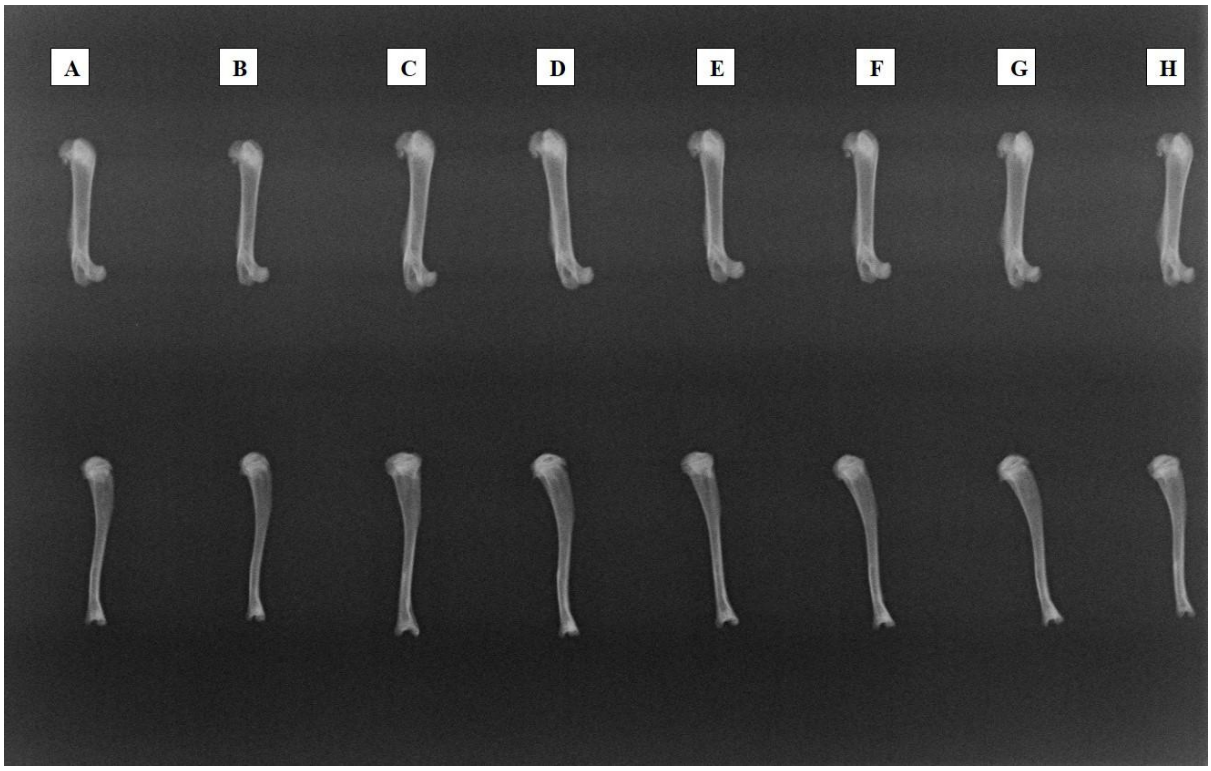

638

639 **Figure 4a:** Representative radiographs showing the effect of a high-fructose diet on the of the

640 femora (top) and tibiae (bottom) of adolescent male Sprague Dawley rats that were

