2- AIM OF THE WORK

The present work will aim to study the effect of maternal malnutrition on the expression of Uncoupling Protein 2 (UCP2) and mitochondrial transcription factor-A (mtTFA) as modulators in the glucose sensing of the different organs (pancreas, liver, muscles and adipose tissues) of the first generation rat offspring.

3. MATERIALS AND METHODS

3.1. Animals and diet

In this study 50 local female Wistar rats (one month age) were used. The animals kept at constant environmental conditions throughout the experimental period under room temperature (21±2 ^{0}C) with a 12 hours light/12 hours dark cycle. The female rats were divided into two experimental groups [171]:

- Group I: Control group (C) consisting of 25 rats feed normal control diet (20% protein)
- Group II: Low–protein group (LP) consisting of 25 rats feed an isocaloric low-protein diet (LP; 8% protein).

All female rats had free access for water and their specific diet for 3 months.

3.1.1 Pregnancy induction and delivery

The protein-malnourished and control virgin females were housed with healthy normal male overnight for the induction of pregnancy. The next day was considered as day 0 of pregnancy (gestational Day 0).

Following mating, all pregnant female rats (control and Low–protein) received their respective diets. Body weights were monitored three times a week. All rats were allowed to give birth naturally. Numbers and sex of pups, birth weight, and nasoanal length was measured for each pup on postnatal day (1) .

Litter sizes were adjusted at birth to ten pups per dam. Pups remained with dams until they were weaned.

Table (2): The diet composition

Macronutrients (g/kg diet)	Control diet (CD)	Low-protein diet (LP)	High-caloric diet (HCD)
Protein • Casein	220	90	244
Carbohydrates • Corn starch	631	761	593
Fat • Lard • corn oil	43	43	48 20
Cellulose	54	54	50
Vitamin mix	10	10	10
Mineral mix	40	40	35
Total energy (kcal/g diet)	3.8	3.8	4.7

3.1.2. Follow up of F1 offspring

The offspring were weaned at postnatal day (22) to either control diet or high-caloric diet. 60 pups from each group (30 males and 30 females) were consuming high-caloric diet ad libitum while the remaining pups were maintained under control diet. From this experimental protocol four groups of offspring were obtained:

- F1 Offspring of control mother under control diet (CF1-CD)
- F1 Offspring of control mother under high-caloric diet (CF1-HCD)
- F1 Offspring of low-protein mother under control diet (LPF1-CD)
- F1 Offspring of low-protein mother under high-caloric diet (LPF1-HCD)

Body weights of the offspring were monitored weekly until postnatal day 210 (30 weeks). Every 5 weeks the glucose tolerance curve was constructed for each subgroup.

At 5, 10, 15, 20, and 25 week of age, 10 pups (5 males and 5 females) of each subgroup were sacrificed by cervical dislocation.

At 30 weeks post-natal, 10 pups (5 males and 5 females) of each subgroup were used for construction of oral glucose tolerance test (OGTT) and then on the second day sacrificed by cervical dislocation.

The blood was obtained immediately and the plasma was obtained for the assessment of glucose, insulin, non-esterified fatty acids (NEFA) and lipid profile. The rats were dissected out to obtain pancreas, liver, muscle and adipose tissues for assessment of insulin receptor, phosphor-insulin receptor (P-IR) and mtTFA and UCP2 gene expression.
A part of the pancreas was fixed and paraffin embedded, sectioned completely (5µm), and mounted for routine histological and Insulin immunohistochemical staining.

3.2. Methods:

3.2.1. Determination of blood glucose:

Plasma glucose levels were determined according to an enzymatic calorimetric method[(172)].

Principle:

Glucose is oxidized in the presence of glucose oxidase. The hydrogen peroxide formed reacts under catalysis of peroxidase with phenol and 4–aminophenazone to a red-violet quinoneimine dye. The intensity of the color is proportional to glucose concentration.

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\text{Glucose oxidase}} \text{Gluconate} + H_2O_2$$

$$H_2O_2 + \text{phenol} + \text{4-aminophenazone} \xrightarrow{\text{peroxidase}} \text{Quinoneimine} + 4H_2O$$

Reagents:

Working reagent

- Phosphate buffer (pH 7.5) 0.1 mol/L

- 4 – Aminophenazone 0.25 mmol/L
- Phenol 0.75 mmol/L
- Glucose oxidase >15 KU/L
- Peroxidase >1.5 KU/L
- Standard: Glucose 100 mg/dl

Procedure:

- 1 ml of enzyme reagent was mixed in test tubes with 10 µl of serum sample or glucose standard.
- The mixture was incubated at 37 °C for 5 minutes.
- Reagent blank was run through the same procedure.
- The absorbance of standard (ΔA standard) and the sample (ΔA sample) were measured against reagent blank at 540 nm.

Calculation:

$$\text{Glucose concentration (mg/dL)} = \frac{\Delta A \text{ sample}}{\Delta A \text{ standard}} \times 100$$

Where, concentration of standard was 100 mg/dL.

3.2.2. Determination of insulin in rat serum:

Rat insulin ELISA kit (Mercodia, Sweden) is used for the non radioactive quantitative of insulin in rat sera [173].

Principle:

This assay is a sandwich ELISA based on capture of insulin molecules from samples to the wells of a microtitre plate coated by a monoclonal mouse anti-rat insulin antibodies and the binding of biotinylated polyclonal antibodies to captured insulin, binding of horseradish peroxidase (HRP) to the immobilized biotinylated antibodies, and quantification of the immobilized antibody-enzyme conjugates by monitoring horseradish peroxidase activities in the presence of 3, 3', 5, 5'-tetramethylbenzidine. The reaction is stopped by adding acid to give colorimetric endpoint. The enzyme activity is measured spectrophotometrically by the increased absorbance at 450 nm.

Reagents:

- Assay buffer: (0.05 M phosphate buffered saline, pH 7.4, containing 0.025 M. EDTA, 0.08 % sodium azide and 1% BSA).
- Wash buffer: (50 mM Tris buffered saline containing Tween- 20).
- Insulin standards in assay buffer: (0.15, 0.40, 1, 3 and 5.5ug/L).
- Matrix solution: (charcoal Stripped pooled mouse serum).
- Insulin detection antibody: (biotinylated anti-insulin antibodies).
- Enzyme conjugate solution: (Streptavidin- horseradish peroxidase conjugates in assay buffer).
- TMB Substrate :(3, 3', 5, 5' - tetramethylbenzidine).
- Stop solution: (0.5 M H_2SO_4).

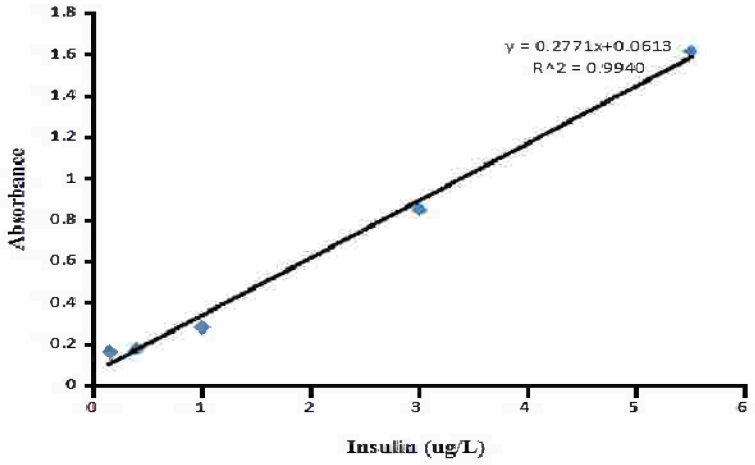
Procedure:

- 10 µl of rat insulin standards were added in duplicate in the order of ascending concentrations. 10 µl of negative and positive control were added to the appropriate wells.
- 10 µl of unknown serum were added to the remaining wells.

- 100 µl of enzyme conjugate were added to all wells and incubated at room temperature on plate shaker for 2 hours.
- Solutions were decanted from the plate and the plate was washed 6 times with 200 µl diluted wash buffer.
- 200 µl of substrate solution (TMB) were added to each well. The plate was covered and shakes in the plate shaker for approximately 15 minutes.
- 50 µl of stop solution were added and shake for 5 seconds to ensure mixing then the absorbance was read at 450 nm in a plate reader within 30 minutes.

Calculation:

The reference curve (Figure:18) was constructed by plotting the difference absorbance unit at 450 nm against the concentration of rat insulin standards.



Figure(18):Standard curve of insulin.

3.2.3. Calclation of HOMA-Insulin resistance index.

The insulin resistance index (IRI) was derived using the homeostasis model assessment (HOMA) as follows[(174)] **:**

IRI= fasting insulin (µU/ml) × fasting glucose (mmol/L)/22.5

3.3.4. Lipid profile Parameters:

3.3.4.1. Determination of total cholesterol:

Serum total cholesterol level was determined on the basis of an enzymatic calorimetric method[172].

