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Reproductive and developmental outcomes, and influence on maternal and offspring tissue mineral concentrations, of (–)-epicatechin, (+)-catechin, and rutin ingestion prior to, and during pregnancy and lactation in C57BL/6J mice

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ABSTRACT

Maternal nutrition can have a significant effect on developmental processes during pregnancy and lactation. While certain flavonoids have been postulated to be beneficial for health, little is known about the effects of ingestion during pregnancy and lactation on the mother and progeny. We report on the effects of maternal consumption of high levels of certain flavonoids on reproductive and developmental outcomes in a mouse model. C57BL/6J female mice were fed a control diet (CT), the CT diet supplemented with 1% or 2% of a mix of epicatechin and catechin (EC1, EC2), or rutin (RU1, RU2) prior to, during pregnancy, and lactation. A subset of dams was killed on gestation day (GD) 18.5 to evaluate fetal outcomes and the remainder was allowed to deliver to evaluate offspring. Maternal food intake, body and tissue weight did not differ among groups. The number of resorptions, implantations, litter size, postnatal survival, body weight, and skeletal development were also similar. Alterations in maternal and offspring liver mineral concentrations were observed. The current results indicate that consumption of high amounts of epicatechin, catechin, and rutin during gestation and lactation is not associated with any marked developmental effects, although changes in liver mineral concentrations were noted.

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Abbreviations: ANOVA, analysis of variance; CT, control diet; EC1, control diet supplemented with 1% mix of (–)-epicatechin and (+)-catechin; EC2, control diet supplemented with 1% mix of (–)-epicatechin and (+)-catechin; EGCG, epigallo-catechin-gallate; GD, gestation day; GTC-H, heat sterilized green tea catechins; GTE, green tea extract; PND, postnatal day; RU1, control diet supplemented with 1% rutin; RU2, control diet supplemented with 2% rutin; SEM, standard error of the means; TBHQ, tertiary butylhydroquinone.

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1. Introduction

Flavonoids are polyphenolic phytochemicals found in a variety of food items and beverages that are grouped into six classes characterized by different chemical structures and physiological properties [1,2]. Data from numerous epidemiological studies support the concept that the chronic consumption of flavonoid-rich diets can be associated with a reduced risk for certain chronic diseases, including cardiovascular, neurodegenerative and select cancers [3–8]. In this context, two subclasses of flavonoids, the flavonols and the flavanols have received particular attention. Flavonols are found in foods and beverages such as onions, leeks, broccoli, cherry tomatoes, red wine, and tea. Rutin (quercetin-3-rhamnosyl glucoside) is a flavonol glycoside composed of quercetin and the disaccharide rutinose. Rutin has been postulated to have anticarcinogenic and vasoprotective activities [9–11]. Flavanols or flavan-3-ols, including the epicatechin and catechin monomers, are widely consumed in teas, red grapes, red wines, apples and cocoa-based products. Similar to flavonols, the flavanols have been reported to have vasoprotective properties including anti-inflammatory and vasodilatory activities [12–16].

With respect to putative health benefits of flavanols and flavonols, results from epidemiological and intervention studies have been largely positive [1,7,15,17–19]. While interest in the potential health benefits of flavonoid intake is considerable, the potential adverse effects of consuming very large amounts of these phytochemicals have not been extensively studied. Many flavonoids are available for purchase as dietary supplements, and it is feasible to achieve levels of intake that markedly exceed what is provided in typical diets. Given that these compounds can have pleiotropic effects, and they have been shown to modulate many cellular activities, including gene expression, cell signaling, enzymatic activity, *etc.*, further examination of their potential negative effects when consumed at high levels is warranted [1,20–22].