641 administered with curcumin during suckling

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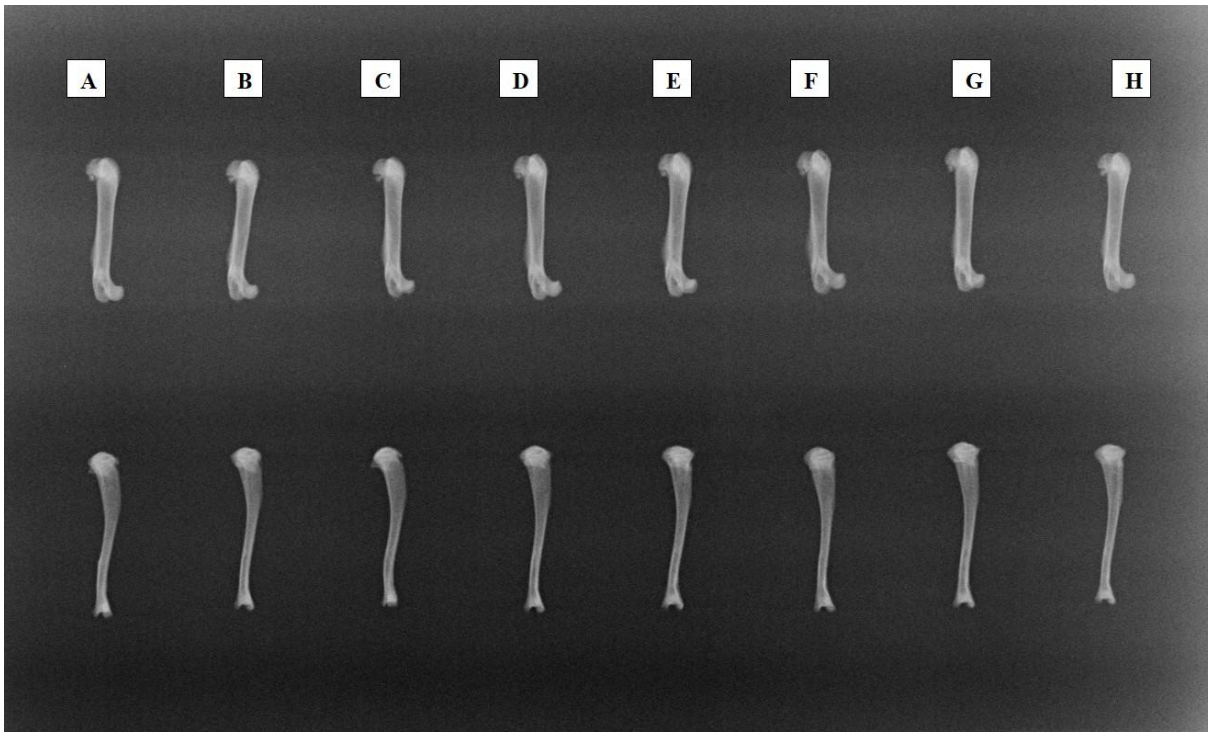

Figure 4b: Representative radiographs showing the effect of a high-fructose diet on the of the femora (top) and tibiae (bottom) of adolescent female Sprague Dawley rats that were administered with curcumin during suckling

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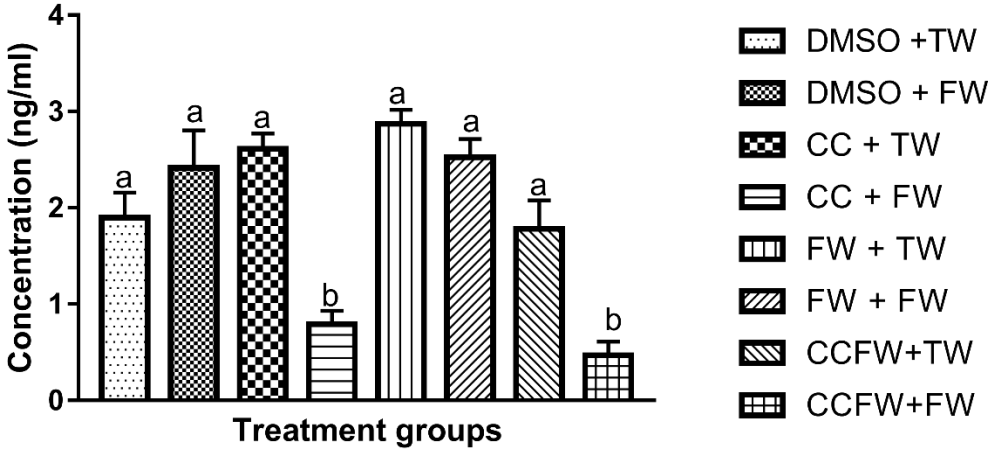

