

Fat and Carbohydrate Distribution in Maternal Diet Programs Liver Sirtuin-1 Expression and Fatty Acid Profile in Mice Offspring

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Abstract

Background: Amount of fat and carbohydrate in maternal diet during gestation and lactation has permanent effects on fetal metabolism. SIRT1 is a nutrient-responsive histone deacetylase that modulates the lipid and glucose metabolism in response to energy stress and extends life span. To study the effects of carbohydrate and fat distribution in a maternal isocaloric diet on fetal gene and protein levels of SIRT1, as well as liver fatty acid profile.

Methods: For this purpose, female mice were inseminated and randomly received the AIN 93G isocaloric pair-fed LF-HC (16% and 64% of calorie as fat and carbohydrate) or HF-LC (48% and 32% of calorie as fat and carbohydrate) diet during gestation and lactation.

Results: SIRT1 gene and protein levels were lower in both sexes born from HF-LC-fed mothers than LF-HC-fed one, significant differences were only observed between males in the gene expression ($p < 0.001$) and females in protein level ($p < 0.001$). Saturated fatty acids and cholesterol were increased while unsaturated fatty acids decreased at the liver of male and female offspring born from HF-LC-fed mothers ($p < 0.001$).

Conclusions: Maternal dietary fat and carbohydrate distribution, regardless of calorie intake, modify the offspring hepatic fatty acid profile, as well as SIRT1 gene and protein expression which effects on life span.

Background

Silent mating type information regulation two homolog 1 (SIRT1) is a nuclear metabolic sensor which is mostly conserved in mammals. It is an NAD⁺-dependent enzyme that regulates epigenetic modifications, as well as gene expression through de-acetylation of histones, transcription factors, and transcription co-factors [1, 2]. Studies reported that [nicotinamide mononucleotide supplementation, as the main coenzyme of SIRT-1 promotes neurovascular rejuvenation; activates SIRT1 at the transcriptional level, protects mitochondria from damage](#), reduces apoptosis and oxidative stress [3, 4]. Recent studies reported that SIRT1 regulates carbohydrate and lipid metabolism including gluconeogenesis, fatty acid oxidation, cholesterol efflux, bile acid synthesis, and lipogenesis at the liver [5-7]. An increase in SIRT1 activity improves liver insulin sensitivity and decreases energy requirements [8]. Recently, attention to SIRT1, as the role player of metabolism is increasing because it is recruited to the damaged sites and promote DNA repair through de-acetylating the repair proteins such as poly (ADP-ribose) polymerase (PARP)-1, Ku70, NBS, and Werner (WRN) helicase and related to lifespan [9].

Interestingly enough, that maternal diet during gestation and lactation creates persistent alterations in fetal metabolism according to the “developmental origins of health and disease” (DOHaD) hypothesis [10]. Hence, early life nutrition and maternal diet can result in developmental adaptations that produce permanent metabolic, physiologic and phenotypic changes without DNA sequence alterations, which predispose an individual to chronic diseases in the adult life [11]. All of these changes are related to the epigenetic marks [12]. Previous studies have shown that type and amount of maternal dietary oil can change fat and bone tissues gene expression at the next generation in a sex-dependent manner [13, 14]. Moreover, one non-human primate study reported that maternal high fat-high calorie diet acetylates histone H3 (H3K14ac) in the liver of offspring via SIRT1 pathway followed by abnormal cytoplasmic lipid accumulation and homeostasis which predispose the next generation to fatty liver disease [15]. Studies have shown that calorie restriction is an inducer for SIRT1 gene expression and protein level, which finally lead to more lifespan [16, 17]. According to our knowledge, all studies focused on the high and restricted calorie diet, and no research was done on the isocaloric diet by considering macronutrients distribution up to now. Also, there is no study to assess this effect in critical periods of life, such as gestation and lactation on the next generation. Therefore, we designed the present study regarding fat and carbohydrate distribution in an isocaloric maternal diet on offspring SIRT1 gene expression and protein level with attention to the profile of fatty acids in the liver.

