Cross fostering to high fat fed dams impact the offspring Leptin sensitivity and obesity

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Abstract- Maternal obesity have important role to develop metabolic diseases in offspring. The aim of the study is to determine the role of postnatal nutrition by using a cross-fostered program to influence on obesity and Leptin resistance in female and male offspring. Female wistar albino rats (97±10 gm) fed either high fat diet (HF45%) or low fat diet (control LF10%) prior (12 weeks) and during gestation period. At lactation pups were cross-fostered either on HF dams (OLF-HF) or on LF dams (OHF-LF) in addition to HF and LF groups. Body weight, biochemical and hormonal parameters for mothers at pregnancy day 18 and for offspring at pubertal age were measured. Prior and through gestation, feeding with high fat diet significantly ($p<0.05$) increased maternal body weight, plasma biochemical and hormonal parameters in dams that effect on the metabolism of their offspring. The cross-fostered on HF dams (OLF-HF group) during lactation period, the offspring had normal range in body weight and pubertal age but significantly elevated fasting plasma glucose (F:14.140±0.331,M:14.06±0.209 mmol/L) total cholesterol (F:0.926±0.025, M:0.960±0.033 mmol/L), Leptin (F:1.248±0.045 ng/ml, M:1.396±0.069 ng/ml) and Insulin concentrations (F:0.350±0.002 ng/ml, M:0.446±0.004 ng/ml) while postnatal feeding on LF dams (OHF-LF group) showed early onset of puberty (F:41.676±0.541, M:41±1.011days), heavy body weight (F:129.276±0.983 gm, M:125.383±1.261 gm) and high levels of biochemical (F: Glu 16.533±0.404, TG 4.706±0.190, T.ch 1.076± 0.146 mmol/L; M: Glu 16.653±0.496, TG 3.333±0.152, T.ch 0.993±0.047 mmol/L) and hormonal parameters (F: Leptin 1.658±0.038 ng/ml, Insulin 0.498±0.001 ng/ml; M: Leptin 1.710±0.080 ng/ml, Insulin 0.481±0.004 ng/ml) in both six offspring but less than in HF group with significant differences ($p<0.05$). The results demonstrated that the postnatal nutrition had influence on offspring metabolic syndromes included obesity and diabetes in sex dependent manner, therefore the obesity can be corrected by changing in postnatal diet.

Index Terms- postnatal nutrition, high fat fed dams, offspring, Leptin level and metabolic syndromes.

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1 INTRODUCTION

Many experimental trials and researches reported the importance of prenatal nutrition in the fetus development [1] [2]. The metabolic abnormalities in childhood were influence greatly with increased prevalence of maternal obesity [3] [4]. Excess nutrition during pregnancy and lactation had adverse effects on offspring by increased risk of obesity [5] [6]. In human diabetic tendency in offspring enhanced by gestational diabetes [7]. The maternal over nutrition caused changes in intrauterine environment effecting on fetus growth, impairing Insulin tolerance [8] abnormal development of hypothalamic neuron included in Appetite regulation [9] and cardiovascular dysfunction [10].Some research showed the maternal nutrition with high fat diet during gestation and suckling periods can induced obese phenotype in male and female offspring independent of postnatal nutrition [11]. The clinical human studies showed that maternal obesity associated with hyperleptenimia, resulted from increased fat deposit, induced gestational complications and enhanced risk of metabolic syndromes in their offspring [12] [3] [4]. Our previous study showed that the obesity induced in female rats by high fat feeding for 12 weeks and then in gestation and lactation periods resulted to increase female offspring body weight, lipid profile, hyperLeptinemia and hyperInsulinemia [13], however some researches induced maternal obesity just during gestation and lactation periods but in our study we induced obesity in female rats before mating (12weeks) and through pregnancy and suckling periods to enhance intrauterine environment and then by using cross-fostering program to explain the role of lactation period (postnatal) to induce or protect offspring from metabolic disorder like Leptin resistance and obesity.

