Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

European Journal of Experimental Biology, 2013, 3(1):191-198

ISSN: 2248 –9215
CODEN (USA): EJEBAU

The effect of maternal diet prior and during pregnancy in rats on obesity development in offspring

Damiah Kasim Suker[1], Sanaa Jameel Thamer[1*] and Taha Jasim Al Taha[2]

[1]*Department of Biology, College of Sciences, University of Basrah, Basrah, Iraq*
[2]*Department of Animal Resource, College of Agriculture, University of Basrah, Basrah, Iraq*

ABSTRACT

Maternal over nutrition may induce long term metabolic complications in offspring. We investigated the effects of fatty acid composition of maternal diets throughout the mothers' life and during pregnancy on postnatal obesity, puberty, leptin, insulin and metabolic parameters in offspring. The obesity was induced by feeding with high fat diet HF (45%) for 12 weeks with control group LF. The obese rats divided to five subgroups HF-HF (45% tallow), HF-P.d (22.5% peanut), HF-O.d (22.5% olive oil),HF-M.d (22.5% tallow), HF-LF(10% tallow) before mating and through gestation period until weaning ,the offspring was maintained on control diet .At puberty, weight and metabolic parameters were recorded. The results indicated that from birth until puberty, pups of HF group showed higher body weight ($p<0.05$) compared to control LF, which reflecting early onset of puberty. The continued feeding on high fat diet (HF-HF), reduced pups weight and increased mortality rate with early age of puberty. The unsaturated fat diets in HF-P.d and HF-O.d regulating pups body weight and reducing mortality rate with normal age for puberty, except in HF-O.d group reach to 47 days .At puberty female offspring of HF and HF-HF groups had higher plasma leptin , insulin and biochemical parameters ,whereas the substituted with unsaturated fat resulting in normal value of leptin and insulin levels while triglycerides levels was increased than the control group LF-LF. The data indicate the importance of maternal nutrition to develop obesity in the offspring, which may be regulated by feeding with unsaturated fat diet.

Keywords: high fat diet, pregnancy, offspring, unsaturated fat diet, leptin sensitivity.

INTRODUCTION

The maternal obesity have a greater influence to increased the risk of disease in their offspring childhood [1, 2, 3, 4] in addition to their pregnancy complication [5] and higher leptin level [6] it documented a problem in human population with transgeneration effect [7]. The nutritional condition during pregnancy influence in utero and fetal growth and disease in adulthood [8] few studies are conducted on the long term effect of excess nutrition during pregnancy or lactation on obesity development in offspring [9]. The epidemic of obesity and childhood disease in development countries related partly to the association between prenatal nutrition and postnatal food consumption [10] some studies investigate the role of maternal rich diet on fetal development [11]. Obesity during pregnancy playing a pathogenetic role in the development of obese phenotype in offspring [12] the children who are born from obese mothers are likely to be obese more than the children from lean mothers [13] . The gestinational diabetes

effected on fetal development and increased the rate of birth weight and enhanced diabetogenic tendency in offspring [14]. Several studies reported the maternal over nutrition lead to cause cardiovascular dysfunction in offspring by increased the tendency of obesity and impaired insulin tolerance [15, 16, 17]. The aim of the study is determined the effect of maternal diet enriched with saturated fat on development obesity , metabolic syndrome and puberty in offspring and the role of unsaturated fat to modulate these effects .

MATERIALS AND METHODS

Animals:
Female Wister albino rats (6 weeks aged and 97± 10 g weight) were acclimatizing on low fat diet for one week before introducing to the experimental diets, the animals either feeding on low fat diet (control diet LF: 10% energy from tallow) n=12 or on a high fat diet (HF 45% energy from tallow) n=36 for 12 weeks (table 1) and assigned as experiment one of the study. All animals were kept in constant room temperature (25-30 c) and 12:12 h light: dark cycle with free access to food and water. in the experiment 2 of the study a group of rats n=6 from the control were continued on low fat diet for additional 8 weeks , while the rats from high fat diet group were divided into the following subgroups (n= 6). The first subgroup was continued to feed on HF diet (45%) for additional 8 weeks (HF-HF). The second subgroup was received moderate fat diet (22.5 % fat) in which tallow was replaced by olive oil (HF-O.d) or peanut (HF-P.d) for additional 8 weeks.. The third subgroup was received a low fat diet (10 % tallow) for additional 8 weeks (HF-LF). The forth subgroup was received a moderate fat diet (M.d: 22.5 % tallow) for additional 8 weeks (HF-M.d).