Principle

Cholesterol esterase (CHE) hydrolyzes esters and H_2O_2 is formed in the subsequent enzymatic oxidation of cholesterol by cholesterol oxidase (CHO) according to the following reaction:

$$\text{Cholesterol ester} + H_2O \xrightarrow{\text{CHE}} \text{Cholesterol} + \text{fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{CHO}} \text{Cholesterol-3-one} + H_2O_2$$

$$2\,H_2O_2 + \text{4-aminophenazone} + \text{phenol} \xrightarrow{\text{Peroxidase}} \text{Quinonimine} + HCl + H_2O$$

Reagents:

Enzyme / buffer reagent

- Phosphate buffer (pH 6.5) — 100 mmol/L
- 4 – Amino phenazone — 0.3 mmol/L
- Phenol — 5 mmol/L
- Peroxidase — >5 KU/L
- Cholesterol esterase — > 150 U/L
- Cholesterol oxidase — > 100 U/L
- Standard: Cholesterol — 200 mg/dl

Procedure:

1 ml of working reagent was mixed with 10 µl of serum sample or cholesterol standard in test tubes. Reagent blank was run through the same procedure. All the tubes were incubated at 37 °C for 5 minutes. The absorbance of standard (ΔA standard) and the sample (ΔA sample) were measured against reagent blank at 546 nm.

Calculation:

$$\text{Cholesterol concentration (mg/dl)} = \frac{\Delta A_{\text{sample}}}{\Delta A_{\text{standard}}} \times 200$$

Where, concentration of standard was 200 mg/dl.

3.3.4.2. Determination of HDL- Cholesterol (HDL-C):

High density lipoprotein (HDL–cholesterol) level was determined by precipitation method[172].

Principle:

When serum is treated with phosphotungustic acid in the presence of magnesium ion, the low density lipoprotein (LDL), very low density lipoprotein (VLDL) and chylomicron are precipitated from serum. The HDL cholesterol remains dissolved in the supernatant. The supernatant then acts as a sample and assayed for cholesterol by an enzymatic method.

Reagents:
Precipitant

- Phosphotungstic acid 0.4 mmol/L
- Magnesium chloride 20.0 mmol/L
- Standard: HDL-cholesterol standard 15 mg/dL

Procedure:

Precipitation:

- 500 µl of precipitant was added to 200 µl of serum sample, mixed well and incubated for 10 minutes at room temperature.
- The mixture was then centrifuged for 10 minutes at 4000 rpm.
- After centrifugation the clear supernatant was separated from the precipitate.
- 1 ml of enzyme reagent of Cholesterol determination was mixed in test tubes with 100µl of sample supernatant, cholesterol standard, or distilled water (as blank).
- The mixture was incubated at 37^0C for 10 minutes.
- The absorbance of standard (ΔA standard) and the sample (ΔA sample) were measured against reagent blank at 546 nm.

Calculation:

$$\text{HDL -Cholesterol concentration (mg/dl)} = \frac{\Delta A_{\text{sample}}}{\Delta A_{\text{standard}}} \times 52.5$$

Where 52.5= Standard cholesterol concentration (15) X Sample dilution factor (3.5)

3.3.4.3. Calculation of LDL- Cholesterol:

The low density lipoprotein cholesterol concentration (LDL–C) was calculated from the total cholesterol concentration (TC), the HDL cholesterol concentration (HDL–C) and the triglycerides concentration (TG)[172].

$$\text{LDL-C} = \text{TC} - (\text{HDL-C}) - \text{TG}/5 \text{ mg/dl.}$$

3.3.4.4. Determination of triglycerides:

The triglycerides level was determined by the enzymatic colorimetric method[172].

Principle:

Glycerol and fatty acids are first formed by the action of lipase on the triglycerides. Glycerol is then phosphorelated by adenosine triphosphate (ATP) to produce glycerol -3-phosphate and ADP in a reaction catalyzed by glycerol kinase.
Glycerol- 3 - phosphate is oxidized by glycerol phosphate oxidase producing dihydroxy acetate phosphate (DAP) and hydrogen peroxide. The latter reacts with 4 aminoantipyrine and 4-chlorophenol under the catalytic influence of peroxidase to form quinoneimine.

$$\text{Triglycerides} \xrightarrow{\text{Lipase}} \text{Glycerol + Fatty acids.}$$

$$\text{Glycerol + ATP} \xrightarrow{\text{Glycerol Kinase}} \text{Glycerol-3 phosphate + ADP.}$$

$$\text{Glycerol-3 phosphate + } O_2 \xrightarrow[\text{oxidase}]{\text{Glycerol phosphate}} \text{DAP + } H_2O_2.$$

$$H_2O_2\text{+4-aminoantipyrine+4-chlorophenol} \xrightarrow{\text{Peroxidase}} \text{Quinnoneimine+HCl + } H_2O.$$

Reagents:

Enzyme/buffer reagent:

❖ Piperazine-N, N′-bis (2-ethanesulfonic acid) buffer(pH 7.5)	50 mmol/L
❖ 4 - chloro phenol	5 mmol/L
❖ 4 – Aminoantipyrine	0.25 mmol /L
❖ Magnesium ions	4.5 mmol/L
❖ ATP	2 mmol/L
❖ Lipase	≥ 1.3 U/mL
❖ Peroxidase	≥ 0.5 U/mL
❖ Glycerol kinase	≥ 0.4 U/mL
❖ Glycerol -3 - phosphate oxidase	≥ 1.5 U/mL
❖ Standard: Triglycerides	200 mg/dl

Procedure:

- ❖ 1 ml of enzyme reagent was mixed with 10 µl of serum sample or triglycerides standard in test tubes.
- ❖ Reagent blank was run through the same procedure.
- ❖ All tubes were incubated at 37 C for 5 minutes.
- ❖ The absorbance of standard (ΔA standard) and the sample (ΔA sample) were measured against reagent blank at 546 nm.

Calculation:

$$\text{Triglycerides concentration (mg/dl)} = \frac{\Delta A_{\text{sample}}}{\Delta A_{\text{Standard}}}$$

Where, concentration of standard was 200 mg/dL.

3.3.5. Determination of NEFA in rat serum

Non-estrified fatty acid (NEFA) ELISA kit, obtained from CUSABIO, employs the competitive inhibition enzyme immunoassay technique [175].

Principle of the assay:

This assay employs the competitive inhibition enzyme immunoassay technique. The microtiter plate has been pre-coated with goat-anti-rabbit antibody. Standards or samples are added to the appropriate microtiter plate wells with an antibody specific for NEFA and Horseradish Peroxidase (HRP) conjugated NEFA. The competitive inhibition reaction is launched between HRP labeled NEFA and unlabeled NEFA with the antibody. A substrate solution is added to the wells and the color develops in opposite to the amount of NEFA in the sample. The color development is stopped and the intensity of the color is measured.

Reagent:

- Standard (0, 16, 40, 96,160,400 nmol/ml)
- Antibody
- HRP-conjugate
- Wash Buffer
- Substrate A & B
- Stop solution
-

Procedure:

1. Blank well was set with distilled water.
2. 50µl of Standard or Sample was added per well.
3. 50µl of HRP-conjugate was added to each well (not to Blank well), then 50µl Antibody was added to each well, mixed well and then incubated for 2 hours at 37°C.
4. Each well was aspirated and washed for three times by Wash Buffer (200µl) for 10 seconds. Then, any remaining Wash Buffer was removed
5. 50µl of Substrate A and 50µl of Substrate B were added to each well, mixed well and incubated for 15 minutes at 37°C in the dark.
6. 50µl of Stop Solution was added to each well and mixed gently
7. The optical density of each well was determined within 10 minutes, using a microplate reader set to 450 nm.

Calculation:

The reference curve was constructed by plotting the absorbance unit at 450 nm against the concentration of NEFA standards.

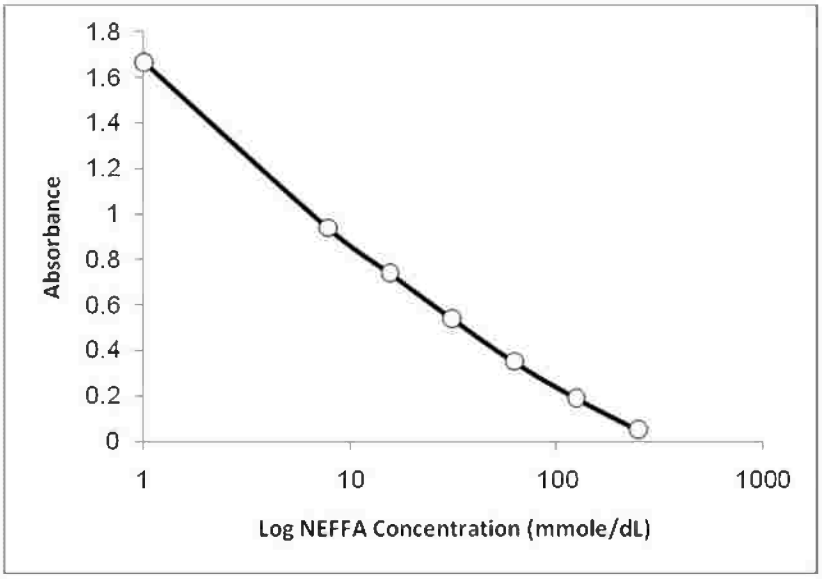


Figure (19): Standard curve of NEFA

3.3.6. Rat Insulin Receptor

Rat insulin receptor ELISA kit (Life Science Inc) is used for the non radioactive quantitative of Insulin Receptor in rat tissues[176].