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2 MATERIAL AND METHOD

2.1 Diets

Diet induced obesity (DIO) in rodents (HF 45 % fat) and its control (LF 10 % fat) were formulated according to the Research Diet INC. [14]. 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)
Casein	200	200
L- cysteine	3	3
Cornstarch	315	72.8
Sucrose	385	272.8
Cellulose powder	50	50
Soy bean oil	25	25
Beef tallow	20	177.5
Mineral mixture	10	10
Dicalcium phosphate	13	13
Calcium carbonate	5.5	5.5

Potassium citrate	16.5	16.5
Vitamin mixture	10	10
Choline bitartarate	2	2
Total weight gm Total Kcal Total Kcal/ gm	1055 4057 3.85	858.1 4057 4.73

2.2 Animals

Female wistar albino rats (weight 97±10 gm) were divided randomly into two weight matched groups, a control group and high fat group (HF). In the control group n= 12, the rats were fed low fat diet (10% energy from fat: beef tallow) while the HF group n=20 were fed on high fat diet (45% energy from fat: beef tallow) for 12 weeks. Food intake and Body weight was recorded weekly. The energy intake was calculated according to [15]: Energy intake = food consumed (gm) × total kcal/gm diet. A group of virgin female rats (n=12) from each dietary experimental groups (LF, HF) were mating to males (aged 17 -18 weeks). Day one of pregnancy was determined by the presence of spermatozoa. Pregnant rats were housed in groups (n=2 each cage) in standard cage and maintained on their experimental assigned diets with free access to water. All rats were kept in constant temperature (25-30C°) and 12:12 h light: dark cycle. During the gestation period, daily food consumption and body weight were recorded. On the day 18 of gestation, three pregnant animals from each dietary group were sacrificed after anesthetizing with pentabarhital sodium 60 mg/kg body weight, blood samples were collected and plasma stored at -78C° in deep freezing for biochemical and hormonal measurements. On the days 21 and 22 of pregnancy, animals were monitored and observed during delivery. After delivery dams and pups' body weight were recorded. Pups in each dam were normalized to 8 to minimize the differences.

At birth half of the control offspring were cross-fostered to high fat fed dams and vice versa to form four groups [control offspring suckling on same dam (OLF), high fat offspring suckling on the same dam (OHF), control offspring suckling on HF dam (OLF-HF) and high fat offspring suckling on control dam (OHF-LF)]. Dams were continued on their assigned diet in each dietary group during the lactation period. In the day 20-21 of lactation, dams from each group were slightly anesthetize and intrapertonial injected with oxytocin (4 IU, Germ) and milk was collected after 15 minutes in class capillary tubes [16] and frozen in -70C° for Leptin analysis. Post weaning, the female and male offspring of each dietary experiment were weighed and fed with control diet (10%fat). Body weight was recorded weekly, the vaginal opining was examined daily until puberty, body weight was recorded and the female offspring (n=3) from each group anesthetized and sacrificed. Blood samples were collected, Plasma stored at -70C° for biochemical and hormonal determination.

2.3 Plasma Biochemical Analysis

Plasma glucose, total cholesterol (T-ch), triglycerides (TG) concentrations were measured by enzymatic method using diagnostic Kit from Randox (UK) and Biolabo companies (France).

2.4 Plasma and Milk Hormonal Analysis

Plasma rat Leptin, Insulin and milk Leptin concentrations (ng/ml) were measured using Rat Elisa kit from CRYSTAL CHEM INC (for Leptin cat no. 90040 USA, for Insulin cat no. 90010 USA).

The homeostatic index of Insulin resistance (HOMA-IR) was calculated according to the equation [17]: HOMA-IR = [Glucose (mmol/L) × Insulin (pmol/L)] ÷155. (Insulin converting from ng/ml to pmol/L: multiplied by 150 [18]).

2.5 Statistical Analysis

Data were analyzed by ANOVA using SPSS version 15 statistic program. Comparisons between means were made using least significant differences (LSD) using Genstat3 statistic program. Differences were considered to be significant at $p<0.05$. Data are presented as means ± standard deviation.