The current literature on the physiological consequences of excessive and chronic consumption, specifically in the context of pregnancy and developmental toxicity of these nutrients is limited. Current literature suggests that there is controversy on whether or not the consumption of all flavonoids during pregnancy is safe or detrimental to developmental processes. Some of these reports come from observations of impaired fetal development (heart, genitourinary and limb anomalies), impaired absorption and metabolism of other nutrients, and persistent lasting effects such as increased risk of early onset of diseases (for example acute childhood leukemia) [23–32]. While the above studies supplemented diets with flavonoid-rich foods or flavonoid preparations, there have been few studies investigating the effects of the chronic consumption of specific flavonoids during pregnancy. In the current paper, we tested the hypothesis that the prolonged consumption of high amounts of three flavonoids (epicatechin, catechin, and rutin) during pregnancy and lactation would result in adverse fetal and neonatal development in a mouse model.

2. Materials and methods

2.1. Materials

Purified (–)-epicatechin, (+)-catechin hydrate, and rutin-hydrate, were purchased from Sigma (St. Louis, MO).

2.2. Animals and diets

Virgin, 5-week old C57BL/6J female mice (Jackson Laboratory, Sacramento, CA) were randomly assigned to one of the five diets ($n = 11–12$ per group): a semi-purified control (CT) diet; the CT diet supplemented with a flavanol mixture of epicatechin: catechin, in a 4:1 ratio at 1% of the diet (8000 mg epicatechin + 2000 mg catechin/kg diet; EC1) or 2% of the diet (16,000 mg epicatechin + 4000 mg catechin/kg diet; EC2); or the CT diet supplemented with rutin at 1% of the diet (10,000 mg rutin/kg diet; RU1) or at 2% of the diet (20,000 mg rutin/kg diet; RU2). The flavanol ratio of 4:1 (–)-epicatechin/(+)-catechin reflects the ratio reported in cocoa-containing products [33,34]. Diets were isocaloric and prepared in small batches containing tertiary butylhydroquinone (Dyets, Bethlehem, PA), to minimize oxidation of the flavonoids.

Female mice were fed their respective diets for 3 weeks prior to breeding. Females were bred overnight with C57BL/6J males fed a commercial stock diet (Purina, Laboratory Rodent Diet 5001). Breeding was conducted daily for a maximum of 2 weeks or until successful mating, as determined by the presence of a vaginal plug in the morning (designated gestation day (GD) 0.5). One group of pregnant dams continued to receive their respective diets through pregnancy until GD 18.5, at which time the dams were killed and their litters collected and examined as described below. The remainder of the dams were transferred to maternity cages on GD 16.5 and monitored for delivery. These dams received their respective diets until postnatal day (PND) 21. During this time, food intake was monitored weekly. Litter size was recorded and litters were randomly reduced to eight pups per dam on PND 4. On PND 21, pups were weaned and housed by sex and dams were killed. PND 21 offspring continued to be housed in the maternity cages and were fed the diets that corresponded to their maternal treatment. Pup growth and mortality were recorded weekly until PND 60. On PND 42, pups were individually housed in suspended stainless steel cages and food intake was recorded weekly until PND 60. The offspring were killed on PND 60 and select tissues (liver, heart, lungs, kidneys, and brain) and plasma were collected. This study was approved by the Animal Care and Use Administrative Advisory Committee of the University of California at Davis.

2.3. Tissue and plasma collection

Dams were killed on GD 18.5, or allowed to deliver and killed on PND 21. Dams were killed by CO₂ inhalation and exsanguination. Blood was collected from the femoral artery in heparinized syringes and the plasma was recovered by centrifugation (1500 × *g* for 15 min at 4 °C), and stored at –80 °C. Tissues were weighed, snap-frozen in liquid nitrogen and stored at –80 °C. For GD 18.5 dams, the

uterus was removed and the number of implantation sites and resorptions were recorded. All fetuses were isolated and weights were recorded. Fetuses were examined for gross dysmorphology. At PND 60, the offspring were killed and blood and tissues were collected as described above.

2.4. Tissue mineral concentrations

Maternal and offspring liver samples were prepared for mineral analysis as described by Clegg et al. [35]. Zinc, iron, copper, manganese, and calcium concentrations were measured using ICP spectrophotometry (ICP-AES, Trace Scan, Thermo-Jarrell Ash, Franklin, MA).

