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# Effects of Putrescine, Spermidine and Spermine on Growth and Serum Lipid Levels in *Sprague-Dawley* Rat Offspring

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## ABSTRACT

Polyamines are short-chain, basic biogenic amines that are essential for cell growth and reproduction. This study was conducted to examine the effects of maternal polyamine intake on growth and serum lipid levels in first generation rat offspring. Female Sprague-Dawley rats (n=35) of 8 weeks old were used in the study. Rats were divided into five groups according to the polyamine they are fed as putrescine, spermidine, spermine, putrescine-spermidine-spermine, and control group. Before pregnancy, during pregnancy and lactation polyamines were administered to rats by oral gavage. After the offsprings were born, weights were measured every two days. Blood samples were taken when they were one month old and serum lipid analyzes were performed. When the groups were compared with the control group, it was shown that spermidine and spermine significantly increased the total cholesterol level, spermidine and putrescine-spermidine-spermine significantly decreased the triglyceride level and significantly increased the HDL level of the spermine. When birth weight averages and final weight averages were compared, it was seen that the group given putrescine-spermidine-spermine had the highest value. In conclusion, this study shows the effects of maternal polyamine intake on growth, total cholesterol, HDL, LDL and triglyceride levels of rat offsprings.

**Key words:** Polyamine, putrescine, spermidine, spermine, lipid profile

## INTRODUCTION

Polyamines are short-chain, basic biogenic amines that are found in almost all living organisms and are necessary for cell growth and reproduction. Putresci-

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ne, spermidine and spermine consisting of arginine, ornithine, and methionine amino acids, are common polyamines found in eukaryotes[1-4].

Putresin, spermidine, and spermine are synthesized in mammalian cells. In addition, it can be taken into the body through diet and intestinal bacteria. Polyamine synthesis in tissues and organs decrease with aging[5]. Polyamine biosynthesis occurs in the G1 phase of the cell cycle and is required for process that initiate cell differentiation[6]. The first synthesis step of polyamines is the synthesis of ornithine, the precursor of polyamines, from the amino acid arginine. Synthesis of three important polyamines found in the cells of all mammals takes place starting from ornithine. Putrescine is synthesized from ornithine by means of ornithine decarboxylase enzyme. Spermidine is synthesized from putrescine by spermidine synthase and spermine is synthesized from spermidine by spermine synthase[4,7-11]. Polyamine levels in cells are altered by synthesis, catabolism, and transport[12,13]. Dietary polyamine, which is one of the important factors determining the amount of polyamine in the body, is absorbed in the small intestine before entering the systemic circulation, passed through the duodenum and proximal jejunum lumen into the blood by passive diffusion to the whole body and used for cell growth[9,14]. High levels of polyamine are found in a variety of foods and beverages such as broccoli, mushrooms, green peppers, rice bran, green tea, mushrooms, soybeans and oranges[14]. Polyamines are also synthesized microbially in the intestine. This synthesis varies according to the type of microorganisms[12].

In fast-growing tissues, metabolic polyamine requirement is quite high during normal growth and development[15]. Polyamine biosynthesis is therefore high in conditions requiring rapid cell growth such as neonatal period and newborn period[11,16]. When polyamine is absent or insufficient in the cell, cell proliferation is inhibited and sometimes results in cell death. In case of inhibition of enzymes involved in polyamine biosynthesis, cell growth slows down[17]. Polyamine deficiency may have negative effects on reproduction. Therefore, it is recommended that diets rich in polyamine be used in reproductive studies. It is recognized that polyamines are essential in reproductive processes and have an important role in embryo and placental development[18]. ODC activity in the placenta is much higher than in the fetus. However, the increase in the amount of polyamine in the fetus is significantly higher than in the placenta. With this increase, the growth rate of fetal tissue increases[13]. Polyamines are important for the uterus during pregnancy during myometrial cell proliferation and development of hypertrophy. Spermine has a relaxing effect on the contraction of the uterus. It also exerts a similar effect by reducing intracellular calcium concentration in vascular smooth muscle[19].

Breast milk, which is the main source of newborns, contains putrescine, spermidine and spermine. While spermidine and spermine are found in similar concentrations in human milk, both decrease during lactation period. In rat milk, spermidine is found more than spermine and increases in lactation period.