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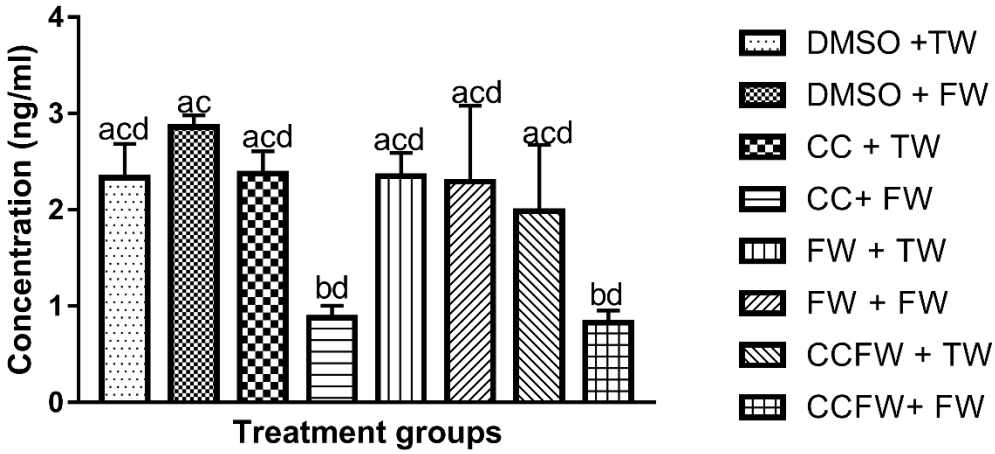

Figure 5: Effect of a high-fructose diet on the plasma concentration of total osteocalcin of male (A) and female (B) Sprague Dawley rats that were administered curcumin during suckling

Table 1a: Effect of a high-fructose diet on the lengths, masses and Seedor index of the bones of male Sprague Dawley rats that were fed curcumin during suckling

	Femur			Tibia		
Treatment groups	**Length (mm)**	**Mass (mg)**	**Seedor index ($mg.mm^{-1}$)**	**Length (mm)**	**Mass (mg)**	**Seedor index ($mg.mm^{-1}$)**
DMSO + TW	32 ± 0.46	479 ± 16	15 ± 0.32	36 ± 0.53	400 ± 13	11 ± 0.23
DMSO + FW	32 ± 0.44	484 ± 11	15 ± 0.17	36 ± 0.29	378 ± 14	10 ± 0.33
CC + TW	33 ± 0.61	488 ± 21	15 ± 0.38	37 ± 0.53	414 ± 14	11 ± 0.26
CC + FW	32 ± 1.20	442 ± 32	14 ± 0.69	36 ± 0.51	364 ± 29	10 ± 0.73
FW + TW	33 ± 1.10	526 ± 29	16 ± 0.37	37 ± 0.57	441 ± 15	12 ± 0.33
FW + FW	31 ± 0.43	468 ± 14	15 ± 0.28	36 ± 0.41	386 ± 12	11 ± 0.29
CCFW + TW	33 ± 0.93	508 ± 21	15 ± 0.45	37 ± 0.56	424 ± 14	11 ± 0.23
CCFW + FW	31 ± 0.70	468 ± 30	16 ± 1.10	36 ± 0.87	394 ± 19	11 ± 0.39

670

671 **Table 1b:** Effect of a high fructose diet on the lengths, masses and Seedor index of the bones of female Sprague Dawley rats that were fed

672 curcumin during suckling

	Femur			Tibia		
Treatment groups	**Length (mm)**	**Mass (mg)**	**Seedor index ($mg.mm^{-1}$)**	**Length (mm)**	**Mass (mg)**	**Seedor index ($mg.mm^{-1}$)**
DMSO + TW	30 ± 0.42	432 ± 16	14 ± 0.35	35 ± 0.33	354 ± 11	10 ± 0.23
DMSO + FW	30 ± 0.30	414 ± 17	14 ± 0.43	35 ± 0.27	335 ± 14	9.7 ± 0.34
CC + TW	32 ± 0.93	438 ± 11	14 ± 0.39	35 ± 0.27	358 ± 9.6	10 ± 0.20
CC + FW	30 ± 0.16	431 ± 9.5	14 ± 0.28	35 ± 0.21	354 ± 4.2	10 ± 0.08
FW + TW	30 ± 0.15	457 ± 6.2	15 ± 0.15	36 ± 0.91	368 ± 6	10 ± 0.31
FW + FW	31 ± 0.53	449 ± 14	15 ± 0.46	35 ± 0.17	370 ± 5.5	11 ± 0.12
CCFW + TW	31 ± 0.32	463 ± 15	15 ± 0.34	37 ± 1.3	369 ± 8.1	10 ± 0.23
CCFW + FW	30 ± 0.56	433 ± 28	14 ± 0.66	35 ± 0.51	343 ± 15	9.9 ± 0.34