3 RESULT

3.1 Dams

3.1.1Maternal Food Consumed and Energy Intake Obese pregnant rats consumed less food and energy intake (100.971±2.170gm, 477.586±5.265 kcal/week) with significant differences than the control group (134.650±1.564gm, 518.402± 4.025 kcal/week) during the three weeks of gestation time (figure 1 and 2).

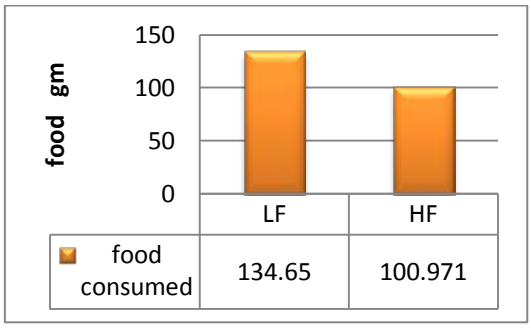

Fig 1: Maternal Food Consumed during Pregnancy Period in the Tested Groups. LF: low fat diet, HF: high fat diet. Means ($p<0.05$).

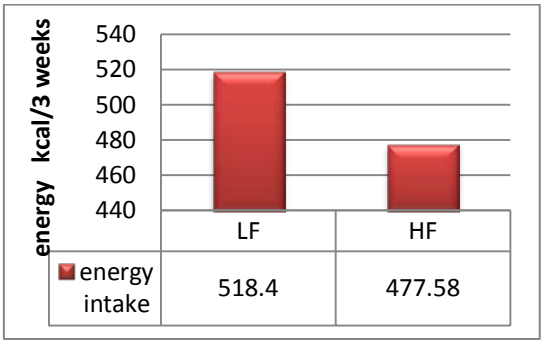

Fig 2: Maternal Energy Intake during Pregnancy Period in the Tested Groups. LF: low fat diet, HF: high fat diet. Means ($p<0.05$).

3.1.2 Body Weight at Gestation Period

The female rats consumed high fat diet prior (12 weeks) and through pregnancy showed significant ($p<0.05$) increasing in body weight especially in the third week (436.353±0.996 gm) compared to the normal weight in the control group (332.913 ±1.123gm) (figure 3).

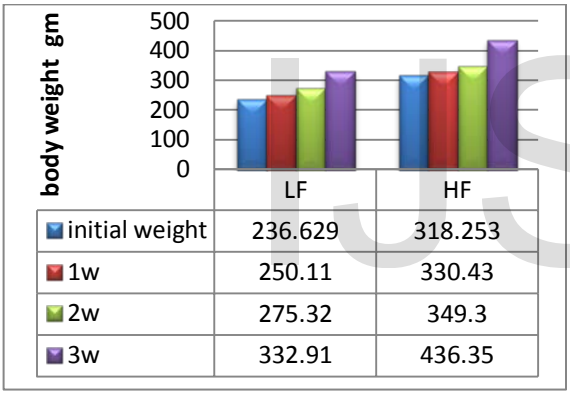

Fig 3: Maternal Body Weight during Pregnancy Period in the Tested Groups. LF: low fat diet, HF: high fat diet. Means ($p<0.05$).

3.1.3 Maternal Biochemical and Hormonal Measurements

The obese pregnant rats from HF group showed significant ($p<0.05$) elevated levels of plasma biochemical parameters at day 18 of gestation, that included glucose 14.972, triglycerides 1.703, cholesterol 1.90 mmol/L compared to control group (table 2). The Insulin sensitivity was decreased in HF dams by increasing HOMI 59.868 comparing to 17.632 in control group, this is also clearing from increased plasma Insulin level 4.132 ng/ml. obsess dams showed higher concentration of plasma Leptin (prenatal) 4.970 ng/ml and milk Leptin (postnatal period: day 21 of suckling) 5.103 ng/ml.

Table 2: Biochemical and Hormonal Parameters of Dams in the Tested Groups. LF: low fat diet, HF: high fat diet. Mean± S.D. ($p<0.05$), different letters referred to significant.