2.5. Skeletal staining

On PND 42, one male and one female from each litter were processed for skeletal examination using a double staining standard protocol [36]. Briefly, the pups were killed by CO₂ inhalation and then skinned, eviscerated with forceps, washed, and dehydrated in 95% ethanol for several days; transferred to acetone for 48 h to remove fat, and then placed in a staining solution (0.3% (w/v) alcian blue in 70% ethanol, 0.1% (w/v) alizarin red in 95% ethanol, acetic acid, 70% ethanol) for 3 days. Skeletons were subsequently cleared in 1% potassium hydroxide for 48 h. Before examination, the samples were transferred to solutions of glycerin at graded concentrations of 20%, 50% and 80% for 1 week each, and stored in 100% glycerol. Skeletal analyses included counting the number of ribs, sternum, vertebrae and examining the arm bones (scapula, humerus, radius, ulna) and leg bones (femur, tibia, fibula) for abnormalities.

2.6. Statistical analysis

Statistical analyses were performed using Small Stata version 11.2 (StataCorp, College Station, TX). Significant effects of maternal dietary treatments on reproductive and postnatal outcomes and on maternal and offspring liver mineral concentrations were determined by analyses of variance (ANOVA). Significant effects of offspring dietary treatments on postnatal body weight were determined by repeated measures ANOVA. *Post hoc* analysis was performed to evaluate the significance of observed differences among the groups using the Tukey method.

Data are expressed as mean ± standard error of the means (SEM); statistical significance was set at $P < 0.05$.

3. Results

3.1. Maternal parameters

Average daily food intake before and during gestation was similar among the treatment groups (data not shown). Weight gain was similar among the groups, with the largest increases occurring during gestation (data not shown). There were no differences among GD 18.5 and PND 21 maternal tissue weights (liver, heart, brain, kidney, lungs) either on a relative (tissue/body weight ratio) or absolute tissue weight basis (data not shown). Liver samples

from dams killed at GD 18.5 were assessed for zinc, manganese, copper, iron, and calcium concentrations (Table 1). Significant differences were observed for zinc copper, and calcium. Specifically, the RU2 group had higher zinc concentrations than the CT, EC1, and EC2 groups, higher copper concentrations than the CT and EC2 groups and higher calcium concentrations than the CT and RU1 groups.

3.2. Reproductive parameters

GD18.5 fetal and placental weights were similar among the treatment groups (Table 2). There were no differences in the number of implantation sites or resorptions per litter across the treatment groups (Table 2). No fetal gross dysmorphology or fetal death was noted in any treatment group.

3.3. Postnatal parameters

There were no significant differences among treatment groups for litter size, number of males and females per litter (Table 3). There was 100% survival from PND 0 until PND 4 and only one EC2 litter and one RU2 litter lost two pups from PND 4 to PND 60.

Although not significant, the EC1 group had the smallest litter size due to one litter only containing two pups.

3.4. Offspring parameters

Weekly food intake of the offspring was similar among groups during each week of food intake data collection (PND 42–48, PND 49–55, and PND 56–60), when expressed per litter as well as per sex (data not shown). Body weights were similar among treatment groups over time from PND 21 through PND 60 (data not shown). There were no differences among PND 60 offspring liver, brain, and lungs tissue weight on a relative (tissue/body weight ratio) or absolute tissue weight basis (Table 4). Relative kidney weight was significantly different among groups ($P < 0.001$), but absolute weights were similar (Table 4). Specifically, EC1 offspring had a larger relative kidney weight than the CT, RU1, and RU2 groups. Absolute and relative heart weight was significantly different between treatment groups (Table 4); specifically, absolute heart weight of the offspring in the RU2 group was lower than the EC1 group. Relative heart weight of the RU2 group was lower than the CT, EC1, and EC2 groups. Liver samples from offspring killed at PND 60 were assessed for zinc, manganese, copper, iron, and calcium concentrations (Table 5). Significant differences were observed for copper, iron and calcium; specifically, the EC1 and EC2 groups had lower copper concentrations than the CT. The EC2 group also had lower liver iron concentrations than the CT group. The RU1 and RU2 groups had lower liver calcium concentrations than the CT group. None of the PND 42 skeletons assessed had any missing or malformed bones (data not shown). At PND42 bone calcification was completed and offspring from all groups showed residual cartilage at the nose, ear, tip of the sternum, and in between the rib bones.