Polyamines have an important role in the growth and development of the digestive system of newborn mammals. It is also important for normal growth and maintenance of the general characteristics of the adult digestive system. Spermidine and spermine have effects such as structural and functional development of the small intestine, enlargement of villi, elongation of crypts, cell proliferation, increase in maltose and sucrose activity, and decrease in lactase activity in rats[20].

Because polyamines are cationic, they tend to interact with anionic structures such as nucleic acid, deoxyribonucleic acid (DNA), ribonucleic acid (RNA), adenosine triphosphate (ATP), some proteins, and phospholipids. These interactions are essential to the biological functions of polyamines. It stabilizes the negative charges of phosphate groups on DNA. It acts on protein synthesis by affecting the secondary structure of RNA and by binding to ribosomes and bringing its subunits together[3,14,21-23]. It also plays an important role in cell growth, proliferation, differentiation, development, immunity, migration, gene transcription, gene expression, and DNA stability as well as nucleic acid and protein synthesis[3,9,13,14,24,25].

Polyamines (3-6%) bind to the membrane with anionic phospholipids and stabilize the membrane. In addition, the polyamines in the serum interact by binding with anionic phospholipids on the surface of proteins and lipoproteins[1]. Thus, lipid peroxidation is inhibited by polyamines. Spermine, spermidine and putrescine, respectively, have a strong antioxidant effect on LDL oxidation. This is due to the ability of polyamines to form complexes with $Cu^{2+}$ and is positively correlated with the number of amine groups of these molecules[26].

According to all this information, polyamines have effects on both growth and development and serum lipid levels. Therefore, in this study, it was aimed to investigate the effects of maternal polyamine intake on growth and serum lipid levels in first generation offspring rats.

## METHODOLOGY

The study was approved by Istanbul Medipol University Animal Experiments Local Ethics Committee, number 38828770-604.01.01-E.706, decision number 10 and dated 14.01.2016, ethical rules were followed during the study.

### Experimental Animals

This study was carried out in Istanbul Medipol University Medical Research Center (MEDITAM). *Sprague-Dawley* rats used in the study were obtained from the same center.

Female *Sprague-Dawley* rats (n=35), 8 weeks old, weighing an average of 156 grams (between 128-182 grams) were used in the study. The number of rats was kept to a minimum in accordance with the literature[27]. All rats were kept at standard environmental conditions (20-22°C temperature, 55-65% humidity and 12 hours night - 12 hours day), and standard pellet feed and fed *ad libitum* with tap water. Marking was made on rats by tail staining method so that each rat could be followed.

### Study Design

After adaptation period, the rats were randomly divided into five groups. Tap water for control was given to the first group, putrescine to the second group, spermidine to the third group, spermine to the fourth group, putrescine, spermidine and spermine to the fifth group through oral gavage. The offspring of the rats given different substances were divided into five groups as follows:

- Group 1 (G1): offspring rats born and fed to mothers given water by oral gavage (control)
- Group 2 (G2): offspring rats born and fed to mothers given putrescine by oral gavage
- Group 3 (G3): offspring rats born and fed to mothers given spermidine by oral gavage
- Group 4 (G4): offspring rats born and fed to mothers receiving spermine by oral gavage
- Group 5 (G5): offspring rats born and fed from mothers given putrescine, spermidine and spermine by oral gavage

### Dietary intervention

The rats in the control group were fed only with pellet feed during the experiment. The rats in the other groups were fed dietary polyamine as well as pelleted feed.

Putrescine [Putrescine dihydrochloride, 98.0% (TLC), 25g, Sigma], spermidine [Spermidine trihydrochloride, ≥99.5% (AT), 5g, Sigma] and spermine [Spermine tetrahydrochloride, ≥ 99.5% (AT), 1g, Sigma] was dissolved in distilled water. The amounts given to rats were calculated as 90 μg/g putrescine, 41.5 μg/g spermidine, 9.5 μg/g spermine. The amount of putrescine, spermidine and spermine given to rats is similar to previous studies and does not exceed the no observed adverse effect level (NOAEL) in experimental animals[27].