Parameters	LF fed dams	HF fed dams
Glu. mmol/L	12.831±2.452b	14.972±3.875a
TG mmol/L	0.735±0.004b	1.703±0.110a
T.ch mmol/L	0.876±0.111b	1.90±0.281a
Leptin ng/ml	2.931±0.430b	4.970±0.380a
Insulin ng/ml	1.420±0.349b	4.132±0.761a
HOMI	17.632±2.218b	59.868±4.210a
Milk Leptin ng/ml	2.531±0.303b	5.103±0.290a

3.2 Offspring

3.2.1 Birth and Weaning Weights

The higher body weight in high fat fed dams was reflected in their pups by increasing their body weight with significant ($p<0.05$) differences (6.77±1.204 gm) than the control group (5.60 ±1.031 gm). After 21 days of suckling, female and male pups of high fat dams characterized by high weaning weight (58.666±3.163 gm; 42.333 ±1.154 gm) with significant ($p<0.05$) than the control group.

The cross-fostered on LF dams (OHF-LF group) or on HF dams (OLF-HF group) showed the same effect. In female pups increasing their weaning body weight (45.666± 0.577, 43.666 ±1.527gm) without significant ($p<0.05$) differences between the two groups, but showed significantly ($p<0.05$) in comparison with female pups of HF group. The male pups also had higher weaning body weight (41.0±1.010, 41.333±1.145 gm) but with no significant ($p<0.05$) differences than the HF group (figure 4 and 5).

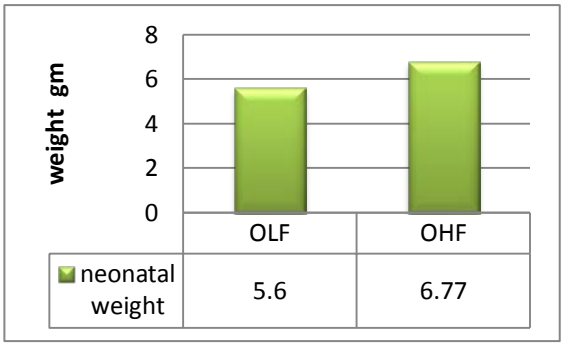

Fig 4: Pups Birth Weights Related to Dams in the Tested Groups. LF: low fat diet, HF: high fat diet. Means ($p<0.05$).

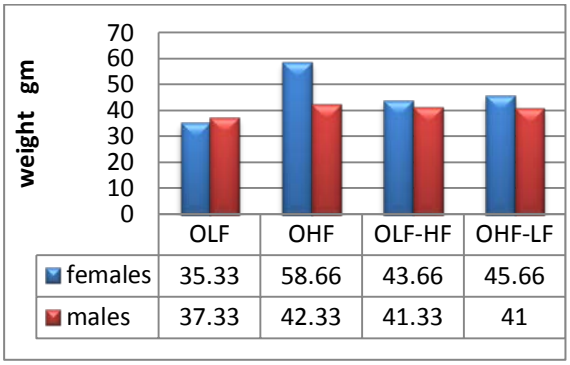

Fig 5: Pups Weaning Weights Related to Dams in the Tested Groups. LF: low fat diet, HF: high fat diet, OLF-HF: offspring of low fat dams suckling from high fat dams, OHF-LF: offspring of high fat dams suckling from low fat dams. Means ($p<0.05$).

3.2.2 Offspring Puberty Weight and Age

At puberty, the female and male offspring of HF and LF groups showed the same pattern of growth as at weaning with significant ($p<0.05$) differences between the two groups (141.156±1.596, 139.38 ±1.061; 125.530±1.22, 121.760±1.702 gm). The cross-fostering on LF dams (OHF-LF) increased body weight in both sex in offspring with significant ($p<0.05$) differences than the other groups (129.276±0.983, 125.383±1.261 gm) while in OLF-HF group the offspring had less weight at puberty (125.340 ±1.055, 122.083±1.457 gm) as the control group with no significantly between them (125.530 ±1.220, 121.760±1.702gm) (figure 6). The HF group showed early age of puberty (38.726 ±0.546; 38.333±1.527 days) compared to the control group (43.660±0.580, 44±1.021 days), followed by OHF-LF group (41.676±0.541, 41±1.011 days). The cross-fostering on HF dams (OLF-HF group) had normal age of puberty as the control group (figure 7).

