A Study on the Effects of Dietary Lactose on Ovarian Function and Body Weight in Normal and Obese Female Albino Rats

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ABSTRACT

This work was done to investigate effects of dietary lactose on ovarian function and body weight in normal and obese female albino rats. 36 female albino rats 150-170gm and 6 weeks old, were divided into two groups:(1)Normal group: composed of 18 rats divided into three subgroups: (A)Control group: received glucose in a dose of 41.9gm/100gm of standard diet for three months, (B)low lactose diet treated group: received lactose in a dose of 10.5gm/100gm of standard diet for three months (C)High lactose diet treated group: received lactose in a dose of 41.9gm/100gm ofthe standard diet for three months.(2)Obese group: composed of 18 rats received high fat diet for one month to induce obesity, then divided into three subgroups: (A) Control group: received glucose in a dose of 41.9gm/100gm of high fat diet for three months (B)Low lactose diet treated group: received lactose in a dose of10.5gm/100gm ofhigh fat diet for three months (C)High lactose treated group: received lactose in a dose of 41.9gm/100gm of high fat diet for three months. At the end of the experiment, rats were weighed and blood collected from retro orbital plexus for determination of estrogen, progesterone, follicle stimulating hormone (FSH), leptin. Vaginal cytology was done regularly to estimate estrus cycles cyclicity. The results showed significant reduction of body weight, estrogen, progesterone, and leptin in both normal and obese rats, and significant increase of FSH in normal and obese rats. Vaginal cytology showed disturbed and irregular estrus cycles. It can be concluded that, administration of lactose in low and high doses for long periods can affect the ovarian function, and caused reduction of body weight due to galactose content. It is recommended that, women with galactosemia and infertility must assess their galactose level which may be the cause of infertility.

INTRODUCTION

Milk contains nutrients necessary for the growth of mammals, and it is the sole food utilized for quiet long period after birth. Most milk consumed by humans is cow's milk, and most dairy products are processed from cow's milk, that contain abundant calcium, which is likely to be lacking in daily life, and the absorbability of calcium from milk product is high[(1)]. Milk and dairy products also contain other nutrients in addition to calcium and used for the prevention of osteoporosis[(2)]. The antiobesity effects of milk and dairy products are due to their calcium content[(3)]. In addition to calcium, the conjugated linoleic acid[(4)] and

leucine[5] are components of milk and dairy products with antiobesity action. Milk contains 4.4% lactose that accounts for 99.8% of saccharides and 40% of the total solids in milk. The biological effects of lactose are including the promotion of calcium absorption and improvement of bone metabolism[6] lactose consists of one glucose molecule and one galactose molecule, which is the primary carbohydrate in milk. The toxic effects of galactose or its metabolites were experienced by individuals who have glactosemia within the ovary[7]. Galactose is also important in the metabolism of healthy ovarian functions, which manifested by the relative abundance of galactose metabolizing enzymes in the ovarian tissue[8]. Subsequently the health problems which affect women concerning high dietary galactose, might increase the risk of ovarian cancer or accelerate the age of onset of ovarian failure[9]. Epidemiological data have suggested that, an earlier age of menopause can be associated with galactose intake, especially in women who have partial deficiencies of the transferase enzyme[10]. It was demonstrated in some women with increased levels of follicle stimulating hormone and an associated increase of galactose consumption early menopause irrespective of transferase activity[11]. Obesity, diabetes, hypertension and hyperlipidemia are independent risk factors of cardiovascular disorder. Obesity is considered to be the main pathological cause of metabolic syndrome[12]. Insulin, leptin and thyroid hormone are not only important metabolic hormones, but also are recognized as signal linking nutrition and growth, development and reproduction[13].

The aim of the present study was to investigate the effect of high and low lactose diet on ovarian functions in normal and obese female albino rats.

MATERIALS & METHODS

36 female albino rats weighting 150-170gm and 6 weeks old. Rats were pair housed at room temperature and had free water access. All control and treatment diets were prepared and remained standard[14] throughout the experiment with exceptions of glucose, lactose and fat content. The rats were divided into two groups each containing 18 rats.

1- Normal group: was divided into three subgroups each containing 6 rats
   a- Control group: received standard diet with glucose (Sigma) 41.9 gm/100gm[8]. of the diet for three months.
   b- Low lactose diet treated group: received standard diet with lactose (Sigma)10.5 gm /100gm [14] of the diet for three months.
   c- High lactose diet treated group: received standard diet with lactose 41.9 gm /100gm of the diet for three months.