Table 1
GD 18.5 maternal liver weight and mineral concentrations.

GD 18.5	CT (n = 5)	EC ₁ (n = 6)	EC ₂ (n = 7)	RU ₁ (n = 7)	RU ₂ (n = 6)
Liver weight (g)	1.8 ± 0.2	1.9 ± 0.1	2.0 ± 0.1	1.7 ± 0.1	1.8 ± 0.1
Zinc (nmol/g)	407.3 ± 16.6 ^a	401.2 ± 9.9 ^a	387.8 ± 18.9 ^a	454.4 ± 21.6 ^{ab}	516.2 ± 31.3 ^b
Manganese (nmol/g)	20.3 ± 1.9	20.1 ± 0.7	18.1 ± 1.4	18.3 ± 1.0	21.2 ± 2.6
Copper (nmol/g)	79.0 ± 7.1 ^a	103.4 ± 12.1 ^{ab}	95.4 ± 13.8 ^a	115.9 ± 8.2 ^{ab}	153.2 ± 15.2 ^b
Iron (nmol/g)	671.6 ± 61.0	679.4 ± 41.4	698.6 ± 51.2	663.9 ± 63.5	735.1 ± 52.0
Calcium (nmol/g)	682.1 ± 35.9 ^a	746.1 ± 36.1 ^{ab}	732.4 ± 31.1 ^{ab}	691.5 ± 32.6 ^a	864.1 ± 52.6 ^b

Values are mean ± SEM; values not sharing the same superscripts are significantly different at $P < 0.05$.

Table 2
Reproductive parameters from GD 18.5 litters.

Diet	Number of implantation sites/litter	Number of resorptions/litter	Fetal weight (g)	Placenta weight (g)
CT	9.3 ± 1.5	1.0 ± 0.6	1.13 ± 0.06	0.11 ± 0.03
EC ₁	8.6 ± 0.4	0.0 ± 0.00	1.07 ± 0.07	0.11 ± 0.01
EC ₂	7.9 ± 1.0	0.9 ± 0.2	1.17 ± 0.03	0.10 ± 0.01
RU ₁	8.0 ± 0.7	0.9 ± 0.5	1.11 ± 0.03	0.14 ± 0.03
RU ₂	9.0 ± 0.5	0.8 ± 0.5	0.98 ± 0.07	0.10 ± 0.01

Values are mean ± SEM.

Table 3
Average litter size and number of males and females per litter, recorded on PND 60.

Diet	Litter size	Number of males/litter	Number of females/litter
CT (n = 3 litters)	7.3 ± 0.7	4.3 ± 0.3	3.0 ± 0.6
EC ₁ (n = 4 litters)	5.8 ± 1.4	2.5 ± 0.5	3.3 ± 1.3
EC ₂ (n = 3 litters)	7.0 ± 0.6	3.0 ± 0.0	4.0 ± 0.6
RU ₁ (n = 4 litters)	7.5 ± 0.3	3.5 ± 0.4	4.0 ± 0.2
RU ₂ (n = 3 litters)	7.3 ± 0.7	3.7 ± 0.4	3.7 ± 0.8

Values are mean ± SEM.

Table 4
PND 60 offspring liver, heart, brain, kidney, and lung tissue weight.