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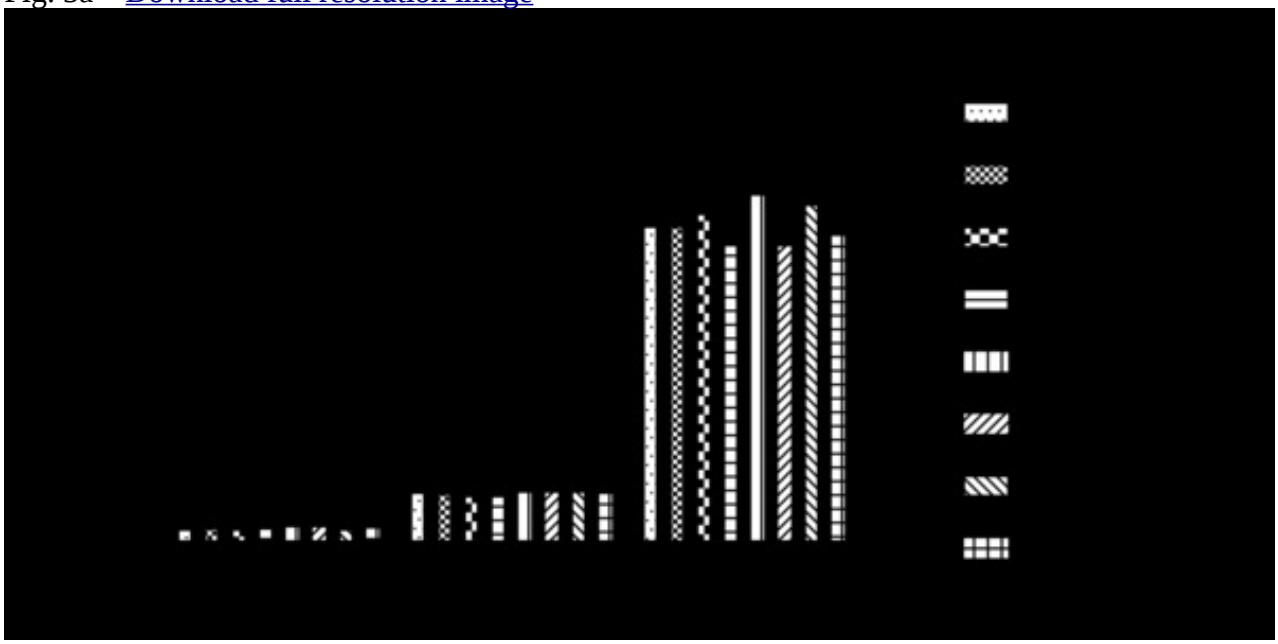

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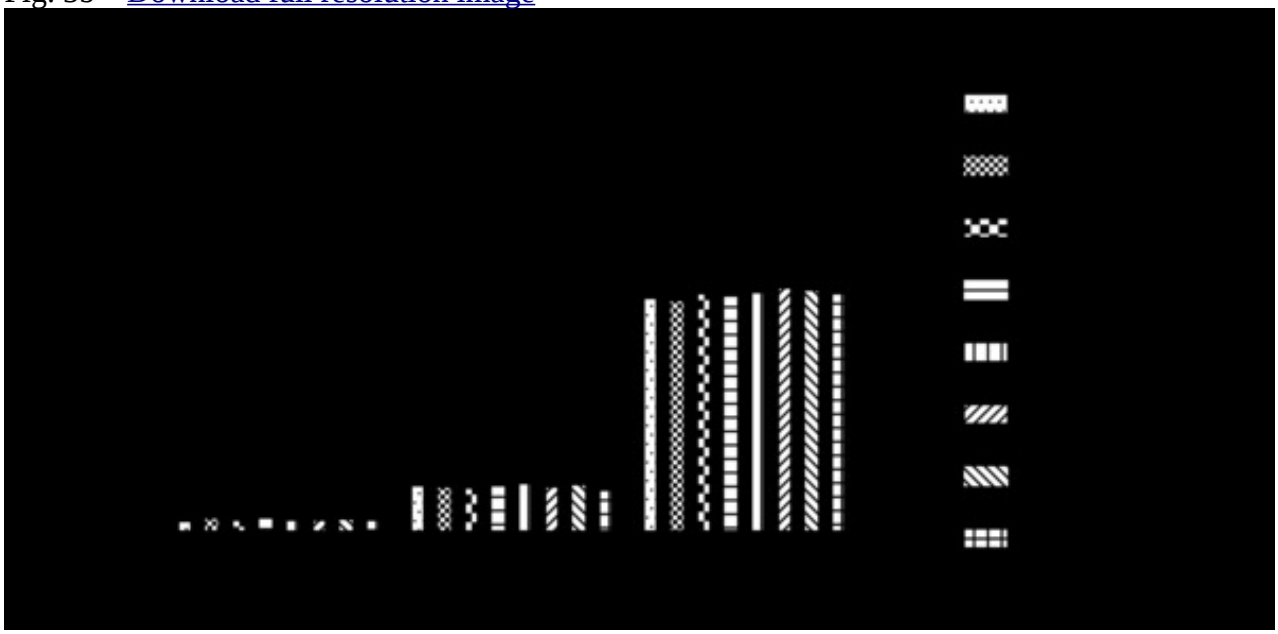