Materials And Methods

Animal experiments

This research conforms to the Institutional and National Guide for the Care and Use of Laboratory Animals. The present study was conducted in accordance with the ARRIVE guidelines and the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978). Twenty female C57BL/6 mice (21 ± 1.5 g) were housed according to the standard protocol for maintenance of laboratory animals ($21-23$ °C; 50 ± 5 % humidity; and 12 h artificial light cycle). After two weeks of adaptation with AIN 93M diet, female mice were mated overnight. The vaginal plug was confirmed, and mothers were randomly divided to the LF-HC and HF-LC diets, which contain 16% and 48% of calories as fat, respectively. LF-HC diet supplies 64% of calorie as carbohydrate compared with 32% in the HF-LC group. Both diets had 3.97 kcal/g. (Table 1) Mothers received LF-HC and HF-LC diets during gestation and lactation periods in a pair-fed model. The number of offspring was equaled in all cages ($n=4$), nursed, and lactated with their mothers for three weeks. Mice were separated according to the sex, and all offspring received LF-HC diet up to 6 weeks. In the end, one male and one female offspring were randomly selected and euthanized by ketamine and xylazine. Liver tissues were removed, snap froze and kept at -80 °C for final gene and protein expression, as well as gas chromatography- mass spectroscopy (GC/MS) analysis at six weeks of age. The study protocol illustrated at figure 1.

SIRT1 gene expression

Frozen liver tissues were powdered in liquid nitrogen (N₂), and total RNA was extracted using TRIzol Lysis Reagent (QIAGEN Inc., Valencia, CA 91355, USA). After tissue washing by PBS, 300 μ l of TRIzol was added to prepare the lysates. Then, chloroform was added, and samples were centrifuged (10000 rpm) for 15 min at 4 °C. To purify the RNA supernatants were collected, isopropanol was added, and samples were stored for 30-60 min at -20 °C. Finally, the samples were centrifuged (10000 rpm) for 15 min at 4 °C. To omit the possible contaminants such as lipids, 700 μ l of alcohol (70-80%) was added and centrifuged (7500 rpm) for 10 min at 4 °C. RNA sediments were dissolved in 20 μ l of distilled water for 5 min at $45-55$ °C. The ND-1000 spectrophotometer (DPI-1, QIAGEN Inc., USA) was used to assess RNA quantity at 260 nm. Quality (integrity) of the extracted RNA was checked by agarose gel electrophoresis. The cDNA was synthesized from one microgram of total RNA using Fermentas protocols (Fermentas Co. USA).

ABI StepOne sequence detection system (Applied Biosystems, California, USA) was used for the real-time polymerase chain reaction (RT-PCR) with 10 pmol of the forward and reverse primers for SIRT1 and glyceraldehyde 3-phosphate dehydrogenase (GAPDH, housekeeping gene), 1 μ l of the synthesized cDNA and SYBR Green I Master Mix (Roche) in duplicate runs. Both primers were designed by the Primer Express software 2.0.0 as bellows: SIRT1 (Forward: GTGTCATAGGATAGGTGGTG; Reverse TATGAAGAGGTGTTGGTGG) and GAPDH gene (Forward: CTATGTTTGTGATGGGTGTGA; Reverse AGTGGATGCAGGGATGATGT).

The copy threshold (Ct) values are calculated and normalized against GAPDH mRNA as an appropriate control [11]. The amplification profile included 40 three-step cycles: 94 °C for 20, $58-60$ °C for 30 s and 72 °C for 30 s. The results were generated and analyzed using the $2^{-\Delta\Delta Ct}$ method in which was computed as follows:

Western blotting

Liver tissues were washed by PBS, powdered on ice, and transferred to a micro tube. Then, 200 μ l of Rippa buffer, as a protease inhibitor, was added and stored for 90 min at 4 °C. The lysates were centrifuged by a refrigerator centrifuge. 50 mg of protein (from each sample) was added to the same amounts of loading buffer for 5 min at 95 °C. Then, denaturated proteins were loaded on wells, inserted in the western pons and running buffer was added at 90-100 mV for 220-230 min. Proteins were separated by 10% SDS-PAGE (PH=6.8) and transferred to nitrocellulose filter membrane at 80 mV for 70-80 min at 4 °C. After overnight incubation in blocking buffer (skim dried milk), diluted SIRT1 antibodies were diluted by TBS-Tween buffer (1:200), added to the membrane and shaken for 120 min. Samples were washed three times with TBS-Tween buffer and shaken for 10 min in each step. Anti-SIRT1 antibody (1:3000) was added and stored on the shaker for 90 min. Then, samples were washed by TBS-Tween for three times and shaken for 10 min. GAPDH antibody was used as the housekeeping protein. Chemiluminescence detection system and ImageJ software were used for detection of protein bands and analyzing the data, respectively.