2- Obese group: received high fat diet composed of 70% fat, 20% carbohydrate and 10% proteins[15] for one month to induce obesity with regular weighing of rats.

Rats were divided into three subgroups each contained 6 rats.
   a- Control obese group: received high fat diet with glucose 41.9 gm/100 gm of the diet for three months.

b- Low lactose treated obese group: received high fat diet with lactose 10.5 gm /100gm of the diet for three months.

c- High lactose treated obese group: received high fat diet with lactose 41.9 gm /100 gm of the diet for three months.

At the end of the experimental period, rats were fasted overnight, weighed and blood samples were collected from retro-orbital plexus and sera were separated for determination of:

Serum estrogen by the method of Ismail et al [16].

Serum progesterone by the method Ismail et al [16].

Follicle stimulating hormone (FSH) by the method of Kulin and Santer [17].

Serum leptin by the method of Spayd et al.[18].

Vaginal cytology: was assessed five days per week The vaginal smears were stained and examined[19]. Rats that exhibited at least 3 consecutive 4 or 5 days/cycle were considered to have regular cycles.

Statistical analysis:

All results were expressed as mean values ± standard deviation (SD). Mean values of different groups were compared using a one way analysis of variance. Mean value of $P<0.05$ was accepted to be a significant difference.

RESULTS

The results of the present study are shown in table (1):

Serum level of estrogen:

The results showed significant reduction of serum estrogen in normal female received low and high lactose diet compared with the control, $P<0.05$. Also, there was significant reduction of serum estrogen level in obese rats treated by low or high lactose diet compared with the control, $P<0.05$, Fig (1).

Serum progesterone level:

The results showed significant reduction of serum progesterone level in normal rats treated by low and high lactose diet compared with the control, $P<0.05$. Also, there was significant reduction of serum progesterone in obese rats treated by low and high lactose level compared with the control, $P<0.05$, Fig. (2).

Serum FSH level: The results showed significant increase of serum FSH level in normal rats treated by low and high lactose diet compared with the control, $P<0.05$. Also, there was significant increase in serum FSH level in obese rats treated by low and high lactose diet compared with the control, $P<0.05$, Fig (3).

Serum leptin level:

The results showed significant reduction of serum leptin level in normal rats treated by low and high lactose diet compared with the control, $P<0.05$. Also, there was significant reduction of serum leptin level in obese rats treated by low and high lactose diet compared with the control, <.05, Fig(4).

Body weight:

The results showed significant reduction of body weight in normal rats treated by low and high lactose diet compared with the control, $P<0.05$. Also, obese rats treated by low and high lactose diet showed significant reduction of the body weight compared with the control, $P<0.05$, Fig (5)

Vaginal cytology:

At the beginning of the experiment, all rats had regular estrous cycles. Afterwards, the incidence of irregular cyclicity appeared and increased. The high lactose treated group of normal and obese rats, had obvious irregularity more than low diets compared with the control. Mild irregularity had been occurred in low lactose treated group.

Table (1): Effects of low and high lactose diet on serum estrogen, progesterone, FSH, leptin, and body weight in normal and obese female albino rats (mean 6±SD)

Parameter	Normal female rate			Obese female rates		
	Control	Low	High	Control	Low	High
Estrogen pg/ml	63.3±096	60.8±1.17*	59.3±0.99*	56.8±1.23	53.01±1.12*	51.3±0.75*
Progesterone pg/ml	12.5±0.55	10.2±0.73*	7.9±0.33*	10.08±0.96	8.5+0.6*	7.05±0.43*
FSH IU	0.468±0.01	0.696±0.03*	0.851±0.03*	0.545+0.01	0.705±0.05*	0.941±0.02*
Leptin ng/ml	33.2±1.07	21.7±2.16*	10.8±1.53*	42.6±1.36	25.4±1.39*	14.3±1.6*
Body weight gm	159.6±7.11	150.1±4.91*	140.8±5.63*	235±7.07	218.6±4.71*	200.5±7.12*