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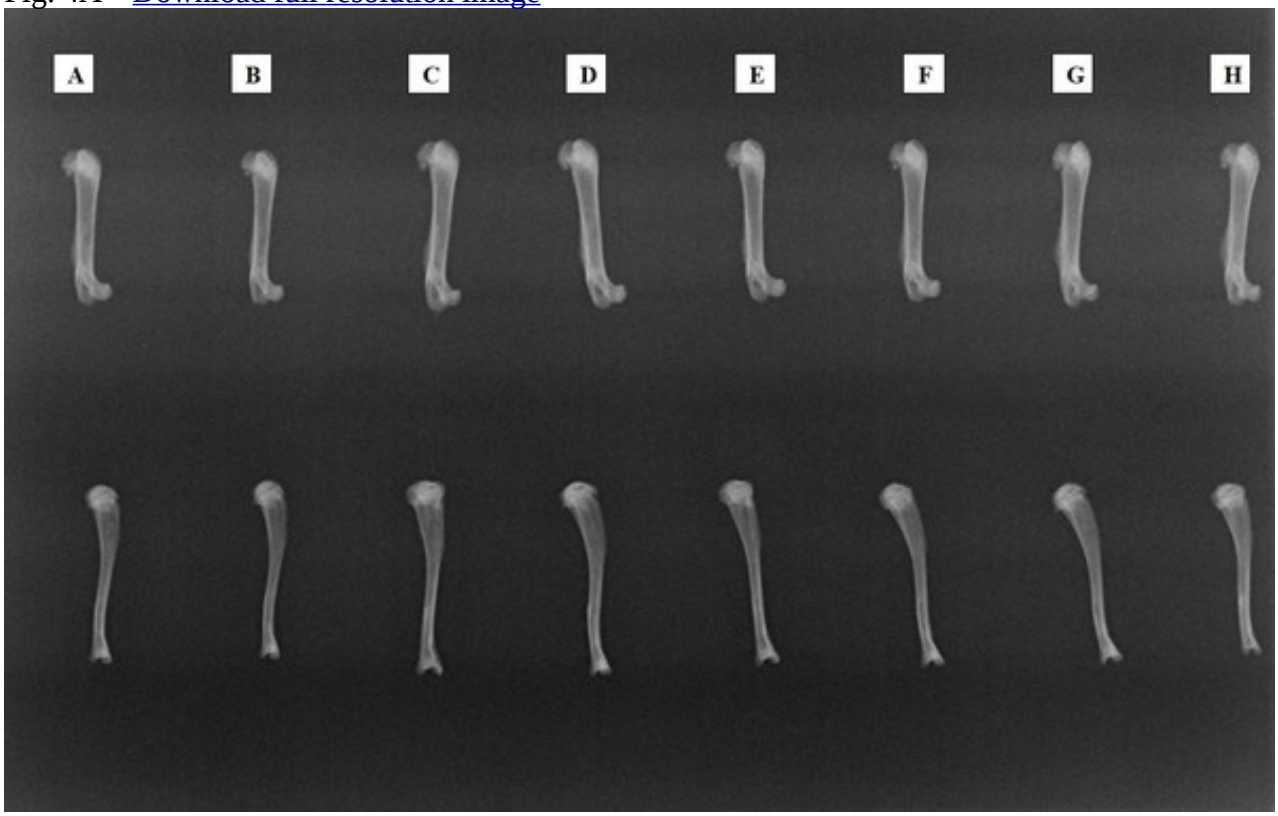