Principle of the assay:

The test principle applied in this kit is Sandwich enzyme immunoassay. The microtiter plate has been pre-coated with an antibody specific to ISR. Standards or samples are then added to the appropriate microtiter plate wells with a biotin-conjugated antibody preparation specific for ISR. Next, Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. After TMB substrate solution is added, only those wells that contain ISR,

biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450nm ± 10nm. The concentration of ISR in the samples is then determined by comparing the O.D. of the samples to the standard curve.

Reagents:

- Pre-coated, ready to use 96-well strip plate.
- Standard (lyophilized).
- Standard Diluent
- Detection Reagent A (green)
- Assay Diluent A.
- Detection Reagent B (red)
- Assay Diluent B.
- TMB Substrate (3,$^{3-}$,5,$^{5-}$ -tetramethylbenzidine)
- Stop Solution
- Wash Buffer (30 × concentrate).

Procedure:

1. All reagents, samples and standards were prepared.
2. 100µL standard or sample added to each well then Incubated for 2 hours at 37°C after covering it with the Plate sealer.
3. The liquid was removed from each well without washing.
4. 100µL of prepared detection reagent A was added. Incubated for 1 hour at 37°C.
5. Solutions were aspirated from the plate and the plate was washed 3 times.
6. 100µL of prepared detection reagent B was added and incubated for 30 minutes at 37°C.
7. Solutions were aspirated from the plate and the plate was washed 5 times.
8. 90µL substrate solution was added. The plate was protected from light and incubated for 15-25 minutes at 37°C.
9. 50µL stop solution added to and the absorbance was read at 450 nm in a plate reader immediately

Calculation:

A reference curve was constructed by plotting the difference absorbance unit at 450 nm against the concentration of rat insulin receptor standards.

3.3.7. Determination of Tissue level of Phospho-Insulin Receptor-β (Tyr1150/1151).

Rat phospho-insulin receptor-β (Tyr1150/1151) ELISA kit (CSTs pathScan) is used for the non radioactive quantitative of Phospho-Insulin Receptor-β (Tyr1150/1151) in rat tissues [176].

Principle:

This assay is a solid phase sandwich ELISA based on detected transfected Phospho-Insulin Receptor-β (Tyr1150/1151) protein. An insulin receptor β mouse mAb has been coated

onto the microwells. After incubation with sample, both phospho- and non phospho-insulin receptor proteins are captured by the coated antibody. Following extensive washing, phospho-IGF-1 Receptor-β (Tyr1135/1136)/ Insulin Receptor-β (Tyr1150/1151) rabbit mAb is added to detect the captured Phospho-Insulin Receptor-β (Tyr1150/1151). Anti-rabbit IgG, HRP-Linked Antibody then used to recognize the bound detection antibody. HRP Substrate, TMB, is added to develop color. The optical density for this developed color is proportional to the quantity of Phospho-Insulin Receptor-β (Tyr1150/1151).

Reagents:

- An insulin receptor β mouse mAb coated microwells.
- Phospho-Insulin Receptor-β (Tyr1150/1151) detection Ab.
- Anti-rabbit IgG, HRP-Linked Ab.
- TMB Substrate: (3,3`,5,5` -tetramethylbenzidine)
- Stop solution.
- Wash buffer.
- Sample diluent.

Procedure:

- 100 µl of sample diluent were added to microcentrifuge tube.
- 100 µl of sample were added to tube and vortex for a few seconds.
- 100 µl of diluted sample were added to the appropriate well then microwells were sealed with tape and pressed firmly.
- Microplate was incubated for 2 hours at 37 °C.
- Microplate was washed four times with 200 µl of wash buffer and 100 µl of detection antibody was added to each well then micro-wells were sealed with tape and the plate was incubated for one hour at 37 °C .
- Microplate was washed four times with 200 µl of wash buffer and 100 µl of HRP-linked secondary antibody was added to each well, the plate was sealed and incubated for 30 minutes at 37 °C.
- Microplate was washed four times with 200 µl wash buffer then 100 µl of TMB substrate was added and incubated for 10 minutes at 37 °C .
- 100 µl of stop solution was added to each well.
- The absorbance was read on a microplate reader at a wavelength of 450 nm.

Calculation:

Concentration of P-IR protein was calculated as a difference absorbance unit/mg protein content of the sample (AU/mg protein) at 450 nm.

3.3.8. Determination of total protein:

Principle:

A modification of the method of Lowry *et al.* was used for the determination of protein in the samples. The color produced is thought to be due to a complex between the alkaline copper- phenol reagent and tyrosine and tryptophan residues of the protein in the sample. The protein concentration in each sample was estimated by referring to a standard curve (Figure: 16) which was constructed using bovine serum albumin [177].

Reagents:

- Sodium hydroxide 0. 1M.
- Sodium carbonate (anhydrous) 2% in 0.1M NaOH.
- K/Na tartarate 2%.

- Copper sulphate 1%.
- Lowry C reagent: prepared immediately before use by mixing volumes of sodium carbonate, K/Na tartarate and copper sulphate reagent in a ratio: 100: 1: 1.
- Folin- Ciocalteau reagent. The working reagent was prepared by diluting the stock reagent 1: 1 (V/V) with distilled water immediately before use.
- Standard bovine serum albumin

Procedure:

The sample was diluted in distilled water (1: 10). Aliquots of 10µl of diluted samples were mixed with 2.5ml of Lowry C reagent. After incubation for 10 minutes at room temperature, 0.25ml of working Folin- Ciocalteau's reagent was added. The tubes were then mixed and incubated in a dark place for one hour at room temperature, after which the absorbance was read at 695 nm using spectronic 21 spectrophotometer.

A blank containing phosphate buffer saline instead of the sample was treated similarly.

The total protein amount was computed with reference to the protein standard curve (Figure:) (2,4,6,8,10,20,30,40,50,60,70,80,90,100 µg protein).

Calculation:

$$\text{The total protein concentration (mg/ml)} = \frac{\text{The total protein amount (mg)}}{\text{The sample volume (ml)}}$$

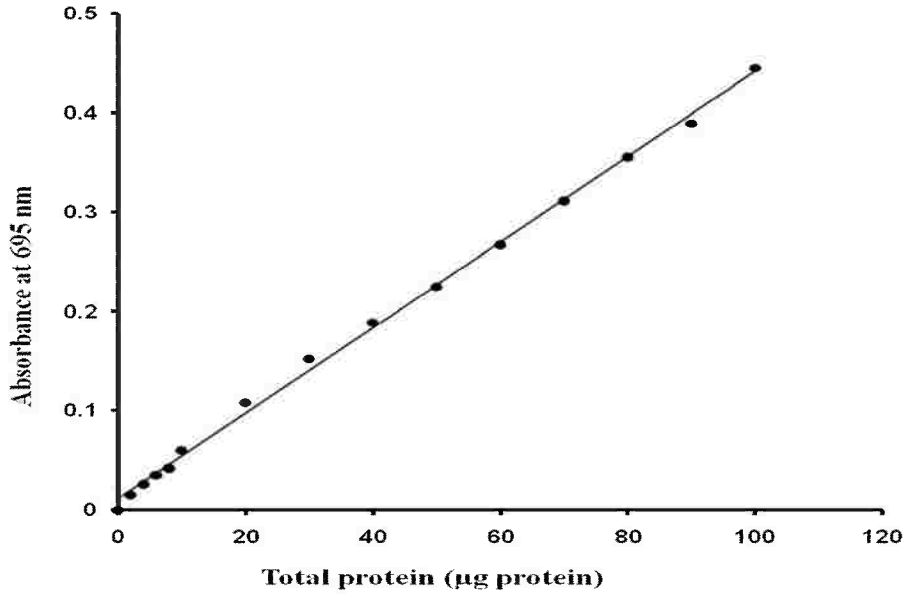


Figure (20): Standard curve of total protein

3.3.9. Semi-quantitative analysis of mTFA and UCP2 gene expression using reverse transcriptase-polymerase chain reaction (RT-PCR:

3.3.9.1. RNA isolation [178]

Total RNA was isolated from tissues using GeneJET™ RNA isolation kit (Fermentas, EU) according to the manufacturer instructions.