**=Denotes statistical significance*

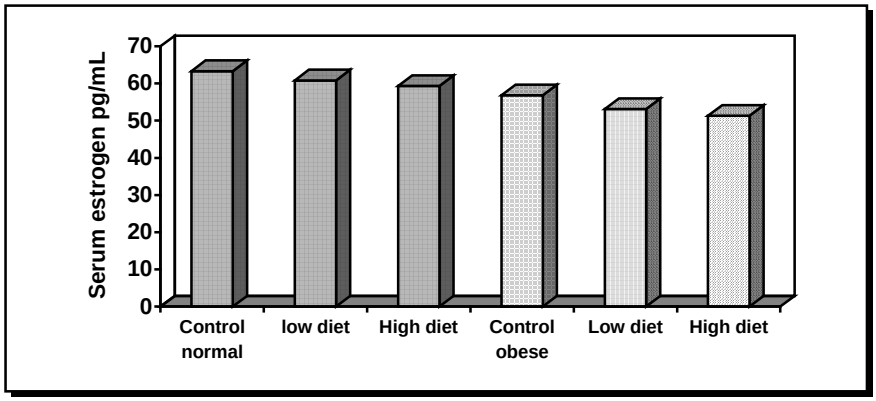

Fig. (1): Effect of low and high lactose diets on serum estrogen (pg/ml) level in normal and obese female albino rats

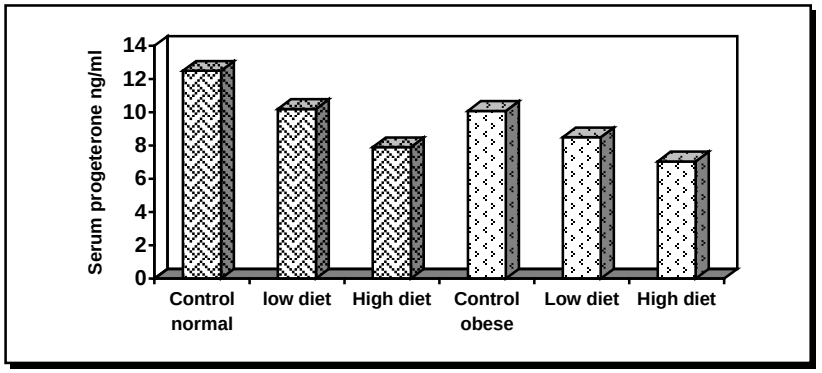

Fig. (2): Effect of low and high lactose diets on serum progesterone (pg/ml) level in normal and obese female albino rats

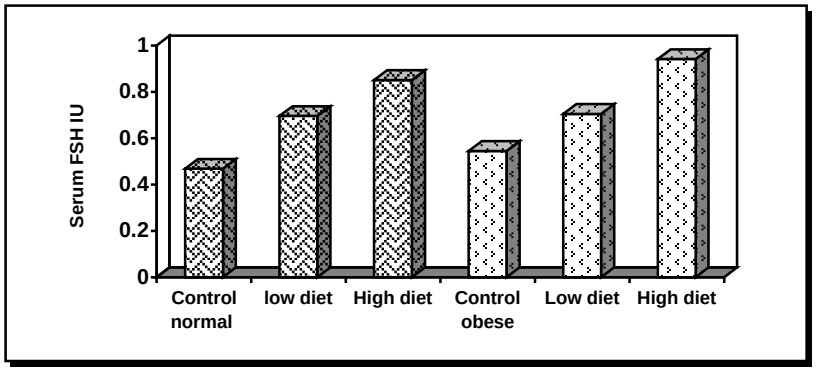

Fig. (3): Effect of low and high lactose diets on serum FSH (IU) level in normal and obese female albino rats

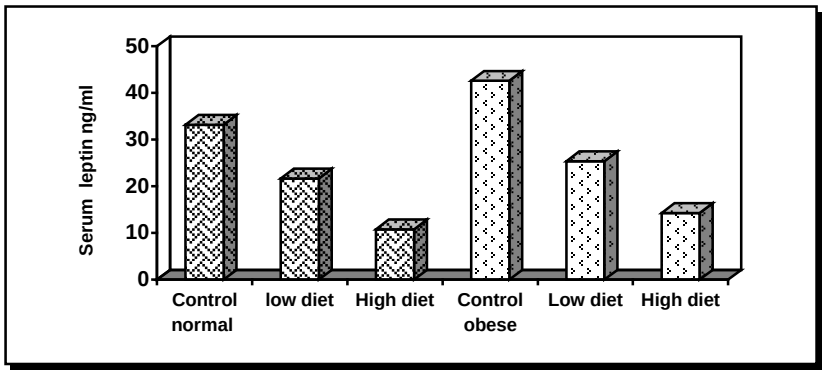

Fig. (4): Effect of low and high lactose diets on serum leptin (ng/ml) level in normal and obese female albino rats