Diet: Diet induced obesity in rodents (HFd 45 % fat) and it´s control (LFd 10 % fat) was formulated according to the research diet [18]. Moderate fat diet M.d (HF-M.d , HF-O.d , HF-P.d) was modifying according to the high fat diet of this study.The composition of the experimental diet shows in table 1.

Table 1: composition of the experimental diets in the study.

Ingredients	Control (10 % fat)	HF (45 % fat)	M.d (22.5%) tallow or olive oil	M.d (22.5%) peanut
Casein	200	200	200	158.1
L- cystine	3	3	3	3
Cornstarch	315	72.8	286.55	273.65
sucrose	385	272.8	286.55	273.65
Cellulose powder	50	50	50	36.22
Soy bean oil	25	25	25	25
Beef tallow	20	177.5	76.4*	0
Peanut	0	0	0	183.8
Mineral mix	10	10	10	10
Dicalicium phosphate	13	13	13	13
Ca^{+2} carbonate	5.5	5.5	5.5	5.5
K citrate	16.5	16.5	16.5	16.5
Vitamin mix	10	10	10	10
Choline bitartarate	2	2	2	2
Total weight gm	1055	858.1	984.5	984.5
Total Kcal	4057	4057	4057.3	4057.3
Total Kcal/ gm	3.85	4.73	4.078	4.078

** use beef tallow or olive oil in the diet.*

Study the reproductive performance: To study the reproductive function, virgin female rats from each dietary groups from the experiment 1 and 2 of the study were used : LFd , Hfd , LF-LF , HF-HF , HF-P.d, HF-O.d, HF-LF and HF-M.d (n=6) .The virgin female rats were time mated by monitored ousters cycle in virginal smear before introducing to the male (one male for each female , aged 17 -18 week) .Day one of pregnancy was determined by the present of sperms after vaginal lavage . Pregnant rats were housed in group (n=3 each cage) in standard cage, containing sawdust and maintained on their assigned diet with free access to water. All animals were kept in constant room temperature (25-30 c) and 12:12 h light: dark cycle with free access to food and water. During the gestation period, daily food intake and body weight were recorded for each groups. On the day 21, 22 of pregnancy, dams were monitored and observed during delivery. After delivery, the pup's weight was recorded. For the next three days, pups mortality was recorded .Dams were continued on their assigned diet in each dietary group during the lactation period until weaning. After weaning, the female and male offspring of each dietary experiment were weighed and then fed on control diet (10 % from fat). The vaginal opining was examined daily, and the vaginal

smears were collected and observed for the presence of cornified epithelial cells (estrus). Body weight recorded and the female offspring were anesthetized and sacrificed. Blood samples were collected, Plasma stored at -78 c.

Hormonal measurements:
Plasma rat leptin and insulin was measured using rat Elisa kit from CRYSTAL CHEM INC (for leptin cat no. 90040 USA, for insulin cat no. 90010 USA).

Biochemical parameters:
Plasma glucose , total cholesterol (T-ch), triglycerides (TG) , high density lipoprotein (HDL), total protein , albumin concentrations were measured by enzymatic method using diagnostic Kit from Randox (UK) and Biolabo companies (France) .

Low density lipoprotein (LDL) was calculated according to the formula Friedewal [19]: LDL cholesterol = T.ch - HDL - (TG/5) , very low density lipoprotein (VLDL) was measured according to the formula Tietz [20]: VLDL = TG/5 . Phospholipids were measured according to the formula Tietz [20]: Phospholipids = 68 + (T-ch × 0.89).

Statistical analysis:
Data were analyzed by one- way and two- way ANOVA using a general liner models procedure using SPSS version 15.0 statistic program. Comparisons between the data were made using least significant differences (LSD). Differences were considered to be significant at $p<0.05$.