Principle:

Samples are lysed and homogenized in lysis buffer, which contains guanidine thiocyanate, a chaotropic salt capable of protecting RNA from endogenous RNases. The lysate is then mixed with ethanol and loaded on a purification column. The chaotropic salt and ethanol cause RNA to bind to the silica membrane while the lysate is spun through the column. Subsequently, impurities are effectively removed from the membrane by washing the column with wash buffers. Pure RNA is then eluted under low ionic strength conditions with nuclease-free water.

Reagents

- Proteinase K
- Lysis buffer
- Wash buffer 1
- Wash buffer 2
- Water, nuclease-free
- GeneJET™ RNA purification columns pre-assembled with collection tubes
- Collection tubes.

Buffer preparation:

- 10 ml of ethanol (96%) were added to 40 ml of wash buffer 1 prior to first use.
- 10 ml of ethanol (96%) were added to 20 ml of wash buffer 2 prior to first use.
- 20 µl of 14.3 M β-mercaptoethanol were added to each 1ml of lysis buffer prior to each RNA purification experiment.
- 10 µl of proteinase K were added to 590 µl of Tris-EDTA buffer (TE buffer: 10 mM Tris HCl, pH 8.0, 1mM EDTA) prior to each RNA purification experiment.

Procedure:

- 300 µl of lysis buffer (supplemented with β-mercaptoethanol) were added to 30 mg tissue and homogenized using glass Teflon homogenizer.
- 600 µl of diluted proteinase K were added to the homogenate and mixed thoroughly using vortex and incubated at 25°C for 10 minutes. The homogenate was centrifuged for 10 minutes at 12000 x g. After centrifugation the supernatant was transferred into a new RNase-free microcentrifuge tube.
- 450 µl of ethanol were added to the supernatant and mixed by pipetting.
- 700 µl of the lysate were transferred to the GeneJET™ RNA Purification column inserted into collection tube. The column was centrifuged for 1 minute at 12000 x g. The flow-through was discarded and column was placed back into the collection tube.
- 700 µl of wash buffer 1(supplemented with ethanol) were added to the GeneJET™ RNA Purification column inserted into collection tube and centrifuged for 1 minute at 12000 x g. The flow-through was discarded and column was placed back into the collection tube.

- 600 µl of wash buffer 2(supplemented with ethanol) were added to the GeneJET™ RNA Purification column inserted into collection tube and centrifuged for 1 minute at 12000 x g. The flow-through was discarded and column was placed back into the collection tube.
- 250 µl of wash buffer 2(supplemented with ethanol) were added to the GeneJET™ RNA Purification column inserted into collection tube and centrifuged for 1 minute at 12000 x g. The flow-through was discarded and column was transferred to into a sterile RNase-free microcentrifuge tube.
- 100 µl of water, nuclease-free were added to the center of GeneJET™ RNA Purification column and centrifuged for 1min at 12000 x g to elute RNA.
- The purification column was discarded and the purified RNA was stored at -20^{0}C.
- The integrity of RNA was confirmed by running RNA on agarose 2% and detection by staining with ethidium bromide.
- The yield of total RNA obtained was determined spectrophotometrically at 260nm, where 1 absorbance unit is equivalent to 40 µg single stranded RNA/ml.

3.3.9.2. Reverse transcriptase-polymerase chain reaction (RT-PCR) [179,180]:

Reagents:

- **Table (3): mTFA and UCP2 Primer sets**

Gene Name		Primer	PCR product
mTFA	**Forward**	5'-GCTTCCAGGAGGCTAAGGAT-3'	**350 bp**
	Reverse	5'-CCCAATCCCAATGACAACTC-3'	
UCP2	**Forward**	5'- CAAACAGTTCTACACCAA-3'	**359 bp**
	Reverse	5'-CGAAGGCAGAAGTGAAGTTGG-3'	
GAPDH	**Forward**	5'AATGTGTCCGTCGTGGATCTGA3'	**117 bp**
	Reverse	5-GATGCCTGCTTCACCACCTTCT3'	

- One-step RT-PCR Master Mix Gold Beads (Bioron, Germany).
- Nuclease Free water
- Agarose
- Ethidium Bromide
- 100 bp DNA ruler (Fermentas)

Procedure:

1- The reaction tube and sample tube were allowed to thaw in the ice rack.

2- The sample was briefly and shortly centrifuged in a microcentrifuge to keep the content down in the tube prior to sampling.

3- The template RNA and specific primer sets were added into the One-step RT-PCR PreMix tubes as the following:
   - One bead (One –step RT-PCR tube)
   - 2ul of forward primer (20 pmole)
   - 2ul of reverse primer (20 pmole)
   - 1ug RNA sample
   - Volume completed to 20 ul with nuclease free water
4- The PCR device was programmed to perform initial reverse transcription to synthesize cDNA then followed by PCR for each gene according to the following table

Table (4): RT PCR protocol

	mTFA	UCP2	GAPDH
cDNA synthesis	42 ^{0}C for 60 minutes (cDNA synthesis) then 94 ^{0}C for 10 minutes (Reverse transcriptase inactivation)		
Number of Cycle	30	30	30
Denaturation	94 ^{0}C for 30 seconds	94 ^{0}C for 30 seconds	94 ^{0}C for 25 seconds
Annealing	58 ^{0}C for 30 seconds	58 ^{0}C for 30 seconds	55 ^{0}C for 30 seconds
Extension	72 ^{0}C for 60 seconds	72 ^{0}C for 60 seconds	72 ^{0}C for 90 seconds
Final Extension		72 ^{0}C for 10 minutes	

5- After the end of the program, the RT-PCR product was run on 1.5% agarose :

- 1.5 gram of agarose was dissolved in 100 ml of TBE buffer (89.0 mM Tris-borate EDTA, pH 8.0), placed in boiling water bath and allowed to boil for 5 minutes. Then 10 µl of 5 mg/ml ethidium bromide solution was added (as DNA stain)
- The warmed agarose (65ºC) was poured carefully into the chamber with casting combs in place. Then allowed to stand for 30 minutes for solidification. The comb was then removed from the set agarose gel.
- RT-PCR product were mixed with the loading dye solution (0.25% bromophenol blue, 0.25% xylene cyanol, 50% sucrose, 10 mM Tris, pH 8.0)
- The agarose gel was immersed in the TBE buffer in the electrophoresis unit and carefully the sample DNA mixture and quantitative DNA markers (DNA mass loader; Fermentas, EU) were loaded into the slots of submerged gel.
- The electrophoresis was run at 100 V for 45 minutes at room temperature. After the termination of the run, the bands were visualized by using UV plate, and were photographed using a digital camera.
- The bands were scanned and the data were analyzed using UVP DOC-ITLS ^{TM} Image & acquisition and analysis software (Ultra-Violet product, Ltd. Cambridge, UK) that analyze the relative band densities of mTFA and UCP2 band to the Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) band (as internal control).

3.3.10. Histopathological study:

The pancreas was selected, weighed, and immediately fixed at room temperature in 10% neutral buffered formalin. The tissue were processed for preparing paraffin sections (dehydrated, cleared , embedded in wax at 60°C and blocked for cutting into 5µm thick sections). They were used as following:

3.3.10.1. Haematoxylin and Eosin [181]

Routine stain was used to study the morphological and pathological changes in all studied groups.

3.3.10.2. Immunohistochemical staining of insulin [182]

Paraffin sections were mounted on coated slides and stained for insulin detection in insulin producing cells. Deparaffinized slides and bring to distilled water, then incubated in citrate buffer solution with pH (6.0) at 100°C. Slides rinsed with large amount of H_2O_2 and washed by PBS. Then incubated with primary antibody (monoclonal insulin antibody) obtained from Cell Signaling@ (USA) at 4°C overnight using a humid chamber. The 2nd day the slides incubated with peroxidase-conjugated secondary antibodies for one hour at room temperature, then incubated in media containing Avidin Biotin complex for 30 min. The slides washed and stained with DAB (3, 3 diaminobenzidine tetrahydrochloride) as chromogen using kits obtained from R&D Systems, Inc. (USA). Then the slides washed, immersed in heamatoxylen as a counterstain, dehydrated, mounted by resin and covered by cover slips for demonstrating under the light microscopy

3.3.10.3. Morphometric Methods [182]

The image for each slide stained by immunohistochemical (insulin +ABC) in both control and experimental groups were captured using a 40 X objective (Bar = 50) with numerical aperture of a high resolution of 16-bit digital camera. Ten images of each group were viewed and recorded using Olympus microscope (Olympus BX 41TF microscope -German) equipped with Spot digital camera, using computer program software image J at histochemical and cell biology department , Medical Research Institute, Alexandria University.

3.3.10.3 .1 β-cell number

The positive insulin stained B-cells were counted (β-cell count) for each pancreatic islets in each image.

3.3.10.3.2 Surface area

All β-cell positive area in pancreatic islet was measured by the area marked at image J software as well as the surface area of the all pancreatic cells (field or figure).