Gas chromatography-mass spectrometry (GC/MS)

Frozen samples were powdered in N₂, and a mixture of chloroform-methanol (1 mL, 2:1; v/v) was added to the 0.5 g of each sample and shaken for 10 min. The chloroform phase containing the lipids was separated, and the aqueous phase was extracted again, similarly. Then, samples were centrifuged (4500 rpm) for 5 min and supernatants collected for the derivatization step. The 2% H₂SO₄-methanol, as a methylation/transesterification solution, was added to the extracted samples and refluxed for 45 min at 80 °C. After neutralization with NaOH (1 M; PH=7), n-hexane was added to each sample, and supernatant was collected for GC/MS analyses.

Analyses were performed on a 5977A MS and 7890B GC (Aligent Co., USA) equipped with the split/splitless column as injector system and HP5-MS (60 m × 0.25 m × 0.25 μm) column for fatty acid profile analysis. The oven temperature was kept at 70 °C for 5 min, then programmed at 15 °C /min to 150 °C and kept for 2 min. Finally, the system programmed at 20 °C /min to 290 °C and kept for 10 min. Electron impact ionization (EI+, 70 eV) was used for all samples. The split ratio was settled on 1:20.

Statistical analysis

By considering a power of 80% and $\alpha=0.05$, sample size was computed according to differences in gene and protein expression of SIRT-1, weight of offspring and lipid profile in the liver through a power analysis using the Mann-Whitney-Wilcoxon test and one-way ANOVA in IBM SPSS statistics software (version 18; IBM Corp). The required sample size was six (for gene and protein expression of SIRT-1), ten (for weight) and five (for lipid profile of liver) offspring in each group to determine the variations. Data were tested for normal distribution using the Kolmogorov-Smirnov test and did not have normal distributions in gene expression even after all of the transformation methods. Then, the differences were measured by the Mann-Whitney-Wilcoxon test. One-way ANOVA was used to compare the lipid profile of liver among the groups. All data are expressed as the means \pm SE. The level of significance was set at $P<0.05$.

Results

Maternal weight gain during gestation

Maternal weight gain was significantly different at week three of gestation. Mothers on the LF-HC diet were significantly heavier than HF-LC-fed one ($p<0.001$). Weight gain of mothers had not significant difference at week 1 and 2. The trend of weight gain is shown in Fig 2 (a).

Effect of the isocaloric LF-HC and HF-LC diets on weight of offspring

Birth and adolescence weight of offspring born from LF-HC-fed mothers was significantly higher in males than females (1.85 ± 0.1 g vs. 1.42 ± 0.14 g, $p<0.001$; and 23.2 ± 1.06 g vs. 19.1 ± 0.7 g, $p<0.001$, respectively). Similarly, adolescence weight was significantly higher in the male offspring born from HF-LC-fed mothers than females (24.2 ± 1.6 g vs. 21.8 ± 1.3 g, $p=0.01$). But birth weight of male offspring was significantly reduced in the HF-LC-fed mothers compared to females (1 ± 0.14 g vs. 1.45 ± 0.1 g, $p<0.001$). Birth weight of male offspring born from LF-HC-fed mothers was significantly higher than HF-LC-fed one ($p<0.001$). Moreover, adolescence weight of female offspring was significantly higher in the HF-LC-fed mothers than LF-HC-fed group ($p=0.005$). The trend of weight gain is shown at Fig 2 (b) & (c).