Diet	Absolute weight (g)				
	Liver	Heart	Brain	Kidney	Lungs
CT	0.85 ± 0.03	0.14 ± 0.01 ^{ab}	0.37 ± 0.01	0.17 ± 0.01	0.16 ± 0.01
EC ₁	0.88 ± 0.07	0.15 ± 0.01 ^a	0.38 ± 0.01	0.19 ± 0.01	0.17 ± 0.01
EC ₂	0.89 ± 0.05	0.14 ± 0.00 ^{ab}	0.39 ± 0.01	0.17 ± 0.01	0.15 ± 0.01
RU ₁	0.91 ± 0.04	0.14 ± 0.01 ^{ab}	0.39 ± 0.01	0.17 ± 0.01	0.16 ± 0.01
RU ₂	0.91 ± 0.08	0.12 ± 0.01 ^b	0.38 ± 0.01	0.18 ± 0.01	0.15 ± 0.01
Diet	Relative weight (%)				
	Liver	Heart	Brain	Kidney	Lungs
CT	4.39 ± 0.12	0.74 ± 0.03 ^a	1.92 ± 0.08	0.85 ± 0.03 ^a	0.81 ± 0.04
EC ₁	4.54 ± 0.36	0.75 ± 0.03 ^a	1.97 ± 0.06	0.99 ± 0.04 ^b	0.85 ± 0.03
EC ₂	4.71 ± 0.23	0.72 ± 0.02 ^a	2.12 ± 0.09	0.91 ± 0.04 ^{ab}	0.81 ± 0.03
RU ₁	4.39 ± 0.18	0.66 ± 0.02 ^{ab}	1.91 ± 0.07	0.80 ± 0.02 ^a	0.75 ± 0.03
RU ₂	4.32 ± 0.21	0.60 ± 0.02 ^b	1.88 ± 0.09	0.82 ± 0.04 ^a	0.76 ± 0.05

Values are mean ± SEM; values not sharing the same superscripts are significantly different at $P < 0.05$.

Table 5
PND 60 offspring liver weight and trace mineral concentrations.

PND60	CT (n = 6)	EC ₁ (n = 8)	EC ₂ (n = 8)	RU ₁ (n = 8)	RU ₂ (n = 6)
Liver weight (g)	0.9 ± 0.0	0.9 ± 0.0	0.9 ± 0.0	0.9 ± 0.0	0.9 ± 0.0
Zinc (nmol/g)	416.2 ± 9.4	382.2 ± 15.7	387.5 ± 8.8	392.4 ± 13.9	324.4 ± 63.9
Manganese (nmol/g)	17.9 ± 0.3	15.8 ± 0.1	18.7 ± 1.8	14.9 ± 1.2	18.0 ± 1.7
Copper (nmol/g)	98.6 ± 6.6 ^a	75.5 ± 6.0 ^b	71.9 ± 3.9 ^b	79.5 ± 2.4 ^{ab}	82.0 ± 4.9 ^{ab}
Iron (nmol/g)	2334.0 ± 244.7 ^a	1948.2 ± 124.6 ^{ab}	1676.3 ± 140.4 ^b	1850.2 ± 55.9 ^{ab}	1901.3 ± 87.0 ^{ab}
Calcium (nmol/g)	989.8 ± 125.5 ^a	787.9 ± 30.8 ^{ab}	818.8 ± 46.4 ^{ab}	724.4 ± 25.7 ^b	731.2 ± 11.3 ^b

Values are mean ± SEM; values not sharing the same superscripts are significantly different at $P < 0.05$.

4. Discussion

The present study explored the extent to which high concentrations of epicatechin and catechin or rutin in the diet during pregnancy, lactation and postnatal periods affects reproductive and developmental processes in an *in vivo* mouse model. The rationale for a focus on these three flavonoids was that these three are being actively studied with respect to their potential health benefits. Current evidence of the metabolism and transfer of flavonoids to the fetus during pregnancy indicates that the flavanols, or more accurately their metabolites, are able to reach fetal tissues, where they could potentially interact with developmental processes [37,38]. Chu et al. [37] measured detectable amounts of catechins and catechin gallate derivatives in most fetal tissues within the first hour following the oral gavage of a moderate dose of a green tea extract (GTE) in GD15.5 rat dams. Maternal plasma concentrations of catechins were approximately 50–100 times higher than that measured in the fetus and fetal catechins were detectable over a 20 h period, suggesting limited excretion capacity.