3.3.10.3.3 β-cell mass

Quantitative evaluation of

- Individual β-cell area (IβA) = $\frac{\text{all Positive β–cell area of islet}}{\text{number of positive β–cells}}$
- Individual β-cell mass= IβA X pancreatic weight
- Total β-cell mass = $\frac{\text{All Positive β cell area of islet}}{\text{Surface area of the all pancreatic cells}}$ X pancreatic weight

4- Results

This chapter contains a summary and statistical analysis of the data on glucose homeostasis parameters, lipid profile and non-esterified fatty acids (NEFA), peripheral glucose sensing parameters (Insulin receptor and Phospho-Insulin receptor) and the expression of mitochondrial parameters (mTFA and UCP2) in different rat tissues (Muscle, Adipose tissue and liver) in the offspring of normal mothers under control or high caloric diet (CF1-CD and CF1-HCD, respectively) and the offspring of protein-malnourished mothers under control or high caloric diet (LPF1-CD and LPF1-HCD, respectively).

4.1 Pregnancy outcome:

The results of pregnancy outcomes were summarized in Table (5). At delivery, the low-protein (LP) nourished mothers showed significantly lower number of pups per litter (by about half) compared to control mothers (Table 5). Also, the pups of LP mothers had significantly lower birth weight compared to control pups of normal mothers (Table 5).

Table (5) : Pregnancy outcome

	Control	Malnourished
No. of litters	29	36
No. of viable pups	362	216
No. of pups/litter	12±3	6±3[a]
Pups weight (gm)	5.9±0.6	5.3±0.38[a]

Data of No of pups/litter and pups weight are presented as Mean±SEM
[a]Significant different from control by ANOVA followed by Fisher's LSD Post hoc test ($p<0.05$)

4.2 Weight of F1 offspring

The results of body weight of all offspring were summarized in Table (6). The body weights of all group of offspring showed age-dependent increase and males are significantly heavier than females (Table 6 and Figure 21 A and B).

While the female offspring of LP mothers under control diet (LPF1-CD) showed no significant change in body weight; male offspring showed significant higher body weight from the 10th week of age and thereafter compared to offspring of control mothers under control diet (CF1-CD) and those control offspring under high caloric diet (CF1-HCD). The male offspring of LP mothers under high caloric diet (LPF1-HCD) showed significant higher body weight from 10th week of age compared to all other groups of offspring (CF1-CD, CF1-HCD and LPF1-CD), however the female offspring (LPF1-HCD) showed higher weight in comparison with CF1-CD and LPF1-CD from the 10th week of age (Table 6 and Figure 21 A and B).

Table (6) : The Body weight (grams) of F1 male and female offspring of control and malnourished mothers under control and high caloric diet at different ages.

Age (week)	Sex	CF1-CD	CF1-HCD	LPF1-CD	LPF1-HCD
5	M	110.6 ± 15	112 ± 10	108 ± 11	118 ± 15
	F	104 ± 8	106 ± 11	102 ± 10	110 ± 12
10	M	250^{*} ± 15	255^{*} ± 14	280^{ab*} ± 12	297^{abc*} ± 12
	F	184 ± 22	191 ± 23	182 ± 17	208^{ac} ± 18
15	M	315^{*} ± 14	330^{*} ± 17	376^{ab*} ± 14	410^{abc*} ± 16
	F	210 ± 20	222 ± 23	205 ± 22	236^{ac} ± 23
20	M	376^{*} ± 15	388^{*} ± 16	441^{ab*} ± 16	465^{abc*} ± 18
	F	228 ± 24	236 ± 20	220 ± 25	257^{ac} ± 21
25	M	405^{*} ± 18	425^{*} ± 22	495^{ac*} ± 20	522^{abc*} ± 20
	F	242 ± 22	253 ± 23	244 ± 23	276^{ac} ± 25
30	M	426^{*} ± 19	448^{*a} ± 20	525^{ab*} ± 21	555^{abc*} ± 22
	F	258 ± 26	269 ± 26	262 ± 26	293^{ac} ± 28

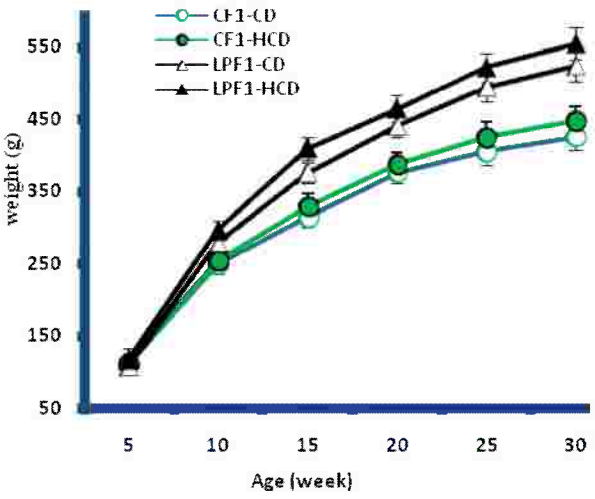


Figure (21 A) Age-dependent change of body weight of male F1 offspring under control or HCD-diet

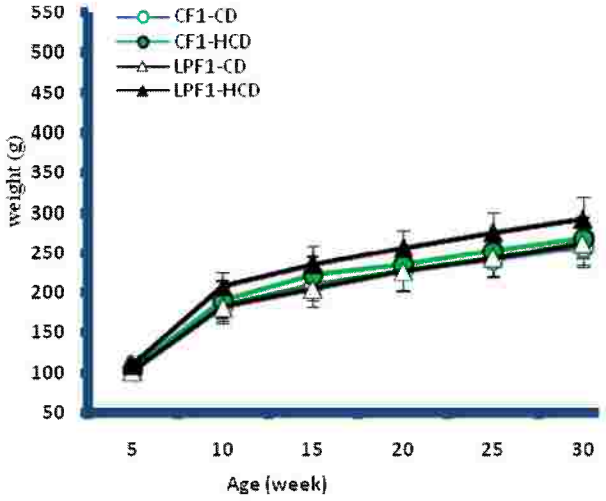


Figure (21 B) Age-dependent change of body weight of female F1 offspring under control or HCD-diet

Data represented as mean ± SD

M: Male **F:** Female **CD:** Control diet **HCD:** High-caloric diet

CF1: Offspring of control mothers **LPF1:** Offspring of low-protein nourished mothers

[a] *Significantly different from CF1-CD group at each age by ANOVA ($p<0.05$).*

[b] *Significantly different from CF1-HCD group at each age by ANOVA ($p<0.05$).*

[c] *Significantly different from LPF1-CD group at each age by ANOVA ($p<0.05$).*

[*] *Significant difference between male and female in the same group at each age by ANOVA ($p<0.05$).*

4.3 Glucose homeostasis parameters:

The results of glucose homeostasis parameters; FBS, insulin, HOMA-insulin resistance and OGTT were summarized in Tables (7-10) and Figures (22-25)

4.3.1 Fasting blood glucose (FBG)

All groups of offspring showed slight age-dependent increase in FBG with no significant difference between male and females (Table 7 and Figure 22 A and B).

The male offspring of LP mothers under HCD (LPF1-HCD) showed a significantly higher FBG compared to control offspring (CF1-CD and CF1-HCD) from 20th week of age, and compared to offspring of LP mothers under CD (LPF1-CD) from 25th week of age. The female offspring of LPF1-HCD showed a significantly higher FBG compared to other groups of offspring (CF1-CD, CF1-HCD and LPF1-CD) only at the 30th week of age (Table 7 and Figure 22 A and B).

4.3.2 Fasting insulin level

All groups of offspring showed age-dependent increase in insulin level. Male control offspring under HCD showed significantly lower insulin level compared to female ones at 20th and 25th week of age while male offspring of LP mothers showed such lower level only at early age (5th and 10th week) (Table 8 and Figure 23 A and B).

The male offspring of LP mothers under CD (LPF1-CD) showed a significantly lower insulin level compared to control offspring (CF1-CD and CF1-HCD) at 15th week of age and this significance was lost thereafter and become significant again at age of 25th week in comparison with male CF1-HCD. The female offspring of LPF1-CD showed significant lower insulin level compared to CF1-HCD at 20th and 25th week of age. In contrast, the male offspring of LP mothers under HCD (LPF1-HCD) showed a significantly higher insulin level compared to control offspring (CF1-CD and CF1-HCD) and offspring of LP mothers under control diet (LPF1-CD) from 15th week of age. The female offspring of LPF1-HCD showed a significantly higher insulin level compared to LPF1-CD females from 10th week of age and compared to CF1-CD from 20th week of age (Table 8 and Figure 23 A and B).

4.3.3 Insulin resistance index by HOMA

The HOMA-insulin resistance index of all groups of offspring showed age-dependent increase and males appear to be less insulin resistant than females in the offspring of control mothers (CF1-CD or CF1-HCD) and offspring of LP mothers under control diet (LPF1-CD) while in the offspring of LP mothers under HCD; at early ages (5th and 10 weeks) males were less insulin resistant than females but at older ages (25th and 30th week) the males become more insulin resistant than females (Table 9 and Figure 24 A and B).