Before oral gavage, the amount of polyamine to be given was calculated by measuring the weights of all rats with precision weighing. Oral gavage was performed every three days for four weeks before pregnancy. At the end of one month, all rats were mated for a week and oral gavage was not performed during this period. Oral gavage was performed every three days during pregnancy (three weeks) and every two days during lactation (four weeks). The reason for not being able to perform oral gavage every day is the possibility that rats may harm their health by irritating the esophagus. The rats in the control group were exposed to the same stress as the rats in the other groups by oral gavage with water. Offspring rats were fed with breast milk for four weeks. Thus, polyamines given to mother rats by gavage were passed on to the newborn through breast milk.

**Analyzing blood samples**

One month after birth, the offspring rats were anesthetized by intraperitoneal administration of Rompun (Xylazine) (2%) and Ketasol (Ketamine) (10%), and 1 mL of blood samples were taken from the jugular vein. The blood samples taken were centrifuged at 3000 rpm for 10 minutes at +4°C within 30 minutes, the serum was separated and transferred into eppendorf tubes and kept at -20°C until analyzed.

**Determination of serum lipid levels**

Serum samples were analyzed in the Biochemistry Laboratory of Medipolitan Sağlık ve Eğitim Hizmetleri A.Ş. by applying standard methods, and total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol and triglyceride (TG) levels were determined. Cholesterol value was analyzed by enzymatic method, HDL and triglyceride value by colorimetric method, LDL value calculation method [LDL=Cholesterol-(TG/5+HDL)][28] Cobas 6000 (Roche, Tokyo, Japan) by working in biochemistry autoanalyzer.

### Termination of the study

The adult and offspring rats were given anesthesia by intraperitoneal injection of Rompun and Ketasol. The study was terminated by performing cervical dislocation as a result of general anesthesia. Adult rats were euthanized after 4 weeks of lactation, and offspring rats 4 weeks after birth.

### Calculation of specific growth rates

The first weight of newborn rats was measured and recorded one day after birth. Weight measurement was continued every two days for a month. Since the offspring in the groups could not be marked individually, weight tracking could not be made individually, so the total weight averages were recorded as group average.

The individual growth rate was calculated as follows[29,30].

Specific growth rate (%) = [(ln w2–ln w1)/days]x100

w1: initial body weight (gram); w2: final body weight (gram); days: number of days between recordings of w1 and w2.

### Statistical analysis

All analysis was performed using IBM SPSS Statistics, v.18.0. The normality of the distribution of the variables was evaluated with the Kolmogorov-Smirnov test. When comparing more than two groups, we used analysis of variance (ANOVA) with one factor or the nonparametric Kruskal Wallis test. Differences between groups were analyzed using the Tukey test when the variances of the groups were homogeneous and the Tamhane's T2 test when they were not homogeneous. P values below 0.05 were considered statistically significant. The data are presented as the mean ± standard deviation (SD).

## RESULTS AND DISCUSSION

The average of the first and last weights and specific growth rates of the offspring rats according to the groups are shown in Table 1. It was determined that the group with the highest first and last birth weight averages was G5, and the group with the highest specific growth rate was G4.

**Table 1.** Weight averages and specific growth rates of offspring rats

| | *Initial weight, gram | *Final weight, gram | Specific growth rate, % |
|---|---|---|---|
| **G1 (n=34)** | 5.77 | 63.61 | 8.00 |
| **G2 (n=36)** | 5.65 | 71.44 | 8.46 |
| **G3 (n=50)** | 5.70 | 63.63 | 8.04 |
| **G4 (n=16)** | 7.02 | 90.30 | 8.52 |
| **G5 (n=9)** | 7.44 | 93.22 | 8.43 |

G1, Group 1; G2, Group 2; G3, Group 3; G4, Group 4; G5, Group 5

*Results are expressed as the mean

### Offspring body weight change

Body weight changes of offspring rats are shown in Figure 1. Growth and development of offspring rats differ according to the polyamines they receive from their mothers via the placenta. When the data are examined, it is seen that the offspring in the G1 group reach the lowest weight and the ones in the G5 group reach the highest weight.