RESULTS

Pups birth and weaning weight: pups of high fat dams give higher birth weight with significantly ($p<0.05$) (6.600 gm) than obtained in control group, (table 2). No mortality was observed in pups after 3 days of lactation in both groups. High birth weight were recorded in the HF-P.d and HF-M.d followed by HF-O.d and HF-Lf groups. The continuous feeding on high fat diet reduced pups birth weight (5.10 gm). During lactation period the pups of HF-HF and HF-M.d groups had mortality rate 1.6 and 1.3 while the groups HF-O.d and HF-LF had rate 0.33. After weaning the female and male offspring showed higher weaning weight in HF group with higher puberty weight in females that reflected the short time for puppetry compared to control group (figures 1,2), Followed by higher weight in HF-P.d group (42.33gm ±1.37 for female and 43.67 gm±3.721 for male) . Normal weaning weight in HF-O.d , HF-Ld and HF-M.d groups . The female offspring of HF-HF group had the less weaning weight (29.67gm ±1.012)with early puberty time whereas the male recorded weight 30.33 gm ± 1.10 as the control with no significant as shown in figure 3 . The results show delayed age of puberty in HF-O.d group (47.67 ±4.4 days). Puberty females' weight were normal in all dietary groups except in HF-HF group which showed the lower puberty weight (118 gm ± 2.64) followed by HF-LF group with 122.67 gm ± 2.51.

Table 2:weight and availability of pups related to dams fed experimental diets

LF:low fat diet, HF:high fat diet; LF-LF: low fat continued on low fat 10% , HF-HF: high fat continued on high fat(45% tallow), HF-P.d: high fat fed peanut 22.5%, HF-O.d:high fat fed on olive oil 22.5% , HF-LF: high fat fed on low fat 10% , HF-Md : high fat fed on tallow 22.5% .Means ±S.D ($p< 0.05$) .

Treatment	Pups weight at birth (g)	Rate number of Live pups at birth	Rate number of Dead pups at lactation day three
Lf (control)	5.608 ± 0.125 b	6 ± 0.011 a	0
HF	6.601 ± 0.142 a	6 ± 0.011 a	0
LF-LF (control)	5.567 ± 0.116 b	6 ± 0.011 a	0
HF-HF	5.100 ± 0.200 c	4 ± 0.80 c	1.6 ± 0.23 a
HF-P.d	6.100 ±0.200 a	6 ± 0.011 a	0
Hf-O.d	5.700 ± 0.240 b	6 ± 0.014 a	0.33 ± 0.1 c
HF-LF	5.533 ±0.271 b	6 ± 0.010 a	0.33 ± 0.1 c
HF-M.d	6.033 ± 0.208 a	4.6 ± 0.260 b	1.3 ± 0.2 b

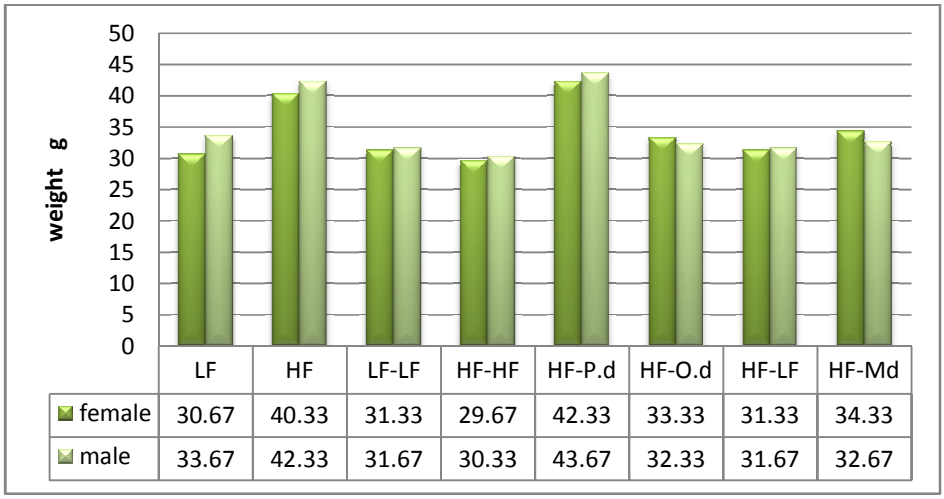

Figure 1: weaning body weight of offspring related to dams fed the experimental diets