Effect of the isocaloric LF-HC and HF-LC diets on SIRT1 gene expression

Although there was no significant difference between the female offspring of the two studied groups, SIRT1 gene expression was significantly higher in the liver of male offspring than females at the LF-HC group ($p<0.01$). Also, SIRT1 gene expression was significantly reduced in the liver of male offspring born from mothers received HF-LC compared with the LF-HC diet ($p<0.001$). (Fig 3a)

Effect of the isocaloric LF-HC and HF-LC diets on SIRT1 protein level

At the offspring born from mothers received LF-HC diet, SIRT1 protein level was significantly higher in the liver of males than females ($p<0.001$). Also, SIRT1 protein level was significantly reduced in the liver of female offspring born from mothers received HF-LC than LF-HC diet ($p<0.001$). (Fig 3b & c)

Effect of the isocaloric LF-HC and HF-LC diets on liver fatty acid profile

Meristic (C_{14:0}) and palmitic (C_{16:0}) acid levels were significantly higher in the liver of female offspring born from mothers received HF-LC compared with LF-HC diet group ($p < 0.001$). Stearic (C_{18:0}), oleic (C_{18:1}), linoleic (C_{18:2}), linolenic (C_{18:3}), dihomo- γ -linolenic acid (C_{20:3}), arachidonic (C_{20:4}) and docosahexaenoic (C_{22:6}) acid were significantly higher in the female offspring of LF-HC than HF-LC diet group ($p < 0.001$). Fatty acid profile in the liver of male offspring was as the same as females. Moreover, eicosapentaenoic acid (C_{20:5}) was significantly higher in the male offspring of LF-HC diet group ($p = 0.001$). Palmitic (C_{16:0}) and stearic (C_{18:0}) acid were significantly lower in the liver of male offspring born from HF-LC-fed mothers than females ($p = 0.03$ and $p = 0.01$, respectively), but oleic acid (C_{18:1}) was significantly higher in the males than females ($p < 0.001$). Liver cholesterol level was significantly increased in the male and female offspring born from HF-LC-fed mothers compared with the LF-HC-fed one ($p < 0.001$, in all cases). Also, cholesterol was significantly higher in the liver of male offspring born from HF-LC-fed mothers than females ($p < 0.001$). (Table 2)

No adverse evidence was shown from the experimental protocol during the study.

Discussion

For the first time, we showed that maternal high fat diet, regardless of calorie intake, during pregnancy and lactation alters fatty acid profile, as well as SIRT1 gene and protein expression in the liver of offspring. Interestingly, female offspring were more susceptible to these changes, compared to the males. Male offspring born from LF-HC-fed mothers were heavier than females, both at the birth and adolescence. Similarly, adolescence weight was significantly higher in the male offspring born from HF-LC-fed mothers than females. However, birth weight of male offspring was significantly reduced in the HF-LC-fed mothers compared to females. SIRT1 gene and protein level were significantly higher in the males than females born from LF-HC-fed mothers compared to the HF-LC-fed group. SIRT1 gene expression was significantly higher in the males at the LF-HC compared with the HF-LC diet group, but these changes were not seen at the protein level. Saturated fatty acids (meristic and palmitic acid) were significantly higher in the liver of male and female offspring born from HF-LC-fed mothers, compared to the LF-HC-fed one. Stearic acid and unsaturated fatty acids level were significantly increased at the liver of male and female offspring of LF-HC than HF-LC group. Liver cholesterol level was significantly higher in males than in females.

Studies conducted to the effect of maternal high fat-high calorie diet on SIRT1 are scarce [15, 18]. Previous studies have shown that SIRT1 expression is reduced in the fetus, especially liver, by maternal obesity, which is related with lower life span [19, 20]. One animal model study on epileptic rats showed that a ketogenic diet (fat: protein+ carbohydrate; 6:1) increases hypothalamic SIRT1 gene expression which was related to brain health [21]. Then, results in this field have inconclusive results, which need more and more precise studies.

According to the DOHaD hypothesis, maternal nutrition has permanent effects on fetal epigenome via epigenetic pathways, which increases susceptibility to chronic diseases at the adulthood [10]. All of these changes can be inherited to the next generation, permanently. A recent study reported that maternal diet have significant effect on litter size and survival, pregnancy rate, as well as body weights which is accordance with our results [22]. At the mentioned study maternal western diet reduced offspring survival to 65% compared with the calorie restricted (75%), sucrose (80%) and standard (95%)-fed groups. Moreover, offspring born from western diet-fed mothers were heavier than other groups. Another study compared the effects of long-term high-fat vs. energy-restricted diet on endothelial nitric oxide synthase (eNOS)-Sirtuin-1 axis and Akt/eNOS phosphorylation in the cavernous tissue. Results showed that long-term energy-restriction increase nitric oxide bioavailability. In addition, long-term high fat diet increases SIRT1 deacetylation to levels, which are detrimental for tissues that is related with rapid aging [23].

