



Natural cocoa improves birth weight and viability of rabbit pups born to hypercholesterolemic mothers



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ABSTRACT

Background: Maternal nutrition affects fetal growth and development in humans and animals. Low birth weight can be caused by maternal hypercholesterolemia and is associated with increased risk of cardiovascular and other metabolic diseases later in adult life. Cocoa powder is rich in flavonoids and reduces plasma cholesterol levels. The study investigated the effect of hypercholesterolemia and natural cocoa intake during pregnancy on the birth weight and viability of rabbit pups.

Methods: Hypercholesterolemic female New Zealand White rabbits were crossed with normocholesterolemic males and randomly grouped into 2 ($n = 4$ each). One group (HCC) received natural cocoa powder in their water in addition to high cholesterol diet during pregnancy, whereas the other (HC) received only water and high cholesterol diet. Litter size, birth weight and viability of pups were assessed and compared to a control group (NC) fed normal rabbit chow ($n = 2$).

Results: Average litter sizes of hypercholesterolemic rabbits (HC and HCC groups) were 3, whereas that of control group, NC was 5. Mean birth weight of pups was significantly lower in offspring of HC (42.73 g, SD 8.47) and HCC (50.0 g, SD 3.54) as compared to NC (73.0 g, SD 5.37). Mortality of offspring was highest in HC group (58.3%) as compared to 50% in HCC.

Conclusions: Maternal hypercholesterolemia during pregnancy reduces litter size, birth weight and viability of offspring in rabbits and these deleterious effects may be minimized by regular intake of natural cocoa. Although further studies in humans need to be conducted, the results of this study suggest the need to monitor maternal cholesterol levels during pregnancy and the effects on pregnancy outcomes and health of offspring later in life.

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Introduction

Intra-uterine environment and nutrition can result in changes that predispose the fetus to cardiovascular and metabolic diseases later in life [1,2] as suggested by the fetal programming hypothesis. Maternal malnutrition and over-nutrition in animal models enhance the development of cardiovascular diseases and metabolic syndrome later in life in the offspring [3]. Exposure to these conditions constitute a disturbance to which cells of the developing fetus adapt in order to survive, leading to irreversible changes or genetic programming that later in life increases susceptibility to diseases [4].

In humans, first evidence for fetal programming *in-utero* showed low birth weight increased the risk for hypertension [5]. Low birth weight is associated with maternal nutrition and women who recorded high carbohydrate intake with low protein and dairy intake in early pregnancy had babies with lower birth weights suggesting impaired intrauterine growth [2,6]. Moreover, epidemiological studies show that death from coronary heart disease is high in individuals born with low birth weight [2]. Putting all these together, maternal nutrition especially early pregnancy may result in priming fetuses to develop disorders that enhances the development of cardiovascular diseases later in life.

Pregnancy, is associated with high levels of cholesterol in maternal circulation and this is a physiological response to increased demands for normal development of the fetus [7]. However, maternal hypercholesterolemia beyond the pregnancy related physiological levels, also known as maternal supra-physiological hypercholesterolemia (MSPH) may predispose the fetus to early development of atherosclerosis and other cardiovascular diseases [8–10]. Although the placenta is known to be restrictive to the transfer of lipids and MSPH may not result in increased cholesterol levels in off spring. There is a correlation between maternal and fetal blood cholesterol levels during pregnancy [11,12] and this may be due to enhanced endogenous synthesis of cholesterol in the fetus. Increased levels of cholesterol in fetal circulation, may enhance atherosclerosis by inducing endothelial dysfunction and intima-media thickening [7,13,14].

Maternal hypercholesterolemia restricts fetal growth which has been shown to be associated with low birth weight and the development of disorders including cardiovascular diseases, later in life [9]. Low birth weight is associated with hypertension and ischemic heart disease as well as overall health outcomes and may be due to restriction of fetal growth and nutrition [6,15,16]. Interestingly, there are no established clinical reference values for maternal hypercholesterolemia and the mechanism of *in-utero* fetal programming is not fully understood [9,10]. Targeting fetal programming and interventions with phytosterols and antioxidants during pregnancy may reduce the adverse effects of maternal hypercholesterolemia on offspring [9,17,18]. Consumption of cocoa is known to reduce oxidative stress and obesity by increasing antioxidant levels [19], high blood pressure as well as risk of coronary heart disease and cardiovascular mortality [20]. This study, aimed at investigating the effect of hypercholesterolemia and natural cocoa intake during pregnancy on the birth weight and viability of rabbit pups. With limited safe lipid-lowering treatments for pregnant women, finding supplements or dietary interventions that inhibit the deleterious effects of maternal hypercholesterolemia on offspring may be beneficial.