LF:low fat diet, HF:high fat diet; LF-LF: low fat continued on low fat 10% , HF-HF: high fat continued on high fat(45% tallow), HF-P.d: high fat fed peanut 22.5%, HF-O.d:high fat fed on olive oil 22.5% , HF-LF: high fat fed on low fat 10% , HF-Md : high fat fed on tallow 22.5% . Means ($p< 0.05$) .

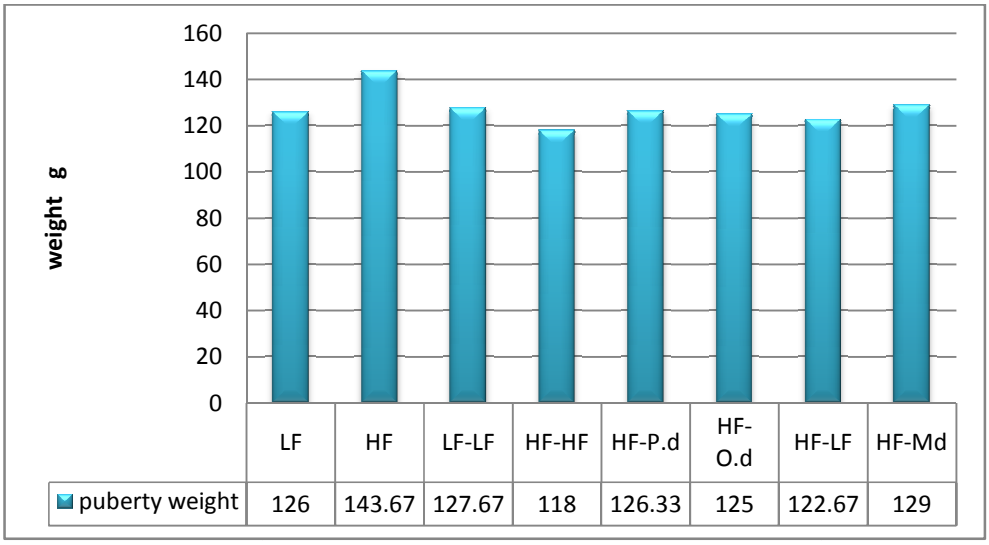

Figure 2: puberty body weight of females offspring related to dams fed the experimental diets

LF:low fat diet, HF:high fat diet;LF-LF: low fat continued on low fat 10% , HF-HF: high fat continued on high fat(45% tallow), HF-P.d: high fat fed peanut 22.5%, HF-O.d:high fat fed on olive oil 22.5% , HF-LF: high fat fed on low fat 10% , HF-Md : high fat fed on tallow 22.5% . Means ($p< 0.05$) .

Offspring plasma leptin and insulin:

Female offspring of high fat diet group (HF,HF-HF) in the onset of puberty , had significantly ($p<0.05$) higher fasting plasma leptin levels (2.31 ng/ml , 2.415 ng/mL) and insulin levels (0.540 ng/ml , 0.87 ng/ mL, respectively) compared to the control group(table 3) . Consequent by higher insulin concentration in female offspring of HF-M.d group (0.98 ng/mL) and plasma leptin was higher (2.155 ng/ml). In HF-P.d group, the concentration of plasma leptin was 1.621 ng/ml and insulin 0.59 ng/ml with no significant from the control groups . In HF-O.d and HF-LF groups, plasma leptin levels in females decreased slightly (1.261 ng/ml ,1.520 ng/ml) than the control group (LF-LF) (1.600 ng/ml) , whereas their plasma insulin did not differ from the control group .