In our study, we did not observe any adverse effects of high-dose epicatechin, catechin or rutin in the maternal diet on fetal growth and development. This absence of observable adverse effects in this *in vivo* study is in seeming contrast with previous *in vitro* reports [39,40]. However, one explanation for a difference in findings between *in vivo* and *in vitro* studies is that flavonoids are extensively and rapidly metabolized by the liver (*i.e.*, methylation, sulfonation, and/or glucuronidation), which likely modulate the cellular bioavailability of these compounds, altering their chemical properties and limiting their biological activity [41–45]. Others have observed nominal reproductive toxicity of flavonoids. Logsdon et al. reported no negative effects on fertility, fetal weight, or prenatal development when CD-1 mice were gavaged 400 or 800 mg/kg/day green tea extract (GTE) alone (contains epigallo-catechin-gallate (EGCG) flavonoid), from gestation days six through thirteen with the exception of an adverse effect on eyelid development; fetal eyelid abnormalities were not observed in our study [46]. In the above study, moderate maternal dosages of GTE (200 mg/kg/day) mitigated the embryotoxic effects of maternal cyclophosphamide intraperitoneal injection on GD10 [46]. Morita et al. [47] also reported no detrimental effects on fertility and fetal growth in rats following the orogastric administration of graded doses of heat-sterilized green tea catechins (GTC-H) in dams between GD 6 to GD 17, daily. Similarly, Isbrucker et al. [48] reported no difference in fetal weight or incidence of external, visceral, or skeletal malformations when Wistar rat dams were fed a standard powdered diet supplemented with epigallo-catechin-gallate (EGCG) daily between GD 6–20 at 0, 100, 300, and 1000 mg/kg/day. A limited number of studies have examined the reproductive consequences of flavonols during pregnancy. Prater et al. [49] reported no significant adverse reproductive effects of low or high dose quercetin (a rutin aglycone) in pregnant C75BL/6 mice. GD14 weights of fetuses from dams fed low or high dose quercetin were similar to those of fetuses of dams fed a control diet. In this study, quercetin seemed to limit the developmental damage caused by the reproductive

toxicant methylnitrosourea. Similar quercetin embryotoxicity preventing effects have been reported in a whole embryo culture model using hydroxurea as the insult agent [50].

Postnatal survival and body weight in our study were not affected. In contrast, Isbrucker et al. [48] reported a significant increase in postnatal death during PND 5–21 in the first and second-generation offspring (compared to the control group) when pregnant rats were fed a standard powdered diet mixed with an EGCG product at approximately 300 and 1000 mg EGCG/kg/day from GD 6–20 for two generations. While a difference in body weight between treatment groups was not observed at any time point in our study, Isbrucker reported that both male and female F₁ rats in the 1000 mg EGCG/kg/day group had lower weight gains and absolute mean body weights compared to the control throughout the course of the study [48]. The differing results for tissue weight observed in our study compared to observations reported by Isbrucker may be attributed to the animal model used (mice *versus* rats) as well as the use of EGCG compared to the specific monomeric compounds used in our study.

The consumption of high amounts of flavonoids has been reported to alter mineral metabolism [1]. This is thought to be due to the ability of the carboxylic and hydroxylic groups of flavonoids to form complexes with metal cations, thus decreasing the amount available for tissue uptake [51]. In addition, flavonoids that can increase metal-binding proteins, such as metallothionein, could have secondary effects on mineral status [30]. An increase in metallothionein in tissue during pregnancy, leading to the sequestration of minerals such as zinc and copper, can reduce the amount of minerals available for uptake by the fetus [52]. We observed increased liver zinc and copper concentrations, in the RU2-fed pregnant dams only. Of interest though is that heart tissue weight (absolute and relative) of the RU2 offspring was smaller compared to some of the other treatment groups. This is important to note as a limited availability of minerals, such as copper, during developmental processes can have lasting, detrimental outcomes [53–55].

In contrast, the observations reported for the offspring showed that compared to CT, copper levels were approximately 25% lower in the EC1 and EC2 groups and iron levels were 28% lower in the EC2 group. Both rutin groups were similar to controls. Kuo et al. [30] reported an interaction between catechin and copper resulting in a decrease in copper concentration in cultured human intestinal cells. This interaction was also observed between catechin and iron concentration. The consumption of flavonoid-rich tea, coffee, and cocoa has also been reported to inhibitor of iron absorption in adults [56]. Previous reports of lower calcium levels in the offspring of RU treated dams were not found.