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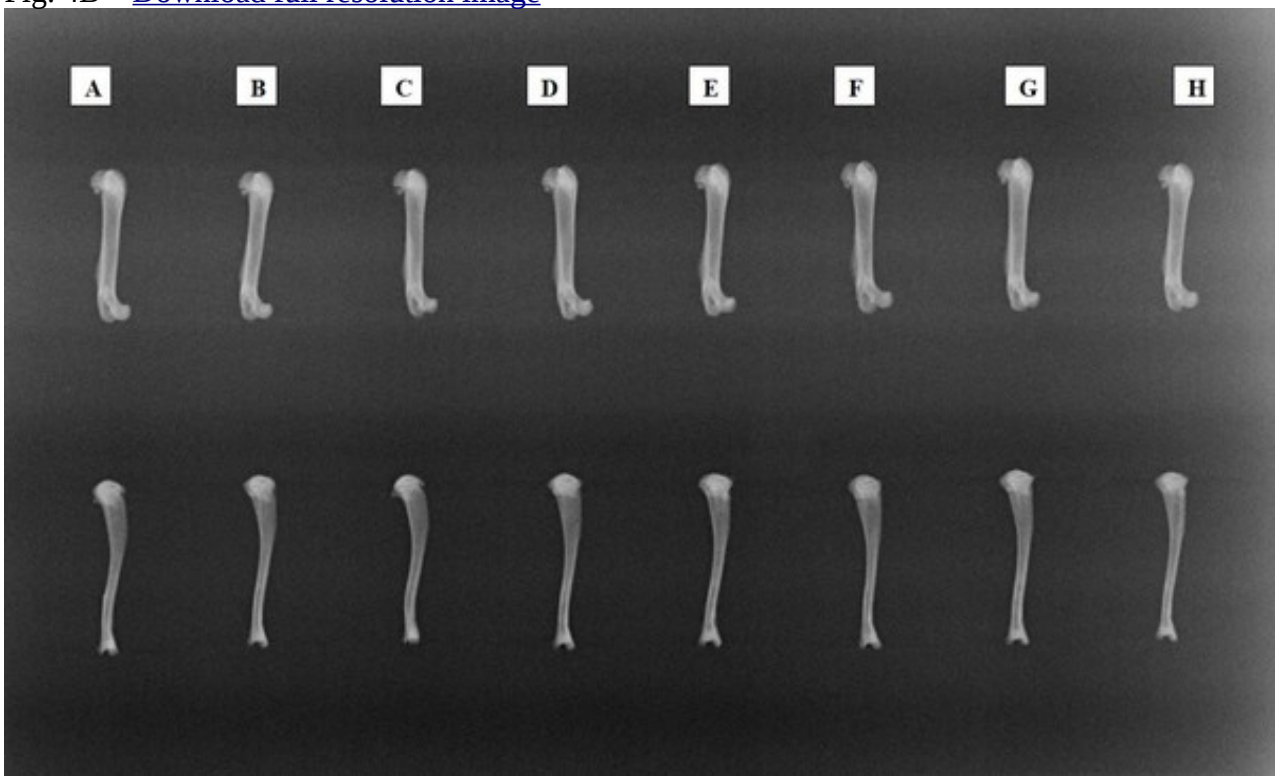