The female offspring of LP mothers under control diet (LPF1-CD) showed significant lower insulin resistance index than control offspring under HCD from the 15th week of age, while male offspring showed no significant difference compared to other groups of offspring. In contrast, the male and female offspring of LP mothers under high caloric diet (LPF1-HCD) showed significant

higher insulin resistance index than all other groups of offspring from 15th week of age for males and 10th week of age for females (Table 9 and Figure 24 A and B).

4.3.4 Oral glucose tolerance test (OGTT)

At age of 30th week, the OGTT indicated that the offspring of LP mothers (under control or high caloric diet) showed impaired glucose tolerance (IGT) after 30 minutes of glucose administration. The LPF1-CD showed normal blood glucose level from 60 minutes after glucose administration and thereafter, while LPF1-HCD showed IGT until the end of the test (Table 10 and Figure 25 A and B). The control offspring under HCD showed IGT after 60 after which the blood glucose levels return to normal values. We can also note that, there are no significant differences between males and females (Table 10 and Figure 25 A and B)

Table (7) : The FBS (mg/dl) of F1 male and female offspring of control and malnourished mothers under control and high caloric diet at different ages.

Age (week)	Sex	CF1-CD	CF1-HCD	LPF1-CD	LPF1-HCD
5	M	75 ± 11	75 ± 12	78 ± 10	82 ± 9
	F	82 ± 14	80 ± 10	85 ± 10	87 ± 9
10	M	78 ± 8	78 ± 11	85 ± 10	86 ± 8
	F	86 ± 13	84 ± 11	88 ± 10	91 ± 8
15	M	83 ± 10	83 ± 10	88 ± 11	94 ± 10
	F	84 ± 10	88 ± 10	90 ± 11	93 ± 10
20	M	83 ± 9	85 ± 10	88 ± 13	98^{ab} ± 11
	F	89 ± 12	94 ± 9	93 ± 13	97 ± 11
25	M	88 ± 8	89 ± 11	88 ± 11	115^{abc} ± 12
	F	91 ± 14	93 ± 12	94 ± 11	104 ± 12
30	M	91 ± 12	94 ± 13	95 ± 11	112^{abc} ± 12
	F	93 ± 12	96 ± 11	93 ± 14	110^{abc} ± 12

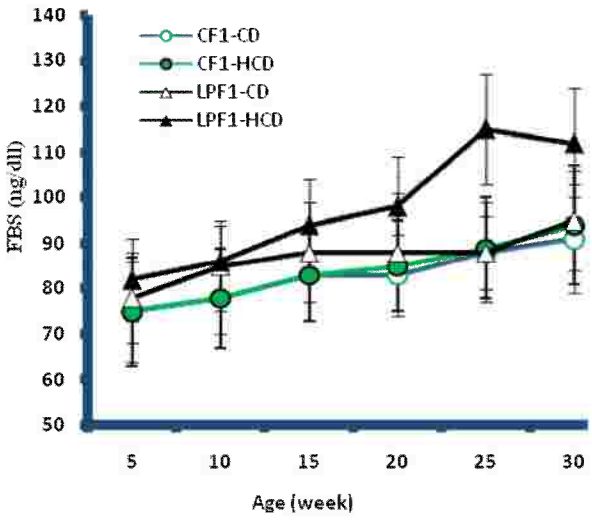


Figure (22 A) Age-dependent change of FBS of male F1 offspring under control or HCD-diet

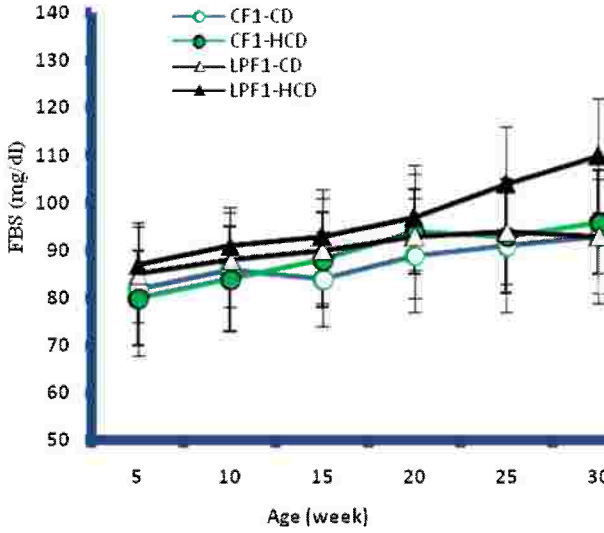


Figure (22 B) Age-dependent change of FBS in fem F1 offspring under control or HCD-diet

Data represented as mean ± SD

M: Male F: Female CD: Control diet HCD: High-caloric diet

CF1: Offspring of control mothers LPF1: Offspring of low-protein nourished mothers

a Signıficandtly dıjferent from CF1-CD group at each age by ANDOVA (p<0.05).

b Signıficandtly dıjferent from CF1-HCD group at each age by ANDOVA (p<0.05).

c Signıficandtly dıjferent from LPF1-CD group at each age by ANDOVA (p<0.05).

Table (8) : The Insulin (ng/ml) of F1 male and female offspring of control and malnourished mothers under control and high caloric diet at different ages.

Age (week)	Sex	CF1-CD	CF1-HCD	LPF1-CD	LPF1-HCD
5	M	0.22 ± 0.02	0.22 ± 0.02	0.2 ± 0.021	0.21^{*} ± 0.02
	F	0.24 ± 0.04	0.25 ± 0.04	0.22 ± 0.021	0.24 ± 0.02
10	M	0.25 ± 0.02	0.25 ± 0.02	0.23^{*} ± 0.024	0.24^{*} ± 0.02
	F	0.27 ± 0.03	0.27 ± 0.03	0.26 ± 0.024	0.29^{c} ± 0.02
15	M	0.3 ± 0.01	0.31 ± 0.016	0.28^{ab} ± 0.02	0.33^{abc} ± 0.019
	F	0.31 ± 0.02	0.32 ± 0.04	0.29 ± 0.02	0.33^{c} ± 0.019
20	M	0.31 ± 0.018	0.32^{*} ± 0.021	0.31 ± 0.02	0.35^{abc} ± 0.02
	F	0.33 ± 0.02	0.35 ± 0.03	0.31^{b} ± 0.02	0.36^{ac} ± 0.02
25	M	0.31 ± 0.015	0.33^{*} ± 0.024	0.31^{b} ± 0.01	0.38^{abc} ± 0.016
	F	0.33 ± 0.03	0.36^{a} ± 0.02	0.32^{b} ± 0.01	0.38^{ac} ± 0.016
30	M	0.33 ± 0.02	0.35 ± 0.02	0.34 ± 0.02	0.41^{abc} ± 0.03
	F	0.35 ± 0.04	0.36 ± 0.03	0.34 ± 0.02	0.39^{c} ± 0.03

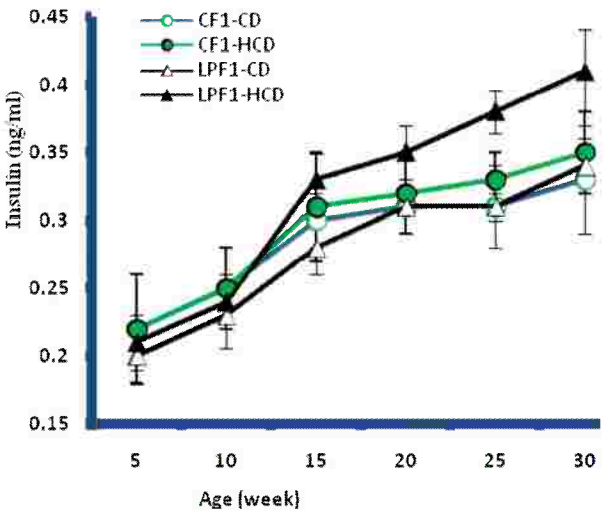


Figure (23 A) Age-dependent change of Insulin in male F1 offspring under control or HCD-diet

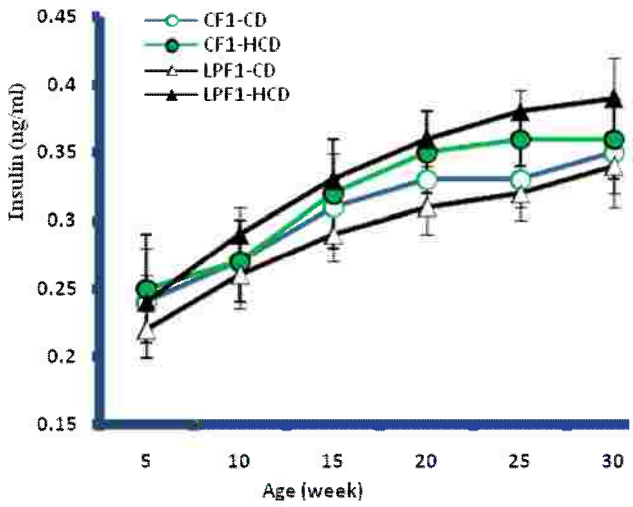


Figure (23 B) Age-dependent change of Insulin in female F1 offspring under control or HCD-diet

Data represented as mean ± SD

M: Male F: Female CD: Control diet HCD: High-caloric diet

CF1: Offspring of control mothers LPF1: Offspring of low-protein nourished mothers

a Signıficandtly dıjferent from CF1-CD group at each age by ANDOVA (p<0.05).

b Signıficandtly dıjferent from CF1-HCD group at each age by ANDOVA (p<0.05).

c Signıficandtly dıjferent from LPF1-CD group at each age by ANDOVA (p<0.05).