Materials and methods

This study was approved by the Ethical and Protocol Review Committee of the College of Health Sciences, University of Ghana.

Animal experiments

Ten adult New Zealand White female rabbits (age: 6 months, body weight: 1.5–2.8 kg) obtained from the Noguchi Memorial Institute for Medical Research (NMIMR) of the University of Ghana were used for this experiment. They were housed under standard conditions of local temperature (28 °C) and relative humidity (80%) and exposed to 12-hour light/dark cycle. Rabbits were randomly assigned by lottery to three groups and housed individually in cages.

Hypercholesterolemia was established as previously described by Blay et al. [14]. In summary, the rabbits were arbitrarily numbered from 1 to 10 and the numbers written on pieces of paper. After mixing the pieces of paper, rabbits were placed in the groups when the numbers were drawn. The first two groups of four rabbits each (HC and HCC) were fed cholesterol-enriched diet which was prepared by mixing standard chow (Kosher Feedmill Ltd, Accra) with 0.5% (w/w) cholesterol (Hopkin and William Ltd, London) and 10% (v/w) coconut oil (open market, Accra). The rabbits were given 24 h unrestricted access to the cholesterol-enriched diet for two weeks, and after a lipid profile test confirmed hypercholesterolemia, they were crossed with normocholesterolemic males. The normal cholesterol range for rabbits is 0.14–1.86 mmol/l. Hypercholesterolemia was defined as a total plasma cholesterol level higher than twice the upper level of the normal range. One group of rabbits given cholesterol-enriched diet was given 2% (w/v) of natural cocoa powder (NCP) (GoodFood brand, Kakawa Enterprise Ltd, Accra) as an aqueous suspension instead of drinking water. This group (HCC) had 24 h access to the NCP suspension, which they drank voluntarily, after being mated until they littered (28–30 days). The second group of rabbits on cholesterol-enriched diet (HC) were given 24 h access to filtered tap water. The third group of rabbits ($n = 2$), designated as normal control (NC), were given standard chow without cholesterol enrichment and filtered tap water throughout the duration of the experiment.

In the previous paper [14], we demonstrated alteration of lipid profile and intima-media thickness of aorta in rabbits born to hypercholesterolemic mothers fed same dose of cholesterol-enriched diet. The present paper focuses on the effect on birth weight and viability of pups born to experimentally-induced hypercholesterolemic mothers.

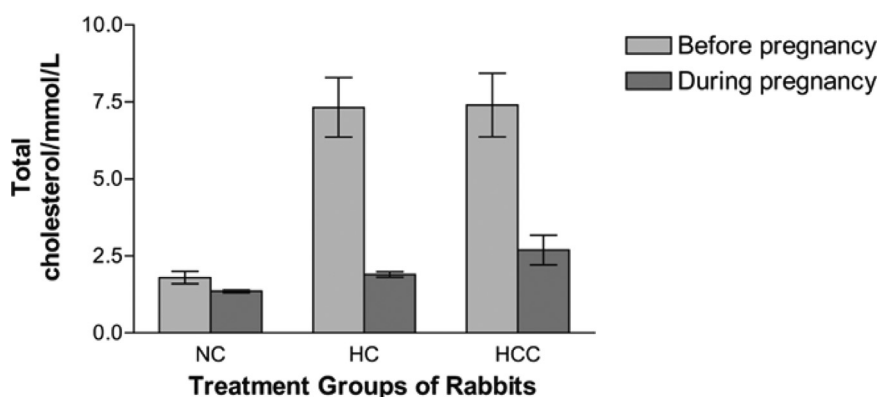


Fig. 1. Mean serum cholesterol just before pregnancy; after hypercholesterolemia had been induced and during pregnancy. NC = Control group, HC = group given cholesterol but not cocoa and HCC = group given cholesterol and cocoa.