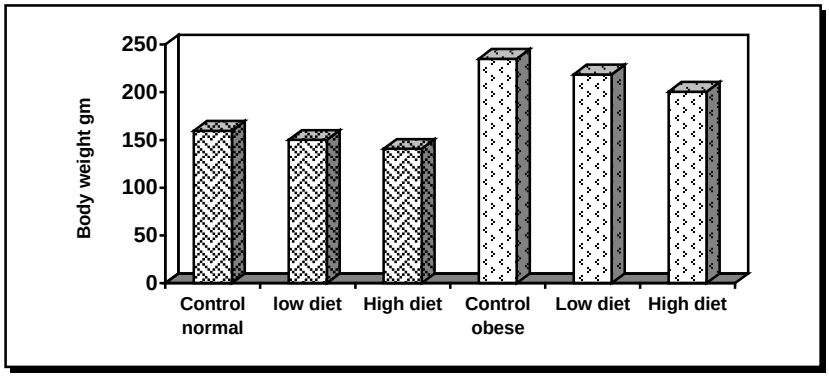

Fig. (5): Effect of low and high lactose diets on body weight (gm) in normal and obese female albino rats

DISCUSSION

Women in western countries are urged to consume dairy products to enhance their calcium intake, with the aim of reduction in risk of osteoporosis[20]. Since lactose is the primary carbohydrate in milk, those who consume large quantities of dairy products ingest large quantities of lactose[21]. Lactose is a disaccharide that is hydrolyzed by enzyme lactase into glucose and galactose. In mammals, including humans, intestinal lactase activity is highest during the time of suckling and declines to lower levels after weaning[22]. The results of the present study showed that, normal and obese female rats fed by low and high lactose diet for three months, showed decreased serum estrogen and progesterone levels. These results could be explained by that, the ovary has relatively abundant levels of three of the major enzymes involved in metabolism of galactose (galactokinase, galactose-1-phosphate uridyl transferase (GALT) and UDP-glactose-4-epimerase)[23]. This fact was proved by the observation that, women who have absent of low GALT activity, have a propensity to develop premature ovarian failure and premature menopause[7]. Also, the deficiency of GALT enzyme induced a lake of UDP-galactose, which is one of the GALT reaction products, and also acting as a substrate for subsequent galactosylation[24]. Moreover, the mechanism of ovarian damage have been hypothesized, by the direct toxicity of galactose and its metabolites within the ovary, and became apparent from the toxic observation of the extremely early ovarian failure (before the age of 30years) in women with galactosemia[25]. Also, it was reported that, women who consume milk and dairy products in large amounts, have higher incidence of developing cancer ovary or premature ovarian failure[26]. In addition, it is possible that, the toxic metabolites of galactose, induced apoptosis of the ovarian tissues, which may be due to an

imbalance between pro-apoptotic and anti-apoptotic factors of the ovary, after binding of pro-apoptotic factors to specific ovarian membrane receptors[27]. The vaginal cytology showed that female rats usually display regular vaginal cycles and after the period of the experiment, they had progressive decrease in the ability to maintain regular estrous cycles[19]. This may be due to the high levels of FSH which explained the failure of ovarian response to pituitary hormones, irregular estrous cycles and progression of ovarian failure [28]. It was observed that 40% galactose diet in rats for two weeks produced inhibition of oocyte maturation manifested by decreased number of coropra lutea, and failure to respond to exogenous gonadotropins. It may be due to an acquired anomaly of the gonadotropin receptors, leading to gonadotropin-resistant syndrome[8]. The results showed significant reduction of serum leptin level and body weight. The reduction of body weight may be due to reduction of serum leptin, which is considered as an indirect parameter positively related to the level of body fat [29]. This effect of leptin may confirm the antiadiposity effect of lactose[15]. Also, reduction of body weight may be due to effect of calcium, the conjugated linoleic acid[4] and leucine[5] content of lactose, with antiobesity action. It was proved by decreased visceral fat accumulation in obese-induced mice with high lactose intake[30]. Also, lack of calcium intake may cause obesity, and lack of calcium in adipocytes was related to obesity [31]. Moreover, lactose may regulate adiposity via promotion of calcium absorption[6]. Also, diets containing 50-60% lactose result in diarrhea[32], it is possible that dietary lactose may reduce the absorption of diet protein and fat[33]. Lactose also reduced plasma lipids, especially triglycerides and hepatic cholesterol accumulation in hamsters[34].