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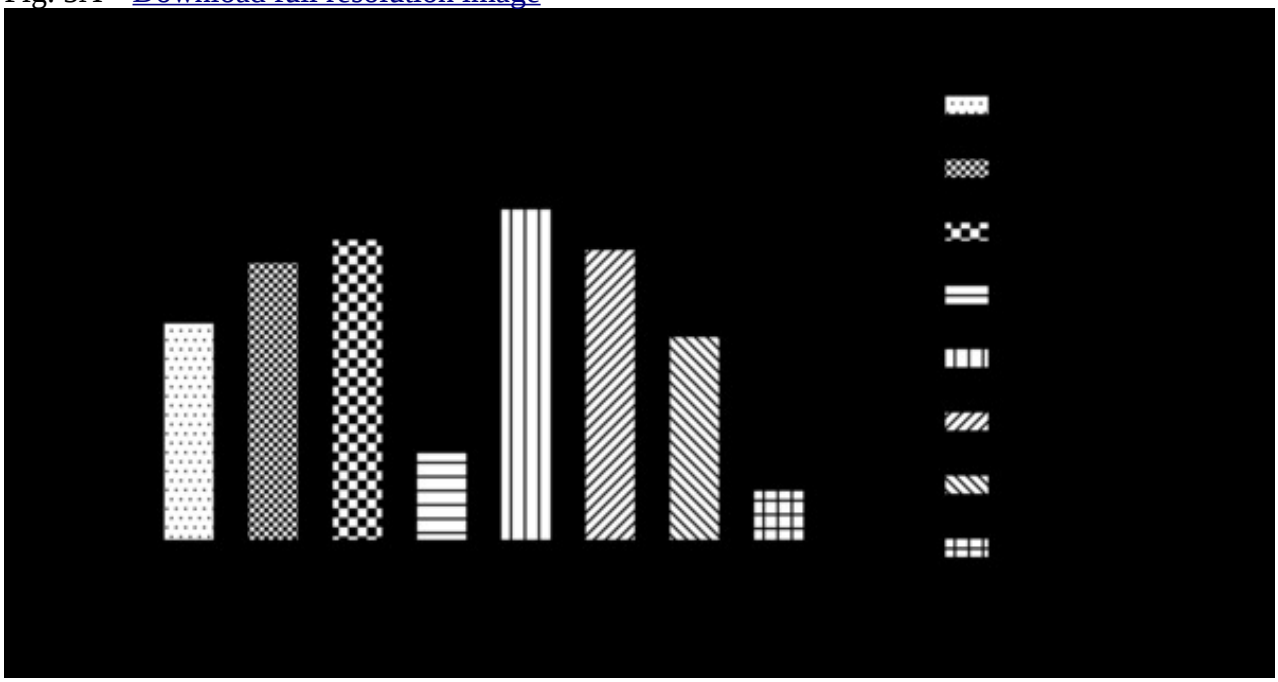

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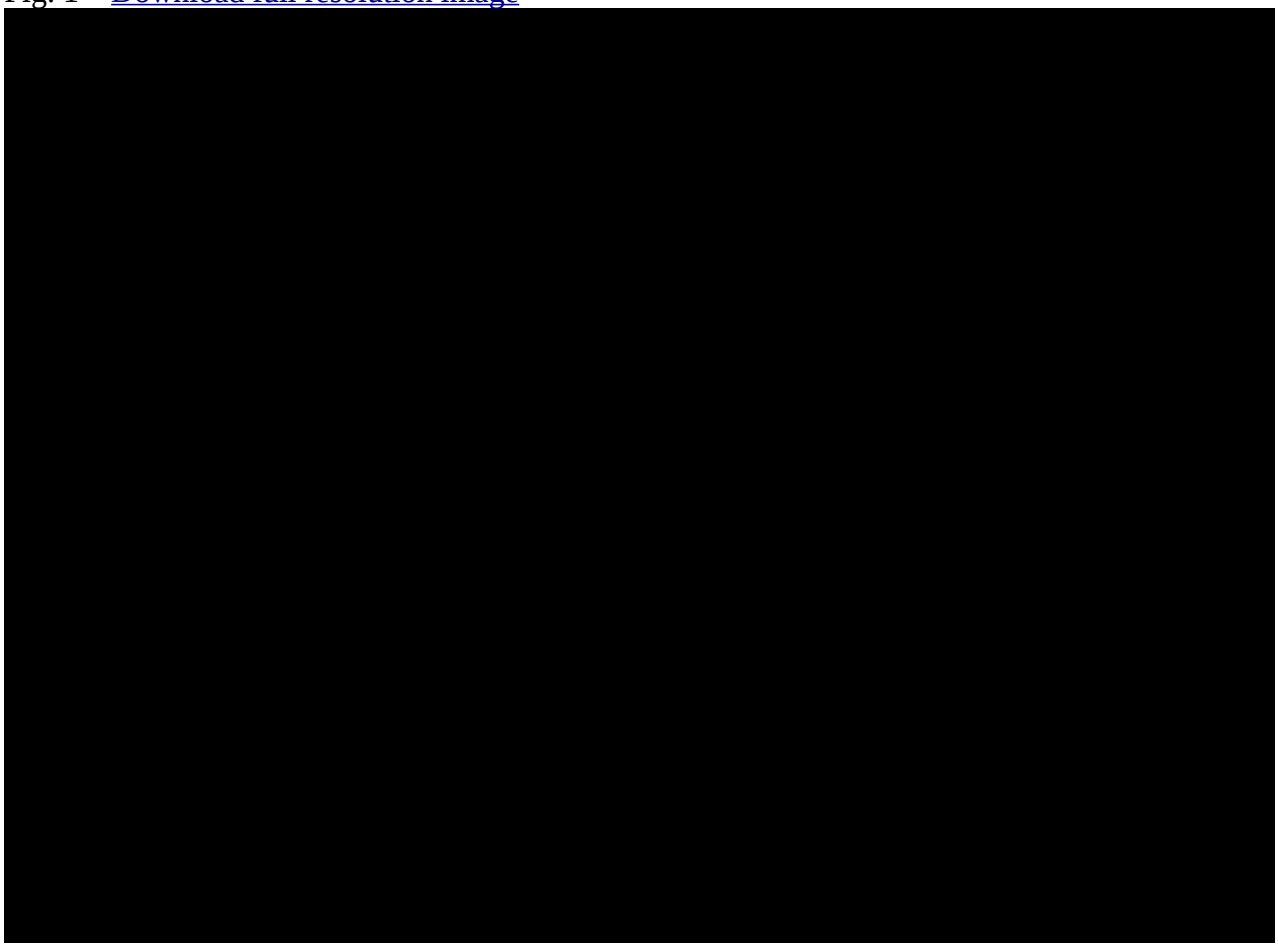