To elucidate the effects of fat and carbohydrate in an isocaloric diet in the next generation, we focused on SIRT1 as a key regulator of cellular metabolism and life span. It is a nutrient-responsive, NAD⁺-dependent histone deacetylase that response to energy availability by inducing macronutrient's catabolic while repressing anabolic pathways, as well as inflammation [24]. At the present study, we showed that high maternal fat compared to high carbohydrate in an isocaloric diet decreases SIRT-1 gene expression and protein level in female and male offspring which increases cholesterol and saturated however decreases omega-

3, -6 and-9 fatty acids production in the liver. Fatty acids are important biological components with various vital roles, and the liver is the main and primary organ for their metabolism. The fetus at the critical stage of development needs substantial amounts of fatty acids, especially omega-3 and omega-6 fatty acids, to support rapid cellular growth and activity [25]. The most biologically important omega-3 fatty acids are eicosapentaenoic acid and docosahexaenoic acid, which are metabolic derivatives of α -linolenic acid via desaturase and elongase enzymes activity. But, an increase in dietary omega-6 fatty acids and intake of high-fat diet block this conversion [26]. Di-homo- γ -linolenic acid (DHGLA) and arachidonic acid (AA) are metabolic derivatives of linoleic acid (LA) as the main omega-6 fatty acids (22). These are precursors of eicosanoids, as signaling molecules, which have important roles in the regulation of inflammation. Intake of high amounts of fat in diet suppresses LA conversion to DHGLA and AA [27]. In general, eicosanoids derived from omega-6 fatty acids are pro-inflammatory while omega-3 derivatives have anti-inflammatory effects. Over the past few decades, the nutritional transition occurred, and there is a notable increase in fat intake, especially sources of omega-6 fatty acids compared to the omega-3 in the world (~15 : 1), which are associated with greater metabolism of these fatty acids and more inflammatory diseases [26, 27]. The main sources of omega-6 fatty acids are safflower, sunflower, soybean, and corn oils [27]. At the present study, we prepared diets by soybean oil, which is conventional and contains more omega-6 compared to omega-3 fatty acids (~7:1). Increase in omega-6 fatty acid intake at the HF-LC diet group leads to more saturated fatty acid and cholesterol and lower unsaturated fatty acids (MUFA and PUFA) production in the liver. These disturbances in omega-6/omega-3 fatty acids and finally derivate eicosanoids lead to increase in insulin resistance and susceptibility to chronic diseases in the next generation [28]. Therefore, one of the reasons for SIRT1 suppression and finally aging process may occur because of liver fatty acid disturbances. We resulted that female offspring are more susceptible to metabolic changes than males, which is in agree with the previous studies [27-29]. Since female is responsible for generating the next generation and because these changes are permanent, maternal diet during gestation and lactation needs striking attention.

Conclusions

In summary, even a normal diet after birth could not delete the effects of maternal dietary changes during critical periods of life and these changes permanently transferred to the next generation and effects on life span. Therefore, any education and intervention to decrease the prevalence and incidence of chronic diseases, as well as extending life span had better start from gestation and lactation in mothers.

List Of Abbreviations

LF-HC; low fat-high carbohydrate, HF-LC; high fat-low carbohydrate, SIRT-1; sirtuin-1

Declarations

Ethics approval and consent to participate: The present study was approved by the Ethical Committee of Tehran University of Medical Sciences, Tehran, Iran (protocol number: 34223-161-01-96).

Consent for publication: Not applicable.

Availability of data and materials: Available. If someone wants to request the data from this study can e-mail to the corresponding author.

Conflict of interests: None.

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Authors' contributions: Authorship

Conceptualization, S.N.M. and F.K.; Methodology, S. Gh., M.S.S.D., S.N.M; Investigation, S.N.M., M.S.S.D and F.K; Writing – Original Draft, S.N.M. and M.S.S.D.; Writing – Review & Editing, S.N.M., M.S.S.D. and F.K.