Conclusion and Recommendation:

It can be concluded that long term exposure to low and high lactose diets, decreased body weight and cause disturbance of ovarian function and induced cycles irregularity due its galactose content. It is recommended that women with galactosemia and infertility must assess their galactose level which may be the cause of infertility.

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دراسة عن تأثير سكر اللبن(اللاكتوز) على وظائف المبيض ووزن الجسم فى إناث الفئران البيضاء السليمة والمصابة بالسمنة

روميساء على الشربينى و محمود عبد الحميد الغريب
قسم الفسيولوجيا – كلية الطب – جامعة طنطا

يهدف هذا البحث إلى دراسة التأثير الناتج من تناول وجبات تحتوى على جرعات صغيرة وكبيرة من اللاكتوز على وظائف المبيض ووزن الجسم فى إناث الفئران البيضاء السليمة والمصابة بالسمنة

وقد أجرى هذا البحث على 36 فارا عمرها حوالى 6 أسابيع ووزنها يتراوح بين 150 – 170 جم قسمت إلى مجموعتين

1- المجموعة السليمة: وتتكون من 18 فارا قسمت إلى ثلاث مجموعات

أ- المجموعة الضابطة: وتغذت على الوجبة الأساسية مضافا إليها 41.9 جم جلوكوز /100جم من وزن الوجبة لمدة ثلاث شهور.

ب- المجموعة المعالجة بجرعة صغيرة من اللاكتوز: وقد تغذت على الوجبة الأساسية مضافا إليها 10.5 جم لاكتوز/100جم من وزن الوجبة لمدة ثلاثة شهور.

ت- المجموعة المعالجة بجرعة كبيرة من اللاكتوز: وقد تغذت على الوجبة الأساسية مضافا إليها 41.9 جم لاكتوز/ 100 جم من وزن الوجبة لمدة ثلاثة شهور.

2- المجموعة المصابة بالسمنة: وتتكون من 18 فارا تغذت لمدة شهر على وجبة عالية الدسم مكونة من 70% دهون، 20% نشويات، 10%بروتين ثم قسمت إلى ثلاثة مجموعات

أ- المجموعة الضابطة المصابة بالسمنة: وقد تغذت على الوجبة عالية الدسم مع 41.9 جم جلوكوز/100 جم من وزن الوجبة لمدة ثلاثة شهور.

ب- المجموعة المصابة بالسمنة و المعالجة بجرعة صغيرة من اللاكتوز: وقد تغذت على الوجبة عالية الدسم مع 10.5 جم /لاكتوز /100 جم من وزن الوجبة لمدة ثلاثة شهور.

ت- المجموعة المصابة بالسمنة و المعالجة بجرعة كبيرة من اللاكتوز: وقد تغذت على الوجبة عالية الدسم من 41.9 جم لاكتوز/ 100جم من وزن الوجبة لمدة ثلاثة شهور.

وقد اجرى فحص مجهري للخلايا المبطنة للمهبل حتى تختبر فترات الطمث وفى نهاية فترة التجربة تم وزن جميع الفئران و جمعت عينات الدم من الشبكة الوريدية خلف العين وذلك لتعيين مستوى كل من هرمون الاستروجين الهرمون المنبه للمبيض والبروجستيرون و اللبتين.

وقد تبين من نتائج هذا البحث أن تناول اللاكتوز بجرعة صغيرة أو كبيرة قد سبب انخفاض ملحوظ ذو دلالة إحصائية في مستوى كل من الاستروجين والبروجستيرون واللبتين ووزن الجسم كما أن هناك ارتفاع ملحوظ ذو دلالة إحصائية فى نسبة الهرمون المنبه للتبويض فى كل من الفئران السليمة والمصابة بالسمنة ، كما وجد من الفحص المجهرى للخلايا المهبلية أن هناك اضطرابات فى الطمث وعدم انتظام الدورة الشهرية.

ويتضح من نتائج هذا البحث أن اللاكتوز بجرعات صغيرة وأيضا كبيرة يسبب اضطرا بات فى وظائف المبيض و يسبب نقص فى وزن الجسم وذلك لاحتوائه على الجلاكتوز.و لذلك ينصح السيدات الاتى تعانى من العقم بقياس مستوى الجلاكتوز لأنه قد يكون سببا لهذه الحالة.