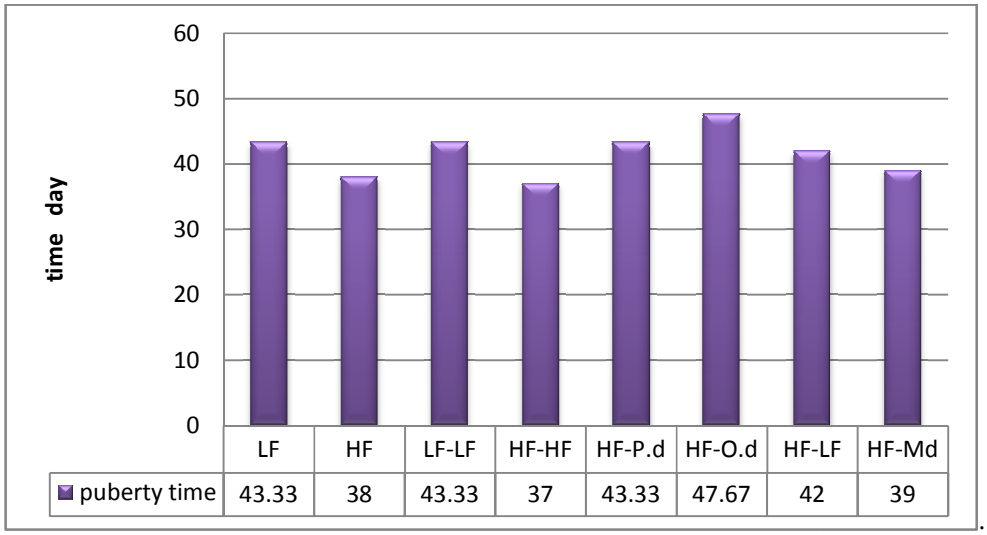

Figure3:puberty time of female offspring related to dams fed the experimental diets

LF- LF:low fat diet, HF:high fat diet;LF: low fat continued on low fat 10% , HF-HF: high fat continued on high fat(45% tallow), HF-P.d: high fat fed peanut 22.5%, HF-O.d:high fat fed on olive oil 22.5% , HF-LF: high fat fed on low fat 10% , HF-Md : high fat fed on tallow 22.5% .Means (p< 0.05) .

Table 3: plasma leptin and insulin concentration in the day of puberty of female offspring related to dams fed the experimental diets

LF:low fat diet, HF:high fat diet;LF-LF: low fat continued on low fat 10% , HF-HF: high fat continue on high fat(45% tallow), HF-P.d: high fat fed peanut 22.5%, HF-O.d:high fat fed on olive oil 22.5% , HF-LF: high fat fed on low fat 10% , HF-M.d : high fat fed on tallow 22.5% . Means ±S.D (p< 0.05) .

Treatments	Female leptin ng/ml	Female insulin ng/ml
Lf (control)	1.400 ± 1.10 b	0.424 ± 0.32 b
HF	2.311 ± 1.20 a	0.540 ± 0.42 a
LF-LF (control)	1.600 ± 1.11 c	0.54 ± 0.33 b
HF-HF	2.415 ± 0.81 a	0.87 ± 0.13 a
HF-P.d	1.621± 0.87 c	0.59 ± 0.164 b
Hf-O.d	1.261± 0.70 e	0.49 ± 0.12 b
HF-LF	1.520 ± 0.92 d	0.40 ± 0.17 b
HF-M.d	2.155 ± 1.05 b	0.98 ± 0.13 a

Table 4 : plasma biochemical concentration in the day of puberty of female offspring related to dams fed the experimental diets. LF:low fat diet, HF:high fat diet

LF-LF: low fat continue on low fat 10% , HF-HF: high fat continued on high fat(45% tallow), HF-P.d: high fat fed peanut 22.5%, HF-O.d:high fat fed on olive oil 22.5% , HF-LF: high fat fed on low fat 10% , HF-M.d : high fat fed on tallow 22.5% . Means ±S.D (p< 0.05) .