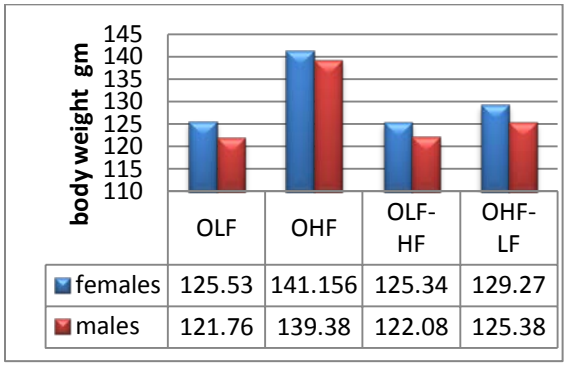

Fig 6: Offspring Puberty Weights Related to Dams in the Tested Groups. LF: low fat diet, HF: high fat diet, OLF-HF: offspring of low fat dams suckling from high fat dams, OHF-LF: offspring of high fat dams suckling from low fat dams. Means ($p<0.05$).

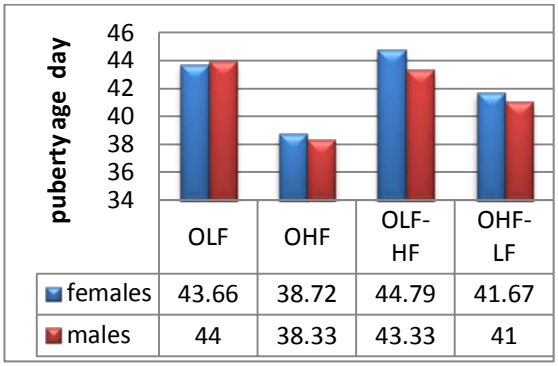

Fig 7: Offspring Puberty Ages Related to Dams in the Tested Groups. LF: low fat diet, HF: high fat diet, OLF-HF: offspring of low fat dams suckling from high fat dams, OHF-LF: offspring of high fat dams suckling from low fat dams. Means ($p<0.05$).

3.2.3 Offspring Biochemical Parameters

Fasting plasma glucose (Glu), triglycerides (TG) and total cholesterol (T.ch) were significant ($p<0.05$) increased in female and male offspring of HF group (HF fed dams) (female: 17.786, 5.530, 1.779 mmol/L; male: 17.186, 3.763, 2.126 mmol/L) compared to the control group (table 3 and 4).

Cross-fostered in OHF-LF group, both sex offspring showed elevation levels of plasma Glu, TG and T.ch (female:16.533, 4.706, 1.076; male:16.563, 3.333, 0.993 mmol/L) compared to low levels in OLF-HF group (female:14.140, 2.833, 0.926; male: 14.063, 2.900, 0.960 mmol/L) with significant ($p<0.05$) differences between them. In comparison with the control group, the levels of the previous parameters in male offspring of OLF-HF group showed significantly ($p<0.05$) than the control group except in TG levels.

Table 3: Biochemical Parameters of Female Offspring Related to Dams in the Tested Groups. LF: low fat diet, HF: high fat diet, OLF-HF: offspring of low fat dams suckling from high fat dams, OHF-LF: offspring of high fat dams suckling from low fat dams. Mean± S.D. ($p<0.05$), different letters referred to significant.