Blood samples were obtained by bleeding of the marginal ear vein of the female rabbits 3 weeks into pregnancy and total cholesterol levels during pregnancy assessed. Total levels of cholesterol were determined after an overnight fast by an enzymatic colorimetric test in a laboratory at the Medical Biochemistry Department (University of Ghana Medical School), using a semi-automated clinical analyser, Microlab 300 (Vital Scientific, the Netherlands). The skin over the ear of the rabbits was anaesthetised using a local anaesthetic cream containing lidocain (Lignocaine 2% Jelly, Purna Pharmaceuticals, Belgium) after the fur over the ear was shaved and the skin sanitised with alcohol. Blood samples were then drawn and stored in sterilised test tubes containing heparin.

Litter size, birth weight and mortality rate of pups

The number of pups or litter size was recorded for each animal after delivery and each pup weighed. Mortality was recorded as number of pups that died within 1 week post-delivery. There were no deaths of the pups beyond 1 week post-delivery.

Statistical analyses

The results were analysed using Graphpad Prism software (5.0). T-test was used to compare the means of two groups while one-way ANOVA was used to compare the means of three groups. A p -value < 0.05 was considered to be statistically significant. Bonferroni's Multiple Comparison Test was done to show actual differences between the three groups.

Results

Hypercholesterolemia in female rabbits during pregnancy

Cholesterol levels in hypercholesterolemic mothers decreased significantly during pregnancy (3 weeks of pregnancy) as shown in Fig. 1. Mean cholesterol level of HC during pregnancy was 1.9 (SD 0.18) mmol/l compared to 7.33 (SD 1.93) mmol/l before pregnancy. An unpaired T-test showed a significant difference ($p < 0.05$, $t = 5.60$ and $df = 6$) between cholesterol levels of HC before and during pregnancy. The HCC group had a mean cholesterol level of 2.7 (SD 0.97) mmol/l during pregnancy and this was significantly lower ($p < 0.05$, $t = 4.14$ and $df = 6$) than the mean cholesterol level of 7.40 (SD 2.05) mmol/l before pregnancy. During pregnancy, mean cholesterol levels of rabbits receiving high cholesterol diet showed no significant difference from control rabbits as shown in Fig. 1.

Litter size and mortality rate

The total number of pups delivered by control group ($n = 2$) was 10, giving an average litter size of 5 pups, whereas HC group ($n = 4$) delivered a total of 12 pups (average litter size = 3) (Table 1). The total number of offspring of the HCC group ($n = 4$) was 6. Two rabbits from the HCC group did not litter at all and so average litter size (3) was calculated using the two that littered. Neonatal mortality was highest among HC group (58.3%), whereas the mortality rate of HCC pups was 50% (Table 1). No death was recorded in the control group.

Birth weight of pups

Differences in birthweight of the neonates of the three groups were statistically significant ($p < 0.0001$, $F = 58.42$ and $df = 2$) as shown in Fig. 2. Offspring or pups of the control rabbits (NC) had the highest mean birthweight of 73.0 (SD 5.37)

Table 1
Litter size and mortality rate of rabbit pups.

Treatment Group	Number of Litter	Average litter size	Mortality rate (%)
NC (<i>n</i> = 2)	10	5	0
HC (<i>n</i> = 4)	12	3	58.3
HCC (<i>n</i> = 4)	6	3 ^a	50

^a 2 mothers did not litter.

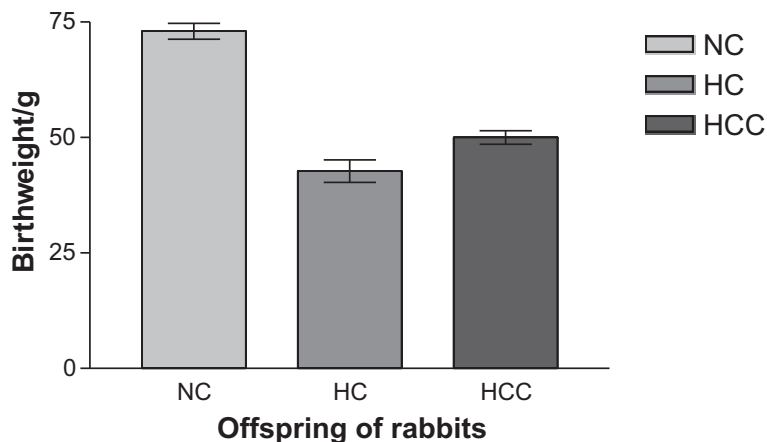


Fig. 2. Mean birth weight of offspring. NC = Control group, HC = group given cholesterol but not cocoa and HCC = group given cholesterol and cocoa.

g followed by that of HCC with 50.0 (SD 3.54) g. Pups from the HC group had lowest mean birthweight of 42.73 (SD 3.54) g. Statistically significant differences existed between NC and HC ($p < 0.001$) and NC and HCC ($p < 0.001$). Mean birth weight of HCC was higher than the mean birth weight of HC but was not statistically different ($p > 0.05$).