Treatments	Glucose	Total cholesterol	Triglycerides
Lf (control)	12.37±1.89 b	0.654±0.23 b	2.710 ± 0.13b
HF	18.30±1.76a	1.667±0.20 a	5.32±0.21a
LF-LF (control)	12.5 ± 3.11 c	2.80± 0.8e	0.661± 0.87e
HF-HF	21.40±1.23 a	5.933± 0.9a	1.774±1.03a
HF-P.d	12.50±0.87 c	3.200±0.31 d	1.206±1.58d
Hf-O.d	11.63 ± 2.2 d	3.400±0.76 c	1.344± 0.92c
HF-LF	14.23±0.95 b	3.300±1.1c	1.376±0.89c
HF-M.d	14.83±1.05 b	3.833± 2.15 b	1.562±0.94b

Female Offspring biochemical parameters:

Fasting plasma glucose ,total cholesterol (TG) and triglycerides (TG) were significantly ($p<0.05$) increased in females offspring of HF diet groups (HF,HF-HF) (18.30 , 1.667 , 5.320 mmol/L ,and 21.40 , 5.933 , 1.774 mmol/L

respectively) compared to control group (LF-LF) (12.5, 2.80,0.661 mmol/L respectively) (table 4) .In HF- P.d group, female plasma glucose was 12.50 mmol/L with no significant difference from the control group, consequent by HF-O.d group with significantly($p<0.05$) (11.63 mmol/L) . In HF-LF and HF-M.d group, high levels of plasma glucose were recorded in females (14.23, 14.83 mmol /L respectively). However, the plasma T-ch and TG were high in all groups with significant differences ($p<0.05$) from the control.

DISCUSSION

Our results demonstrate that the higher fetal weight of high fat fed dams (fed for 15 weeks) may be caused by changes in nutritional transport though placenta ,either up –regulation of specific nutrition's like glucose and amino acids indicating from higher protein gene expression of glucose trans port Glut 4 and sodium coupled neutral amino acid transport SANAT 2 [21] or either to the ability of placenta to take up chylomicron remnants core lipids by increasing mRNA expression of fatty acid oxidation protein PPAR rather than fatty acid transport [22] . The reduction in fetal growth in dams fed high fat diet for 23 weeks (HF-HF group) may explained by decreased sensitivity of body tissue to insulin action as a resulted from highest insulin and glucose concentration that may have an effect on placental nutrition transport, that could have an influence on the numerous events in fetus growing process or on reproductive hormones including prostaglandin synthesis [23]. Diets supplemented with USFA (MUFA or PUFA) prior and though pregnancy resulted in normal fetus weight, especially with peanut supplementation. The possible explanation either to the changes in maternal nutritional status during ovum maturation and in early embryonic development which had greater effect than the last trimesters of pregnancy due to placental barrier development [24] or the fatty acid composition of the diet may influencing on the umbilical plasma phospholipids composition that may cause a competition between fatty acid types which became more effective in altering the neonatal essential fatty acids status [25] in addition to the beneficial role of oil/fat antioxidants to increase fertility and reproductive performance [26 ,27]. Another factor may be attributed to the activation role of USFA diet on reproductive process, via providing a precursor for prostaglandin synthesis and can modulate the expression pattern of many enzymes involved in both prostaglandin and steroid metabolism, also the proportion of different PUFA in tissue of the reproductive tract reflects dietary consumption [28]. Our finding on peanut effect was similar to the findings of Tchokonte & Longo [29], they found that intraperitoneal injection of peanut oil in female rats increased endometrial receptivity; pregnancy rate and fertility by triggering decidulisation and deliveries than progesterone stimulate rats.