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Tables

Table 1 Composition of diets (per 1 kg)

Ingredients (g/kg)	Casein	Corn starch	Sucrose	Soybean oil	Fiber	Mineral mix	Vitamin mix	L-cysteine	Choline tartrate	tert-butyl hydroquinone	
Diets											
AIN 93M	140	630	100	40	50	35	10	1.8	2.5	0.008	
AIN 93G	LF-HC	200	530	100	70	50	35	10	3	2.5	0.014
	HF-LC	200	217	100	210	222.5	35	10	3	2.5	0.014

AIN 93M; diet during maintenance, AIN 93G; diet during growth, LF-HC; low fat-high carbohydrate diet, HF-LC; high fat-low carbohydrate diet

Ingredients were prepared as follows: L-cysteine (W326305, Sigma Aldrich, Germany), AIN 93 M mineral mix (296040002, MP Biomedicals, USA), AIN 93 vitamin mix (296040201, MP Biomedicals, USA), choline bitartrate (C1629, Sigma Aldrich, Germany), tert-butyl hydroquinone (112941, Sigma Aldrich, Germany). Casein lactate, corn starch, sugar, soybean oil and fiber were prepared from local products

Table 2 Amount of liver fatty acids in the studied groups

Groups	LF-HC	LF-HC	HF-LC	HF-LC
	Female	Male	Female	Male
Fatty acids				
Meristic acid (C_{14:0})	0.36±0.04	0.31±0.03	3.5±0.35	3.2±0.2
Palmitic acid (C_{16:0})	2.83±0.2	2.4±0.1	7.13±0.32	6.44±0.29
Stearic acid (C_{18:0})	15.38±0.81	19.45±1.4	5.6±1.1	2.5±0.55
Arachidic acid (C_{20:0})	1.53±0.4	0.35±0.1	0.54±0.08	0.04±0.2
Oleic acid (C_{18:1})	3.84±0.29	6.7±0.71	0.52±0.07	2.93±0.45
Linoleic acid (C_{18:2})	2.62±1.64	27.7±1.95	9.4±0.08	10.75±1.32
Linolenic acid (C_{18:3})	0.43±0.06	0.3±0.04	0.1±0.015	0.06±0.02
Dihomo-γ-linolenic acid (C_{20:3})	2.83±0.24	1.88±0.28	0.61±0.47	0.06±0.02
Arachidonic acid (C_{20:4})	13.7±1.36	10.9±0.6	2.94±0.1	2.4±0.5
Eicosapentaenoic acid (C_{20:5})	1.36±0.56	2.8±0.82	0.22±0.03	0.1±0.04
Docosahexaenoic acid (C_{22:6})	13.67±2.07	8.97±0.61	2.21±0.3	1.3±0.18
Cholesterol (C₂₇H₄₆O)	1.13±0.22	2.43±0.44	45.24±1.87	69.5±1.08