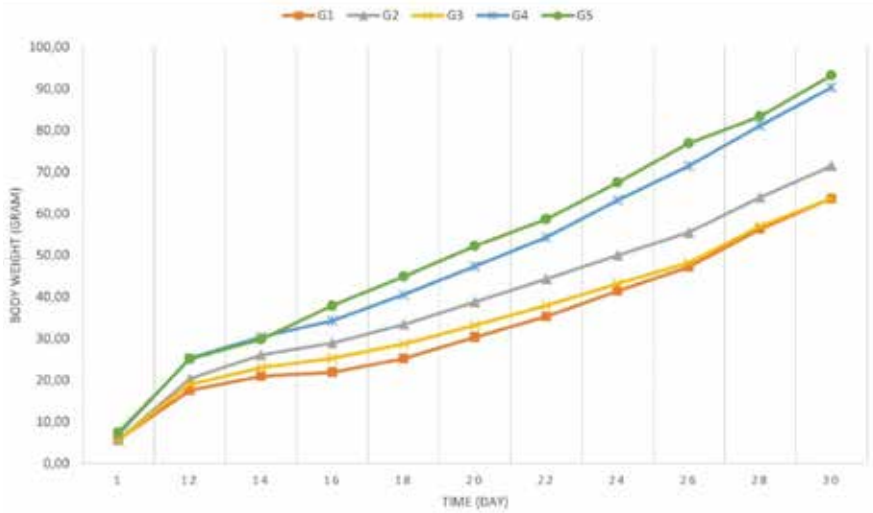


**Figure 1.** Body weight change of offspring during growth

G1, Group 1; G2, Group 2; G3, Group 3; G4, Group 4; G5, Group 5

Body weights are expressed as the mean

### Changes in serum lipid levels

Total cholesterol, triglyceride, HDL, and LDL cholesterol levels of offspring rats according to the type of polyamine taken from both placenta and breast milk are shown in Table 2. When the data obtained were examined, it was found that there were significant differences between groups are shown in Table 3 in terms of total cholesterol, HDL cholesterol and triglycerides.

**Table 2.** Results of serum lipid levels of offspring

| | Total cholesterol mg/dL | Triglyceride mg/dL | HDL cholesterol mg/dL | LDL cholesterol mg/dL |
|---|---|---|---|---|
| **G1** | 51.77±6.23$^{ab}$ | 64.21±17.12$^{fg}$ | 45.69±5.27$^{j}$ | 0.07±0.26 |
| **G2** | 48.28±6.43$^{cd}$ | 55.54±13.27$^{h}$ | 41.73±5.80$^{klm}$ | 0.34±1.07 |
| **G3** | 56.90±8.02$^{ac}$ | 44.02±13.31$^{fhi}$ | 48.46±6.28$^{kn}$ | 1.55±2.77 |
| **G4** | 62.16±6.51$^{bde}$ | 60.20±12.64$^{i}$ | 57.57±5.72$^{ilno}$ | 0.50±1.37 |
| **G5** | 50.86±5.23$^{e}$ | 49.12±10.09$^{g}$ | 48.16±5.78$^{mo}$ | 0.00±0.00 |
| ***p*** | <0.001* | <0.001* | <0.001* | 0.055$^{£}$ |

G1, Group 1; G2, Group 2; G3, Group 3; G4, Group 4; G5, Group 5; HDL, high-density lipoprotein; LDL, low-density lipoprotein

Results are expressed as the mean ± SD

$^{a, b, c, d, e, f, g, h, i, j, k, l, m, n, o}$: Means that have superscript in common are significantly different from each other.