As shown in HF-HF and HF-M.d groups At birth, Pups of high fat fed dams had higher body weight than their low fat counterparts, the possible explanation that increase in milk preference from HFd dams, Therefore, the increased food intake was due to increased milk production in high fat fed dams [30], or due to permanent increase in galanin expression that induced a preference for fat ingestion in neonatal overfed dams [31]. The increased body weight in HF-HF and HF-M.d groups caused higher levels of plasma cholesterol as the fat deposition that have positive association with blood lipid [32] and higher plasma glucose may be related to impair insulin sensitivity, also diabetes association obesity increased glycosylated haemoglobin HbA1C [33]. Some of the following studies conducted on the effect of extreme obesity to reduce offspring birth weight and body size [7] or to increase pups mortality [34] in dams fed a high fat diet, and other researchers reported that obesity during pregnancy have long term effect on offspring development [17] , and the metabolism of offspring was effected and altered by overweight and gestational diabetes which have greater propensity to develop diabetes and / or obesity [35]. The peanut group (HF-P.d), olive oil (HF-O.d) low and moderate saturated diet groups ,their offspring weight as the control , this data demonstrate that different fatly acids had different effect on body weight , composition and metabolism [36]. The alteration in activity of hypothalamus gonadotropin releasing hormone (HGRH) neurons and consequent increase in the release of gonadotropin from pituitary gland cause the release of gonadal hormones from gonads and initiate puberty [37]. Our results indicate that fetal exposure to HFd and HF-M.d diet influence the timing of puberty in rat offspring. Body fat mass may contribute (HF group) [38], and the fat rich diet also effect [39] , or it occur independent on change in total body weight as shown in HF-O.d group and HF-HF group [40] .

High fat diet groups (HF, HF-HF) and HF-M.d female offspring had higher leptin concentration ,this may related to the quality of dietary fat during the suckling period influence the leptin levels and the responsiveness of the hypothalami-pituitary –adrenal axis in rat pups [41] or may the high fat diet through gestation cause hypothalamic leptin resistance in offspring ,increase leptin levels and developing obesity [42]. Offspring normal plasma leptin in HF-P.d and HF-O.d may caused by the effect of maternal fatty acid composition on leptin level in dams and in their pups [43] or because sucking pups on unsaturated fatty acids may lower leptin as their mothers. The hyperleptinemia

and hyperinsulinemia in offspring may cause by reduced sensitivity to circulating leptin and / or insulin in critical receptor region in hypothalamus [44] by increasing mRNA and protein levels of leptin receptor and insulin in HF fetus hypothalami and increased mRNA levels of neuropeptide y and agouitrelated polypeptide indicating orexigenic in offspring [12] these effects may be modulated by activity of unsaturated fatty acid on leptin and insulin receptors and increased their sensitivity and caused their action by decreasing insulin concentrations to normal levels .

REFERENCES

[1] Catalano PM , *J Clin Endocrinol Metab* , **2003**, 88,3505.
[2] Ehrenberg HM, Mercer BM, Catalano PM, *Am J Obstet Gynecol*, **2004**, **191**, 964.
[3] Oken E, Taveras EM, Kleinman KP, Rich-Edwards JW, Gillman MW, *Am J Obstet Gynecol*, **2007**, **196**, 322, 1.
[4] Gillman MW, Rifas-Shiman SL, Kleinman K, Oken E, Rich-Edwards JW , Taveras EM , *Obesity (Silver Spring)* ,**2008**, **16**, 1651.
[5] Omeni AA, Otamere HO, Adisa WA , Osifo UC, Iyamu OA, Ediangbe AP , Akpamu U , Odiase DE , *European Journal of Experimental Biology*, **2012**, 2 ,1974.
[6] Gholamreza F, Shahram S, Rahman S, *European Journal of Experimental Biology* , **2012**, 2 ,1226.
[7] Howie GJ , Sloboda DM , Kamal T, Vickers MH , *J Physiol*, **2009**, 587, 4 ,905 .
[8]Siemelink M, Verhoef A, Dormans JAM, Span PN, *Diabetologia* ,**2002**, 45,1397.
[9] Vickers MH, Krechowec SO, Breier BH, *Curr Drug Targets*, **2007**, 7, 923.
[10] Hill DJ, Duvillie B , *Pediatr Res*, **2000**, 48, 269.