Data are expressed as means ± SD

Figures

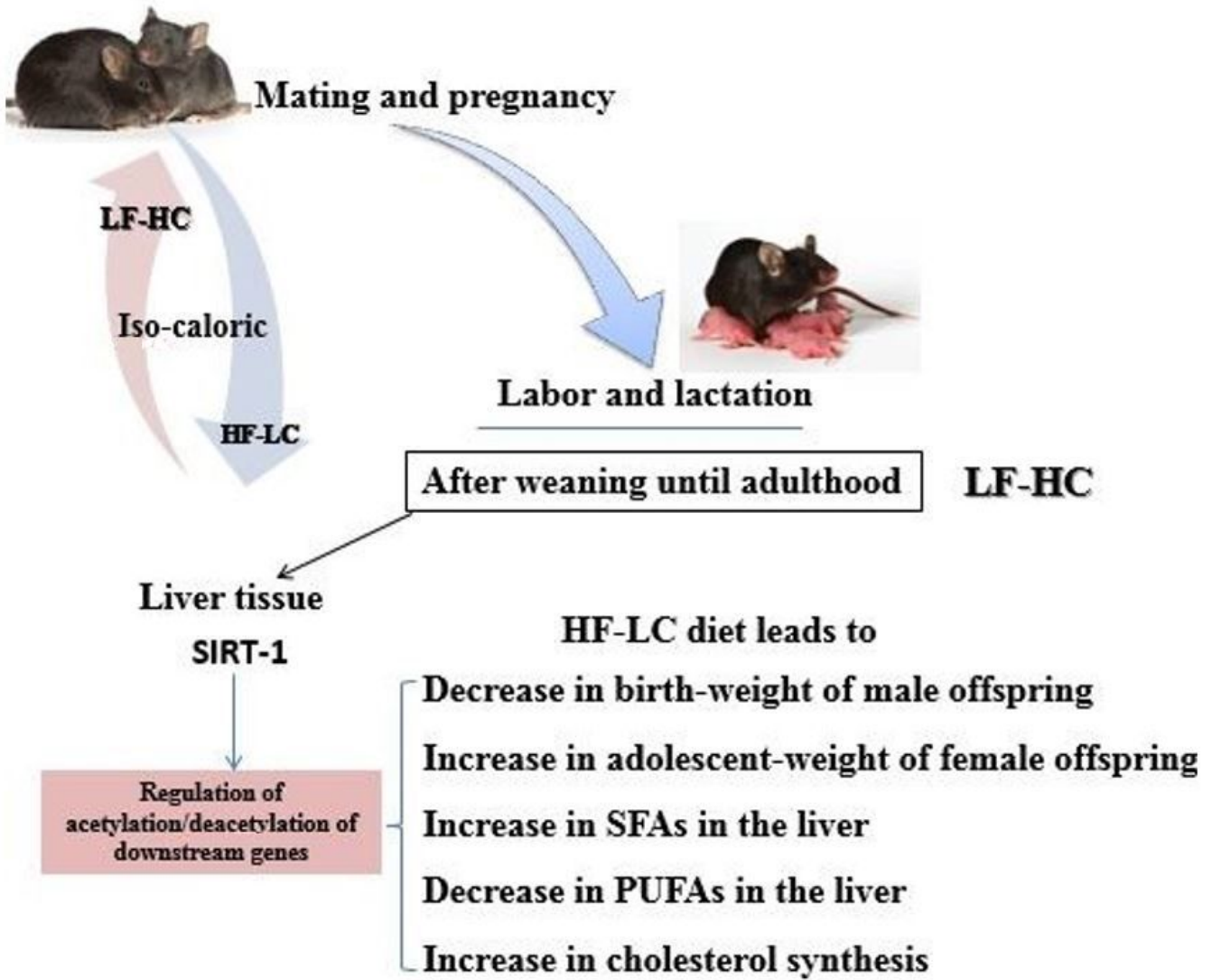
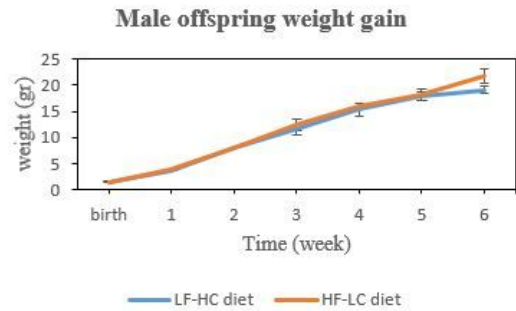


Figure 1

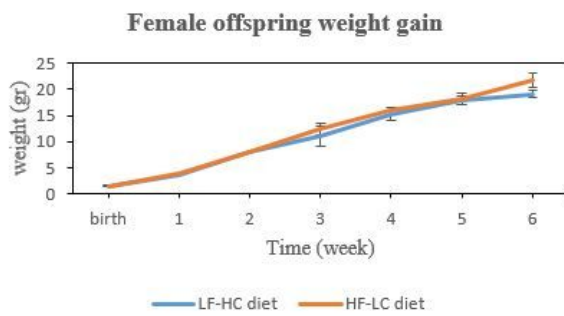
Study protocol



(a)



(b)



(c)

Figure 2

a) Trend of maternal weight gain during gestation; b) Trend of male offspring weight gain from birth up to the adolescence; c) Trend of female offspring weight gain from birth up to the adolescence