** Signıficant dıjference between male and female in the same group at each age by ANDOVA (p<0.05).*

Table (9) : The HOMA-IR of F1 male and female offspring of control and malnourished mothers under control and high caloric diet at different ages.

Age (week)	Sex	CF1-CD	CF1-HCD	LPF1-CD	LPF1-HCD
5	M	$1.0^{*} \pm 0.09$	$1.0^{*} \pm 0.11$	$0.9^{*} \pm 0.12$	$1.0^{*} \pm 0.11$
	F	1.2 ± 0.12	1.2 ± 0.12	1.1 ± 0.13	1.2 ± 0.15
10	M	$1.2^{*} \pm 0.09$	1.2 ± 0.11	$1.2^{*} \pm 0.1$	$1.2^{*} \pm 0.09$
	F	1.4 ± 0.14	1.3 ± 0.13	1.4 ± 0.14	$1.6^{abc} \pm 0.14$
15	M	$1.5^{*} \pm 0.08$	$1.5^{*} \pm 0.1$	1.5 ± 0.12	$1.8^{abc} \pm 0.11$
	F	1.5 ± 0.12	$1.7^{a} \pm 0.14$	$1.5^{b} \pm 0.15$	$1.8^{ac} \pm 0.14$
20	M	$1.5^{*} \pm 0.11$	$1.6^{*} \pm 0.12$	1.6 ± 0.1	$2.0^{abc} \pm 0.12$
	F	1.7 ± 0.14	$1.9^{a} \pm 0.15$	$1.7^{b} \pm 0.13$	$2.1^{abc} \pm 0.13$
25	M	1.6 ± 0.09	$1.7^{*} \pm 0.1$	$1.6^{*} \pm 0.15$	$2.6^{abc*} \pm 0.1$
	F	1.8 ± 0.04	$2.0^{a} \pm 0.13$	$1.8^{b} \pm 0.16$	$2.3^{abc} \pm 0.15$
30	M	1.8 ± 0.09	1.9 ± 0.15	1.9 ± 0.12	$2.7^{abc*} \pm 0.11$
	F	1.9 ± 0.13	2.0 ± 0.16	1.9 ± 0.15	$2.5^{abc} \pm 0.17$

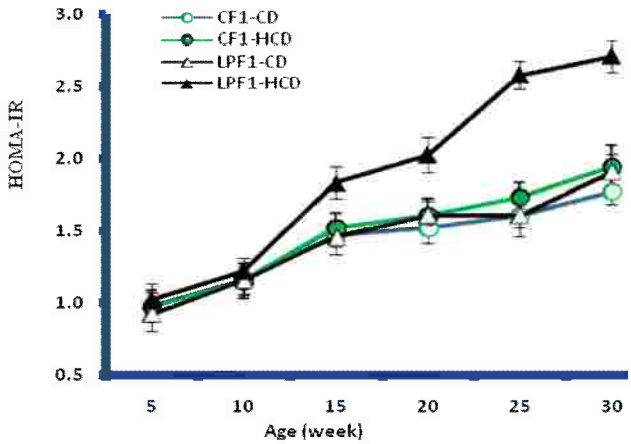


Figure (24 A) Age-dependent change of HOMA of male F1 offspring under control or HCD-diet

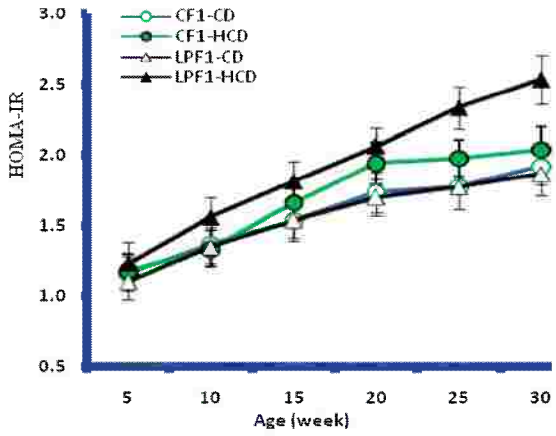


Figure (24 B) Age-dependent change of HOMA i female F1 offspring under control or HCD-diet

Data represented as mean ± SD

M: Male **F:** Female **CD:** Control diet **HCD:** High-caloric diet

CF1: Offspring of control mothers **LPF1:** Offspring of low-protein nourished mothers

[a] *Significandtly different from CF1-CD group at each age by ANDOVA (p<0.05).*

[b] *Significandtly different from CF1-HCD group at each age by ANDOVA (p<0.05).*

[c] *Significandtly different from LPF1-CD group at each age by ANDOVA (p<0.05).*

[*] *Significandt difference between male and female in the same group at each age by ANDOVA (p<0.05).*

Table (10) : The OGTT (mg/dl) of F1 male and female offspring of control and malnourished mothers under control and high caloric diet at 30 weeks of age.

Time (min)	Sex	CF1-CD	CF1-HCD	LPF1-CD	LPF1-HCD
0	M	91 ± 11	94 ± 11	95 ± 7	112^{abc} ± 9
	F	93 ± 10	96 ± 11	93 ± 6	110^{abc} ± 6
30	M	116 ± 10	137^{a} ± 12	156^{ab} ± 8	167^{abc} ± 9
	F	120 ± 11	141^{a} ± 11	150^{a} ± 10	164^{abc} ± 10
60	M	129 ± 12	156^{a} ± 11	126^{*} ± 6	139^{bc*} ± 7
	F	134 ± 12	148^{a} ± 12	156^{a} ± 5	148^{ac} ± 5
90	M	106 ± 11	116 ± 9	112 ± 6	127^{abc} ± 5
	F	111 ± 10	123^{a} ± 10	113^{b} ± 6	125^{ac} ± 6
120	M	84 ± 10	94 ± 12	98^{a} ± 6	118^{abc} ± 7
	F	95 ± 12	101 ± 13	96 ± 7	114^{abc} ± 7

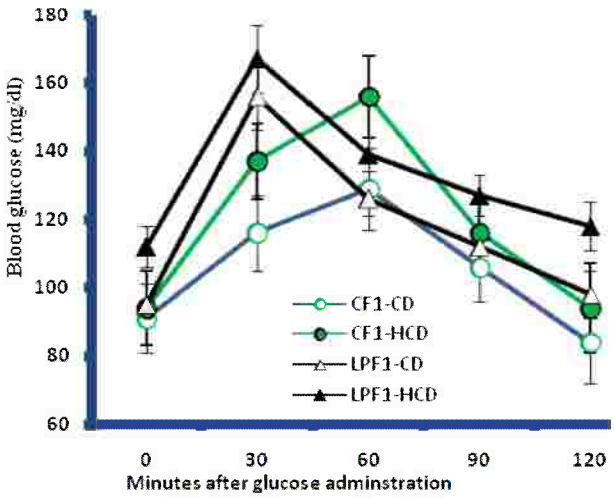


Figure (25 A) OGTT in male F1 offspring under control or HCD-diet at 30th week age

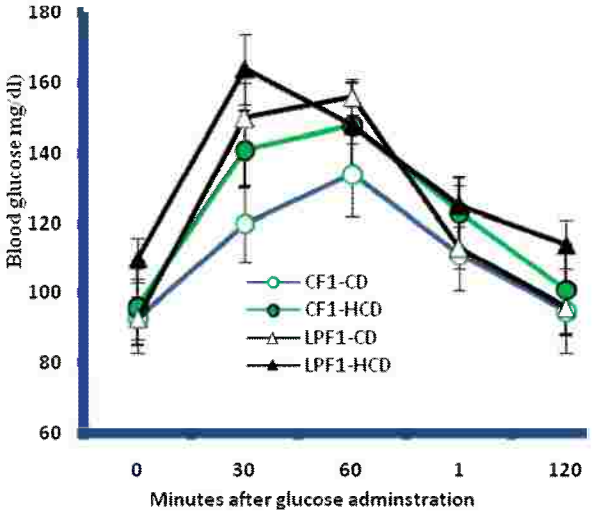


Figure (25 B) OGTT in female F1 offspring under control or HCD-diet at 30th week age

Data represented as mean ± SD

M: Male **F:** Female **CD:** Control diet **HCD:** High-caloric diet

CF1: Offspring of control mothers **LPF1:** Offspring of low-protein nourished mothers

[a] *Significandtly different from CF1-CD group at each age by ANDOVA (p<0.05).*

[b] *Significandtly different from CF1-HCD group at each age by ANDOVA (p<0.05).*

[c] *Significandtly different from LPF1-CD group at each age by ANDOVA (p<0.05).*

[*] *Significandt difference between male and female in the same group at each age by ANDOVA (p<0.05).*

4.4 Lipid Profile and Non-esterfied fatty acids (NEFA)

4.4.1 Total cholesterol

The results of total cholesterol in males and females were represented in Table(11) and Figure (26 A and B). Total cholesterol level showed a slight age-dependent increase in all groups of offspring with highest levels observed in LPF1- HCD group. Also, there were no significant differences between males and females.