[11] Armitage JA, Taylor PD , Poston L, *J Physiol*, **2005**, **565**, 3.
[12] Gupta A, Srinivasan M , Thamadilok S, Pate MS, *Journal of Endocrinology* , **2009**, 200, 293.
[13] Whitaker RC, *Pediatrics* , **2004**, 114 , 29.
[14] Van Assche FA, Holemans K , Aerts L, *British Medical Bulletin*, **2001**, 60, 173.
[15] Khan IY, Dekou V, Douglas G, Jensen R, Hanson MA, Poston L , Taylor PD, *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology* , **2005**, 288 ,127.
[16] Muhlhausler BS, Adam CL, Findlay PA, Duffield JA & McMillen IC, *FASEB Journal* , **2006** , 20,1257.
[17] Taylor PD & Poston L, *Experimental Physiology*, **2007**, 92, 287.
[18] Research diet INC, USA. www.researchdiets.com.
[19] Friedewald WT, Levy RI , Fredrickson DS, *Clin Chem* ,**1972**, 18, 499.
[20] Tietz NW, *Fundamentals of clinical chemistry*, W. B. Saunders Company, **1976** , pp 1263.
[21] Jones HN, Woollett LA , Barbour N , Prasad PD , Powell TL , Jansson T , *FASEB J*, **2009**, 23, 271.
[22] Sandra LR , Katie TB, Qing Y, Patrick TSO, Laura AW , *Am J Physiol Endocrinol Metab*, **2011**, 301,416.
[23] Graham J, Franks S, Bonney RC, *Fatty Acids*, **1994**, 50, 321.
[24] Wynn M, Wynn A, *The case for preconception care of men and women*. Bichester, United Kingdom: AB Academic, **1991**,pp 84.
[25] AlMDM, van Houwelingen AC, Hornstra G, *Am J Clin Nutr*, **2000**, 71, 285.
[26] Juneja SC, Williams RS, Farooq A, Cegini N, *J Asst Reprod Genet*, **1996**, 13, 578.
[27] Paumgartten FJ, De- Carvalho RR, Souza CA, Madi K, Chahoud I, *Braz J Med Biol Res* , **1998**, 31, 955.
[28] Wathes CD , Abayasekara RE , Aitken RJ, *Biology of reproduction* , **2007**, 77, 190.
[29] Tchokonte-Nana V, Benjamin Longo-Mbenza M.D, *Iranian Journal of Reproductive Medicine* , **2011**, 9, 301.
[30] Del Prado M, Delgado G, Villalpando S, *J Nutr* ,**1997**, 127,458.
[31] Plagemann A, Harder T, Rake A, Voits M, Fink H, Rohde W, Dorner G., *Brain Res*, **1999**, 836,146.
[32] Zamani A, Beni MA , Abadi MAZN , *European Journal of Experimental Biology*, **2012**, 2 ,1509.
[33] Murugan K, Shrivastava DK , Patil SKB, Sweety L, Debapriya G, Bharti A , Kumar LS , *Advances in Applied Science Research*, **2010**, 1 , 112.
[34] Bue, JM , Hausman, DB, Berdanier, CD , *Am. J. Obstet*., **1989**, 161, 234.
[35] Gluckman PD, Hansom MA , Beedle AS, *Biossays*, **2007**, 29,145.
[36] Pellizzon M, Buison A, Ordiz F, Ana LS, Catherine KL, *obesity research* , **2002** ,10, 947.
[37] Ojeda SR, *Perspect. Biol. Med*, **1991**, 34**,** 365.
[38] Frisch R E & McArthur JW, *Science*, **1974**, **185,** 949.
[39] Clarke R , Van Den Berg HW, Murphy RF, *J. Natl. Cancer Inst.*, **1990**, 82**,** 1702.
[40] Henderson BE, Ross R , Bernstein L, *Cancer Res.*,**1988**, **48,** 246.
[41] Trottier G, Koski KG , Brun T, Toufexis JD , Richard D, Walker CD , *Endocrinology.*, **1998**,**139,** 3704.

[42] Ferezou-Viala J, Roy AF, Serougne C, Gripois D, Parquet M, Bailleux V, Gertler A, Delplanque B, Djiane J, Riottot M , Taouis M , *Am J Physiol Regul Integr Comp Physiol*, **2007**, **293**, 1056.
[43] Korotkova M, Gabrielsson B, Hanson LA, Strandvik B, *J. Lipid Res.* ,**2001**, 42, 359.
[44] Mitra A. , Ph.D. thesis. Florida University (Gainesville,USA, **2008**).