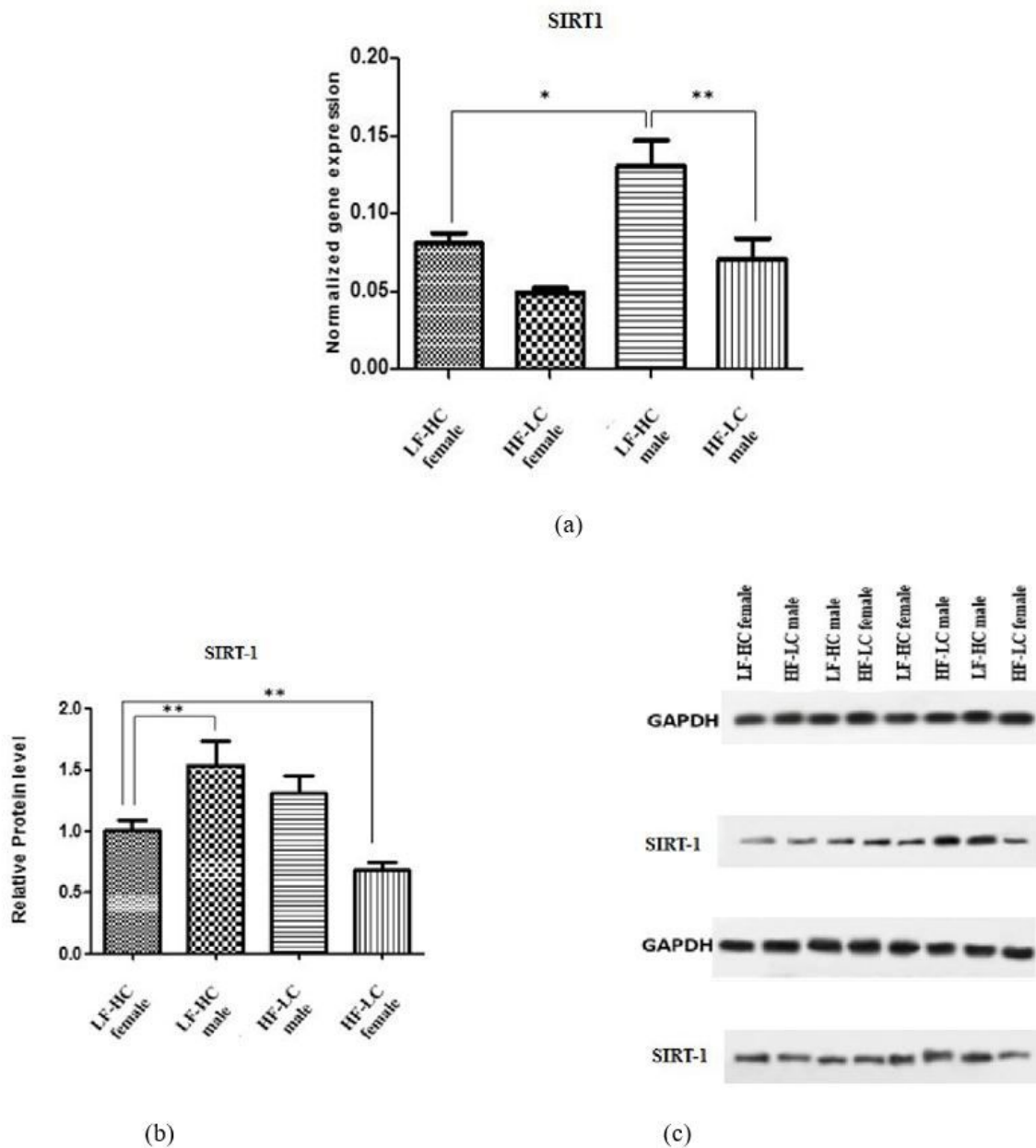


Figure 3

a) Normalized SIRT1 gene expression against GAPDH in the liver of studied groups (* $p < 0.01$ and ** $p < 0.001$); b) Quantitative analysis of SIRT-1 protein normalized against GAPDH in the liver of studied groups (** $p < 0.001$); c) Western-blot analysis of liver tissue lysates from the same groups. GAPDH was used as the housekeeping protein. The grouping of gels/blots cropped from different parts of the same gel