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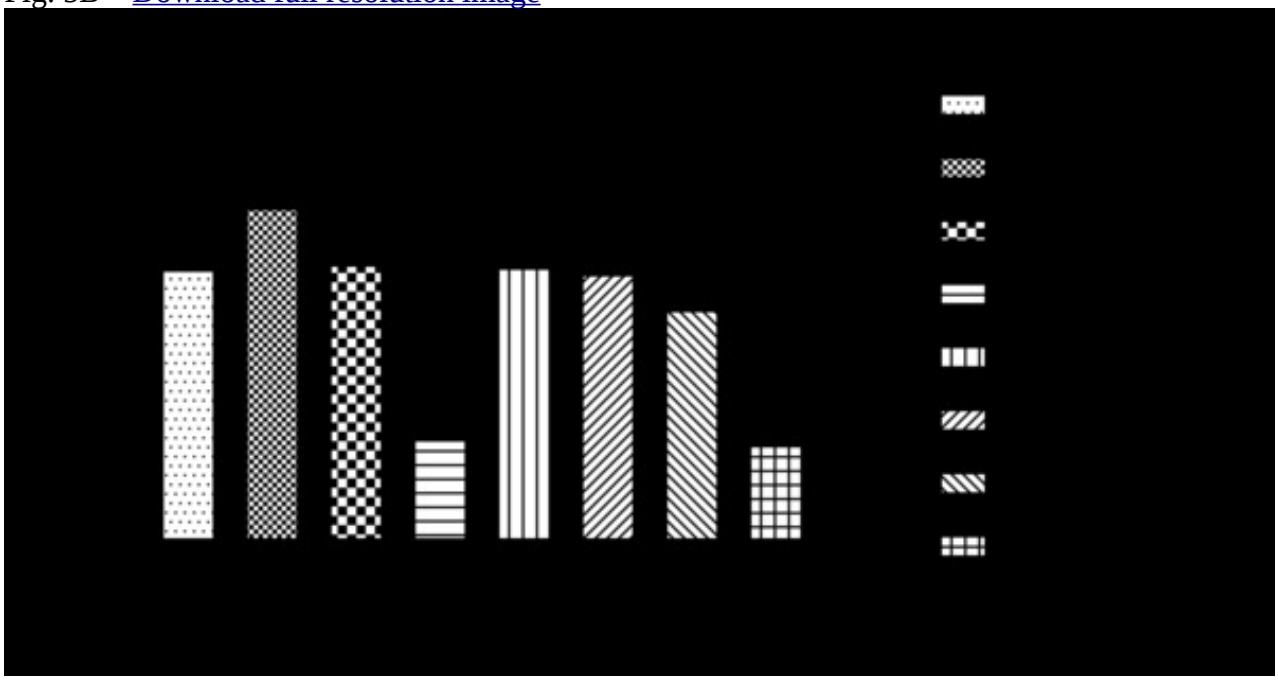

Fig. 2 Download full resolution image