******One-Way-Anova Test*

**£***Kruskal Wallis Test*

**Table 3.** Statistical results (p values) of total cholesterol, triglyceride, and HDL levels between groups

| | | G1 | G2 | G3 | G4 | G5 |
|---|---|---|---|---|---|---|
| Total Cholesterol | **G1** | - | 0.294 | **0.018*** | **<0.001***** | 0.997 |
| | **G2** | 0.294 | - | **<0.001***** | **<0.001***** | 0.863 |
| | **G3** | **0.018*** | **<0.001***** | - | 0.074 | 0.125 |
| | **G4** | **<0.001***** | **<0.001***** | 0.074 | - | **0.001**** |
| | **G5** | 0.997 | 0.863 | 0.125 | **0.001**** | - |
| Triglyceride | **G1** | - | 0.117 | **<0.001***** | 0.888 | 0.043* |
| | **G2** | 0.117 | - | **0.004**** | 0.812 | 0.742 |
| | **G3** | **<0.001***** | **0.004**** | - | **0.001**** | 0.853 |
| | **G4** | 0.888 | 0.812 | **0.001**** | - | 0.322 |
| | **G5** | **0.043*** | 0.742 | 0.853 | 0.322 | - |
| HDL | G1 | - | 0.069 | 0.271 | **<0.001***** | 0.805 |
| | G2 | 0.069 | - | **<0.001***** | **<0.001***** | **0.034*** |
| | G3 | 0.271 | **<0.001***** | - | **<0.001***** | 1.000 |
| | G4 | **<0.001***** | **<0.001***** | **<0.001***** | - | **0.002**** |
| | G5 | 0.805 | **0.034*** | 1.000 | **0.002**** | - |

G1, Group 1; G2, Group 2; G3, Group 3; G4, Group 4; G5, Group 5; HDL, high-density lipoprotein

*p* *<0.05 **<0.01 ***<0.001

Polyamines are essential for various reproductive processes such as early embryogenesis, implantation, placental growth, and angiogenesis[9,10,18]. Polyamines found in mammalian placentas are key regulators of DNA synthesis, protein synthesis, cell proliferation and cell differentiation. Beneficial changes in the metabolic profiles of polyamines during pregnancy can have a long-term effect on postnatal growth and metabolism. Maternal protein intake during pregnancy and lactation is an important factor affecting fetal and neonatal development, child health and diseases in all life[11,18,25].

Putrescine functions as a growth factor in the rat intestine and can directly induce DNA, RNA, and protein synthesis. Spermine regulates growth responses[14]. Spermidine is the unique hypusine ($N^{\delta}$-4-amino-2-hydroxybutyl(lysine)) of eukaryotic translation initiation factor 5A (eIF5A), which is necessary for protein synthesis and growth is included as a substrate for the modification[9,14].

During pregnancy, polyamine levels are high in plasma and urine as well as in amniotic fluid. Especially the 11-14th week of pregnancy. Putrescine, spermidine and spermine levels are at the highest level in amniotic fluid[31]. Plasma putrescine, spermidine and spermine levels gradually increase by the third trimester of pregnancy and reach the highest concentration at the end of pregnancy[32].

Ornithine decarboxylase (ODC) is the rate limiting enzyme in polyamine biosynthesis. ODC activity is also increased to increase polyamine concentration, especially during embryogenesis and cell growth. This enzyme activity changes rapidly against hormonal stimulus. Progesterone an important hormone in pregnancy, controls the increase in ODC activity associated with cell proliferation activity[9,33]. ODC level is found more in the placenta than the fetus[13]. The role of putrescine synthesis in the fetus is important because putrescine is required for spermidine and spermine conversion. ODC and polyamine synthesis are very important for embryo development as it increases the growth rate in tissues.

In a study with mice[13], when the polyamines found in mouse placenta, yolk sac and fetus in the second half of gestation were examined, it was determined that spermidine was synthesized the most. In maternal deficiency of arginine, which is the precursor of polyamines, supplementation with dietary arginine is effective in positive change in embryonic and fetal survival and growth[9,11,34]. In our study, when we look at the first weights measured after birth (Table 1), it was determined that the offspring born from rats that received three polyamines together with the diet were the largest. This suggests that this may be different from polyamine synthesis under normal conditions since dietary polyamine is taken during pregnancy.

Putrescine, spermidine and spermine are found in breast milk, which is the main food source of newborn. While spermidine and spermine are found in similar concentrations in human milk, both decrease during the lactation period. While there is more spermidine than spermine in rat milk, its amount increases during the lactation period. Polyamines play an important role in the growth of newborn mammals. It is also important for normal growth and maintenance of the general characteristics of the adult digestive system[20,36-37].