The high flavonoid diets were well tolerated among all study treatment groups and there were no differences in the amount of food consumed per treatment group. In order to compare flavonoid intake in our study to that of other species including humans, we calculated intake based on metabolic body size [57,58]. In our study, the flavonoid intake during gestation was approximately 693.9 mg/kg^{3/4}/day (EC1), 1215.7 mg/kg^{3/4}/day

(EC2), 665.2 mg/kg^{3/4}/day (RU1), and 1243.8 mg/kg^{3/4}/day (RU2). The amount of flavonoids ingested during gestation in our study is high compared to the amount provided in other studies [47,48]. Morita et al. gavaged-administered GTC-H to Sprague Dawley rats during the second week of gestation. The flavanols, catechin and epicatechin, contributed 12% of the GTC-H that was administered, resulting in a flavanol intake of 57.7 mg/kg^{3/4}/day, 176.9 mg/kg^{3/4}/day, and 297.8 mg/kg^{3/4}/day for the 200, 600, and 2000 mg/kg/day dose groups, respectively [47].

Using flavonoid intake based on metabolic body size, the human equivalent consumption of the amount of flavonoids consumed by the mice during gestation in this study would be approximately 0.09 mg/kg^{3/4}/day (EC1), 0.18 mg/kg^{3/4}/day (EC2), 0.08 mg/kg^{3/4}/day (RU1), and 0.17 mg/kg^{3/4}/day (RU2) for a human weighing 70 kg. It is difficult to compare the intake of flavonoids in this study to typical human intake, as there is considerable variability in reported intakes. Estimated total flavanol intake has been reported at approximately 0.53–2.6 mg/kg^{3/4}/day and total flavanol intake at approximately 0.16–4.96 mg/kg^{3/4}/day [1]. In the Iowa Women's Study, Mink et al. reported flavanol intake of participants at the highest quintile to range from 0.62 to 1.07 mg/kg^{3/4}/day and flavanol (including procyanidins) intake to range from 5.58 to 43.39 mg/kg^{3/4}/day. Even if these are estimations that include all flavonoids, the flavonoid intake in our study greatly exceeds these reports of human ingestion.

5. Conclusions

In summary, the data from this study show that the chronic consumption of maternal diets containing high amounts of flavanols (epicatechin and catechin) or flavonols (rutin) throughout gestation and lactation did not affect litter size, fetal development, postnatal survival, skeletal development or postnatal body weight of the offspring. Flavonoid intake at these high levels were associated with alterations in maternal and offspring liver mineral content as well as lower offspring heart tissue weights in the RU2 group compared to the EC1 group. The reduction in liver copper and iron concentration in the offspring supplemented with epicatechin is important to note since long-term deficiency can result in adverse developmental consequences. Similarly, the functional consequences of lower hepatic calcium concentrations in offspring supplemented with rutin need to be ascertained. We note that the flavonoid intake level in our study is approximately 28 times the highest reported human intake. While dietary intake of flavonoids in humans is unlikely to reach these high levels, we purposely chose to examine the chronic intakes of high amounts of flavonoids because the use of flavonoid supplements could reach or exceed these levels. The results from this study indicate that high intakes of the flavanols epicatechin and catechin and the flavanol rutin are not associated with any overt developmental toxic effects but may interact with the metabolism of certain metals. Additional studies investigating the dose-response curve of flavonoids on hepatic mineral content and the effect on iron, copper, and

calcium-dependent processes in the offspring are warranted. The absence of developmental toxicity and the embryo protective effects of flavanol and flavanol ingestion against known reproductive toxicants in rodents shown by us herein and by others, combined with previous reports of potential health benefits of flavonoid ingestion in complicated pregnancies, support that flavonoid consumption as part of a regular diet is largely beneficial to pregnant women [46,49,50,59–62]. Translating the findings of this animal study into recommendations for pregnant women are limited but a cautious interpretation of the data from this study indicates that the consumption of flavanols and flavanols by pregnant women does not represent a risk.

Transparency document

The [Transparency document](#) associated with this article can be found in the online version.

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