Treatments	Gluc mmol/L	TG mmol/L	T.ch mmol/L
OLF	12.51±0.215d	2.827±0.102c	0.661±0.018d
OHF	17.786±0.467a	5.530±0.200a	1.779±0.103a
OLF-HF	14.140±0.331c	2.833±0.115c	0.926±0.025c
OHF-LF	16.533±0.404b	4.706±0.190b	1.076± 0.146b

Table 4: Biochemical Parameters of Male Offspring Related to Dams in the Tested Groups. LF: low fat diet, HF: high fat diet, OLF-HF: offspring of low fat dams suckling from high fat dams, OHF-LF: offspring of high fat dams suckling from low

fat dams. Mean± S.D. ($p<0.05$), different letters referred to significant.

Treatments	Gluc mmol/L	TG mmol/L	T.ch mmol/L
OLF	12.91±0.534d	2.803±0.100c	0.667±0.020d
OHF	17.186±0.664a	3.763±0.055a	2.126±0.127a
OLF-HF	14.063±0.209c	2.900±0.100c	0.960±0.033c
OHF-LF	16.653±0.496b	3.333± 0.152b	0.993±0.047b

3.2.4 Offspring Hormonal Parameters

Female offspring of HF dams showed elevated levels of fasting plasma Leptin, Insulin and HOMI concentrations (2.116 ng/ml, 0.529 ng/ml, 9.107) compared to the low levels in control group OLF (1.402 ng/ml, 0.404 ng/ml, 4.882) with significant ($p<0.05$) differences between them (table 5 and 6). The cross-fostered on LF dams (OHF-LF group): female and male offspring showed higher Leptin and Insulin concentrations (female: 1.658 ng/ml, 0.498 ng/ml; male: 1.710 ng/ml, 0.481 ng/ml) followed by OLF-HF group (female: 1.248 ng/ml, 0.350 ng/ml; male:1.396 ng/ml, 0.446 ng/ml) with significant ($p<0.05$) differences between them. The Insulin sensitivity was enhanced in cross-fostered groups especially in OLF-HF group without significant differences than the control group.

Table 5: Hormonal Parameters of Female Offspring Related to Dams in the Tested Groups. LF: low fat diet, HF: high fat diet, OLF-HF: offspring of low fat dams suckling from high fat dams, OHF-LF: offspring of high fat dams suckling from low fat dams. Mean± S.D. ($p<0.05$), different letters referred to significant.

Treatment	Leptin ng/ml	Insulin ng/ml	HOMI
OLF	1.402±0.068c	0.404±0.003c	4.882±0.116c
OHF	2.116±0.026a	0.529±0.002a	9.107±0.240a
OLF-HF	1.248±0.045d	0.350±0.002d	4.789±0.121c
OHF-LF	1.658±0.038b	0.498±0.001b	7.966±0.231b

Table 6: Hormonal Parameters of Male Offspring Related to Dams in the Tested Groups. LF: low fat diet, HF: high fat diet, OLF-HF: offspring of low fat dams suckling from high fat dams, OHF-LF: offspring of high fat dams suckling from low fat dams. Mean± S.D. ($p<0.05$), different letters referred to significant.

Treatment	Leptin ng/ml	Insulin ng/ml	HOMI
OLF	1.470±0.026c	0.465±0.005b	5.809±0.234c
OHF	1.875±0.056a	0.485±0.001a	8.068±0.361a
OLF-HF	1.396±0.069d	0.446±0.004c	6.068±0.322c
OHF-LF	1.710±0.080b	0.481±0.004a	7.708±0.405b

4 Discussion

The data of our study showed that the obese dams of high fat group consumed less food during gestation period than the control group (LF diet) this finding may be explained by the effect of the high value of fat in the HF diet (45%) compared to LF diet (10%). Previous studies supported this results and referring to the role of fat diet content [19]. Despite decreasing in maternal caloric intake, HF dams gained more body weight during pregnancy, this may be contributed to increase body fat store resulted from saturated fatty acids consumption which characterized by low oxidative rate [20], this result also supported by Howie [11] that increased body weight in HF dams through gestation and lactation compared to control group.