Analysis of growth data shows that arginine plays a very important role in postnatal development and that arginine in the diet is responsible for growth in infancy. Arginine deficiency in the diet can restrict the growth and development of newborn animals. Because the amount of arginine obtained from breast milk and its own synthesis often cannot meet nutritional requirements[34,38,39]. In a study in which neonatal rats were given oral spermidine and spermine[20], no significant difference was observed between the control group and the body weight of rats given polyamine It is thought that this result may be caused by the lack of polyamine synthesis as a result of feeding a diet containing insufficient spermidine and spermine. When the last weight measurements of the offspring in our study are examined **(**Table 1**)**, it was observed that the offspring born from rats that received three type of polyamine in the diet during lactation had the highest weight, and the offspring born from rats that did not take polyamine had the lowest weight. When the specific growth rate was examined, it was determined that the offspring born from rats that did not take polyamine was the lowest. This shows how important dietary polyamine intake is in neonatal growth. However, since the group averages were taken for each weight measurement in our study, it could not be determined whether the result we obtained was significant.

Polyamines that are polycationic are bound with phospholipids that are polyanionic at physiological pH. Polyamines stabilize the membrane by binding to the membrane with anionic phospholipids. The amount of spermine (2.3 mol/100 mol) bound to phospholipids is greater than the amount of spermidine (0.23 mol/100 mol)[1,23]. Cationic polyamines in serum interact by binding with anionic phospholipids on the surface of lipoproteins called HDL and LDL[40]. In recent years, polyamines have been shown to be powerful antioxidant agents as they protect cellular components such as polyunsaturated fatty acids in membranes from oxidative damage[14,41]. Of the polyamines, especially spermine has a strong antioxidant effect on human LDL oxidation compared to α-tocopherol, followed by spermidine and putrescine[26]. In our study, it was determined that the HDL level of the group given spermine was significantly higher than the other groups, and it is in accordance with the literature. However, it was shown that there was no statistically significant difference in LDL levels between the groups.

Methionine and arginine are required for polyamine biosynthesis. In a study examining the serum lipid profiles of broiler chickens fed with different levels of arginine[42], it was found that the triglyceride level was significantly lower compared to the control group. In another study with broiler chickens[43], serum

triglyceride levels were found to be high in 40-day-old broiler chickens fed a diet containing insufficient methionine. Similarly, Ebrahimi et al.[44] observed that the expression of the gene encoding FAS was decreased when L-arginine was added to the diet of broiler chickens. In addition, in the study by Filho et al. [45], it is estimated that the effects of dietary L-arginine on lowering serum triglyceride and total cholesterol levels are associated with decreased gene expression of FAS and HMG-CoA. These results are in line with our study. In other words, the triglyceride level of the G1 group is significantly higher than that of the G3 and G5 groups.

In a study with treated diabetic rats, a significant increase in triglyceride, HDL and LDL levels was found as a result of feeding spermine for five months[46]. In our study, when the groups that were given spermine and control were examined, a significant increase in total cholesterol and HDL levels (respectively $p < 0.001$, $p < 0.001$), a decrease in triglyceride level ($p = 0.888$) (Table 3) and an increase in LDL ($p = 0.927$) were observed. This result suggests that rats with diabetes may have been using spermine for a much longer time. Also, maternal spermine intake and direct dietary spermine intake are likely to have different results on serum lipid levels.

In conclusion, this study demonstrated the efficacy of maternal polyamine intake on growth and development and serum lipid levels in first generation offspring rats. Positive effects on growth and development of rat offspring were observed as a result of using polyamines both separately and together. In addition, when the serum lipid levels of the offspring were examined, significant differences were observed between groups and the effectiveness of polyamines on total cholesterol, triglyceride, HDL, and LDL was demonstrated. These results are proof that the dietary polyamines are passed on to offspring through the placenta and breast milk.

## STATEMENT OF ETHICS

The study was approved by Istanbul Medipol University Animal Experiments Local Ethics Committee.

## CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

## AUTHOR CONTRIBUTIONS

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Fatma Mert-Biberoğlu and Nihal Büyükuslu. The first draft of the manuscript was written by Fatma

Mert-Biberoğlu and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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