Discussion

Maternal hypercholesterolemia during pregnancy and low birth weight (LBW) predispose offspring to developing cardiovascular diseases later in life. Cocoa consumption is known to be protective against cardiovascular diseases. This study shows maternal hypercholesterolemia during pregnancy reduced significantly birth weight and viability of rabbit pups and consumption of natural cocoa minimized this effect.

Studies in humans show that low birth weight increases rate of illness and reduces significantly the probability of survival within the first year [16,21,22]. LBW is also associated with coronary heart disease, stroke, hypertension, type 2 diabetes, obesity and hyperlipidaemia later in life [23,24]. The present study showed that maternal hypercholesterolemia is associated with low birth weight and increased mortality in rabbit pups. Mortality rate was highest in the group with the lowest birth weight, while no death was recorded in the control group that had the highest birth weight. Low birth weight in this study may have been the result of exposure to high levels of cholesterol in circulation resulting in an abnormal intra-uterine environment, leading to restricted growth and hence reduced weight at birth [25–28].

The mechanism by which maternal hypercholesterolemia restricts fetal growth is yet to be fully understood but increased inflammation and oxidative stress that lead to impaired vascular function may be implicated [29–32]. Ingestion of cocoa by hypercholesterolemic mothers increased birth weight slightly although the change was not statistically significant. Regular intake of natural cocoa also decreased the mortality from 58.3% to 50%. Altering the dosage of natural cocoa may enhance the beneficial effects on birth weight and viability [33]. Reduced litter size in this study may be attributed to inhibited intra-uterine development [34]. Further investigations is required to elicit the mechanisms through which hypercholesterolemia reduces growth and survival, however, evidence from previous studies suggest natural cocoa through its antioxidant activity may reduce oxidative stress and inflammation and hence preserve vascular function and growth during development [35].

An interesting observation of this study was that although pregnant rabbits continued to receive high cholesterol diet, maternal cholesterol levels significantly reduced during pregnancy. In humans, cholesterol levels during pregnancy has shown conflicting results with some studies indicating lower levels during pregnancy, while other studies showed higher levels [36]. Maternal cholesterol levels may reduce during pregnancy due to the high demand for cholesterol for development of the fetus [37] and differ during the course of pregnancy [38,39]. In the early stages, cholesterol levels are high due to increased synthesis, but in later trimesters there is an increased break down of lipid storage for the growing fetus [37]. Another possible explanation why maternal cholesterol levels were reduced is that, there may have been increased cholesterol transport into fetal circulation [40]. Cholesterol is an essential component of cell membranes and is therefore in high demand during processes like cell proliferation, cell differentiation and cell-to-cell communication as well as metabolism

during fetal development [38]. Although the transfer of cholesterol into the fetus is physiologically necessary to enhance growth, high cholesterol levels in the mothers at the beginning of pregnancy may have led to higher levels of cholesterol in fetal circulation. Increased cholesterol transfer beyond physiologically normal levels may therefore have restricted growth, resulting in low birth weight and decreased survival of pups.

Natural cocoa intake minimized to an extent the effects of high cholesterol during pregnancy. The beneficial effects of cocoa may be dose-dependent and as such may account for the reason why a significant reduction in birth weight was not observed in this study [33,41]. Cocoa may reduce the deleterious effects of maternal hypercholesterolemia on birth weight and viability when consumed at the appropriate dose. Further studies will be required to determine the right dose. The mechanism by which cocoa protects against pathological changes also needs further investigations, but experimental evidence supports the fact that improvement of vascular function and antioxidant activity are benefits of cocoa intake [42]. Consumption of cocoa increases serum high density lipoprotein (HDL) and decreases oxidation of low density lipoprotein (LDL) and these effects are associated with decreased cardiovascular diseases [43].

Conclusions

Maternal hypercholesterolemia during pregnancy reduces litter size, birth weight and viability of rabbit pups and ingestion of unsweetened natural cocoa may improve birth weight and viability. The results of this study provide evidence for further study both in animals and humans to understand cholesterol metabolism in pregnancy and to monitor the effect of increased maternal cholesterol on offspring.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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