The high levels in biochemical parameters in obese pregnant rats may be related to effect of saturated fatty acids to promote hepatic triglycerides synthesis [21] that suppressed LDL receptor and elevate cholesterol levels [22] while the hyperLeptinemia and hyperInsulinemia in obese dams may be related to reduced their action in hypothalamus by abnormal signaling transduction [23] which showed by increasing HOMI levels that indicating Insulin resistance that explained the hyperglycemia of these groups, in addition to the role of saturated fat to impair receptors of glucose transport (GLUT 1 and 4) in the cell membrane[24].

The neonate born from HF dams showed high birth weight than the control group, this may be related to changes through embryonic development which include alteration in gene expression of nutritional transport like glucose (Glu T4) [25] and fatty acids oxidation factors proliferator activated receptor (PAR) that accelerate fetal [26] or increase milk preference and fat ingestion as a resulted from permanent increase in galanin expression [27].

Our results demonstrated that female offspring of cross-fostered OLF-HF group increased body weight at weaning compared to LF group, this reflect the effect of type of feeding during lactation period which caused change in body weight, which was also clear in pups of OHF-LF group that showed high body weight but less than of HF group. The possible explanation either related to increase fat level in HF dams milk, since it correlate with dietary fat [28] or related to Leptin effect that can be transmitted to offspring via maternal milk [16] which showed high level in milk of HF dams.

Sex differences in offspring was reflected in male pups at weaning that showed less body weight compared to female

pups with the same pattern of growth in HF and cross-fostered groups. The differences in gender offspring may response to maternal HF diet which also reported by other studies [16] [29].

At puberty, female offspring of cross-fostering OHF-LF group showed high body weight compared to OLF-HF group, this reflecting the role of gestation period in HF dams rather than the feeding type in lactation time to cause alternations in hypothalamic development of growing fetus which increased mRNA expression of neuropeptide Y, agoutirelated polypeptide, Leptin and Insulin receptors in HF fetus hypothalami [9] that responsible of orexigenic effect and contributed to initiate puberty in early age in both six of offspring in OHF-LF group in addition to the effect of body fat mass [30] and fat rich diet [31] especially in HF group. The same effect was also shown in male offspring of HF, LF and cross-fostered groups.

Plasma glucose and lipid profile were increased in both sex offspring of HF group that may be related to that gestational diabetes caused change in metabolism of the offspring [32] and as reported previously alternation in fetus hypothalamus during gestation lead to orexigenic effect [9].

In cross-fostered group OLF-HF the postnatal on HF dams enhanced susceptibility to metabolic abnormalities by increasing plasma glucose and lipid profile, while in OHF-LF group the exposure to LF dams led to decrease these parameters suggesting correct metabolic syndromes in this group that may developed during prenatal period.

The prenatal feeding with HF diet was sufficient to impair Leptin sensitivity by increasing plasma Leptin level even when exposure to postnatal LF dams that showed in OHF-LF group. This elevation either related to responsiveness of the hypothalami-pituitary-adrenal axis in offspring to level of fat milk through lactation period [28] or as resulted of hypothalamic Leptin resistance [29] while the hyperInsulinemia resulted from hypothalamic Insulin resistance [33] by increased Insulin receptor gene expression and reduced sensitivity [9] that indicating from the results by increasing HOMI level in offspring.

In OLF-HF group, the high concentration of Leptin may resulted from transmitted the hormone via maternal milk to offspring which have influence especially in the later postnatal period [16]. Also the alternation in Leptin secretion from dams during suckling period caused changes in hypothalamic development of neonate, as Leptin is important trophic factor influence on neurons development in hypothalamus [34] and then metabolic syndrome [35], the study of Sun [16] showed high Leptin level in HF dams milk in the first postnatal week, therefore, in this study, the biochemical and hormonal parameters of OLF-HF pubs were changed by alternation in the development of pups hypothalami.

5 Conclusion

This study examine the effect of postnatal feeding on metabolic features of rats offspring by use cross- fostering procedure. The results showed that the type of dams feeding during lactation period induced change in offspring body weight, biochemical and hormonal parameters in sex dependent manner that may cause alternation in pups hypothalami presented by initiate puberty, therefore the Leptin resistance, obesity and diabetes can be corrected by change the postnatal diet.

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