The offspring of LP mothers under HCD (LPF1-HCD) showed significantly higher total cholesterol level than CF1-CD only at age of 30th week (Table 11 and Figures 26 A and B).

4.4.2 High Density Lipoprotein-Cholesterol (HDL-C)

The results of HDL-C in all offspring were represented in Table (12) and Figure (27A and B). HDL-C level in all groups of offspring showed an age-dependent decline. The male offspring of control mothers at age of 10,15 and 20 weeks showed lower HDL-C levels than female ones, also male offspring of LP mothers under HCD (LPF1-HCD) showed significantly lower level than female offspring from 15th week of age and thereafter.

The male offspring of LPF1-HCD showed significantly lower HDL-C level compared to other groups of offspring from the 25th week of age (Table 12 and Figure 27 A and B).

4.4.3 Low Density Lipoprotein- Cholesterol (LDL-C)

The results of LDL-C in all offspring were represented in Table (13) and Figure (28A and B). LDL-C level in all groups of offspring and during the study period showed similar pattern of changes to total cholesterol, however no significant differences were detected between different offspring or between males or females (Table 13 and Figure 28 A and B).

4.4.4 Triglycerides

Table (14) and Figure (29 A and B) represent the results of triglycerides (TG) in offspring groups at different ages. While the TG levels in male CF1-CD offspring showed no change with age, it's levels in the other male offspring groups and all female offspring groups showed an age-dependent increase. No significant difference observed between males and females in all offspring groups except in LPF1-HCD offspring which showed that from 20th week of age the males have higher triglycerides level than females.

The results indicated that only male offspring of CF1-HCD and LPF1-CD showed significantly higher triglycerides level than control offspring CF1-CD. Also, male offspring of LPF1-HCD showed significant higher levels compared to all other groups from the 20th week of age and thereafter (Table 14 and Figure 29 A & B).

4.4.5 Non-esterfied fatty acids (NEFA)

Table (15) and Figure (30 A and B) represents the results of NEFA in all groups of offspring. While the male offspring of control groups (CF1-CD and CF1-HCD) showed no change in NEFA levels with age, other males (LPF1-CD and LPF1-HCD) and all female offspring showed an age-dependent increase in NEFA levels (Table 15 and Figure 30 A and B). The male offspring of CF1-CD and LPF1-CD showed lower NEFA level than female offspring at age of 20 week and thereafter for CF1-CD group and 25 week and thereafter for LPF1-CD group.

The male control offspring under HCD showed higher NEFA level than control offspring under CD from the 10th week of age. With respect to offspring of LP mothers; the male offspring under

CD (LPF1-CD) showed no significant change compared to CF1-CD but showed significantly lower NEFA levels than CF1-HCD from the 10th week of age and thereafter. In contrast, the male offspring of LPF1-HCD showed significantly higher NEFA levels than CF1-CD and LPF1-CD from the 10th week of age and thereafter, and the female offspring of LPF1-HCD showed this significance only at 30th week of age. The highest level of NEFA was observed in the male and female LPF1-HCD offspring at 30 week age (Table 16 and Figure 31 A and B).

Table (11) : The Total Cholesterol (mg/dl) of F1 male and female offspring of control and malnourished mothers under control and high caloric diet at different ages.

Age (week)	Sex	CF1-CD	CF1-HCD	LPF1-CD	LPF1-HCD
5	M	122 ± 15	126 ± 31	121 ± 20	128 ± 19
	F	128 ± 20	132 ± 22	126 ± 18	132 ± 20
10	M	154 ± 31	164 ± 33	152 ± 27	159 ± 22
	F	151 ± 23	156 ± 25	148 ± 23	162 ± 26
15	M	143 ± 33	155 ± 25	142 ± 29	166 ± 27
	F	156 ± 22	163 ± 25	153 ± 27	166 ± 24
20	M	147 ± 25	158 ± 27	156 ± 22	172 ± 30
	F	160 ± 25	168 ± 27	156 ± 24	174 ± 27
25	M	160 ± 27	173 ± 21	162 ± 31	180 ± 24
	F	165 ± 27	183 ± 21	164 ± 31	188 ± 31
30	M	166 ± 21	189 ± 23	174 ± 30	208[a] ± 25
	F	171 ± 21	195 ± 25	178 ± 26	211[a] ± 28

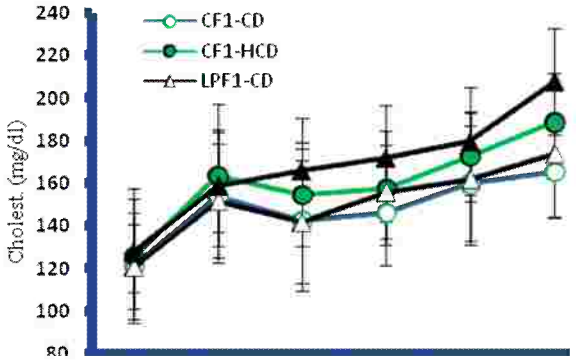


Fig (26 A): Age dependent change in serum level of cholesterol in male F1 offspring under control and HCD-diet

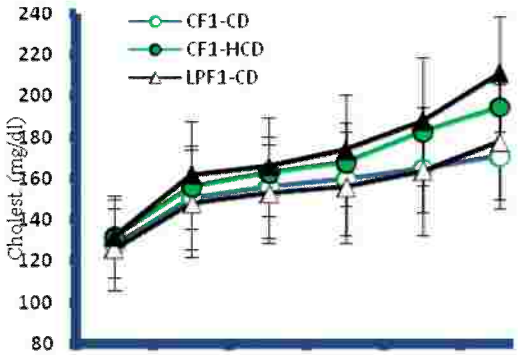


Fig (26 B): Age dependent change in serum level of cholesterol in female F1 offspring under control and HCD-diet

Data represented as mean ± SD

M: Male **F:** Female **CD:** Control diet **HCD:** High-caloric diet

CF1: Offspring of control mothers **LPF1:** Offspring of low-protein nourished mothers

[a] *Significandtly different from CF1-CD group at each age by ANDOVA ($p<0.05$).*

Table (12) : The HDL-C (mg/dl) of F1 male and female offspring of control and malnourished mothers under control and high caloric diet at different ages.

Age (week)	Sex	CF1-CD	CF1-HCD	LPF1-CD	LPF1-HCD
5	M	49 ± 5	51 ± 4	52 ± 5	48 ± 4
	F	54 ± 5	52 ± 4	55 ± 5	52 ± 5
10	M	49* ± 3	46 ± 4	50 ± 4	45 ± 4
	F	53 ± 3	48 ± 4	51 ± 5	48 ± 4
15	M	46* ± 4	43* ± 3	48 ± 4	42* ± 5
	F	51 ± 4	47 ± 3	50 ± 4	47 ± 4
20	M	44* ± 3	42 ± 4	46 ± 5	38* ± 4
	F	49 ± 3	45 ± 4	48 ± 3	45 ± 3
25	M	44 ± 4	41 ± 5	43 ± 5	36ac* ± 4
	F	48 ± 4	44 ± 5	46 ± 3	43 ± 5
30	M	43 ± 3	39 ± 4	42 ± 3	32abc* ± 3
	F	46 ± 3	42 ± 4	43 ± 4	40a ± 4

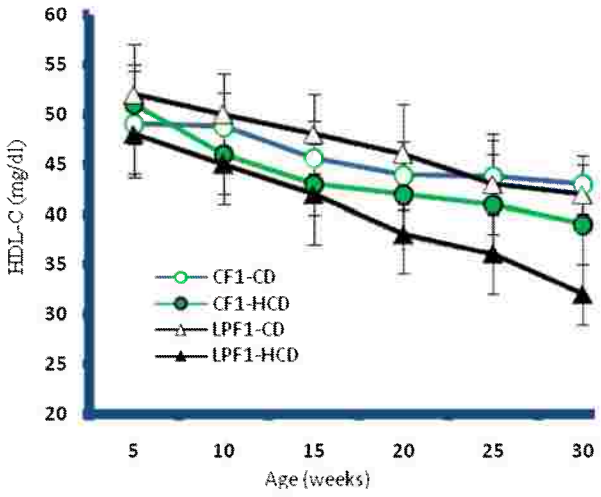


Fig (27A): Age dependent change in serum level of HDL-C in male F1 offspring under control and HCD-diet

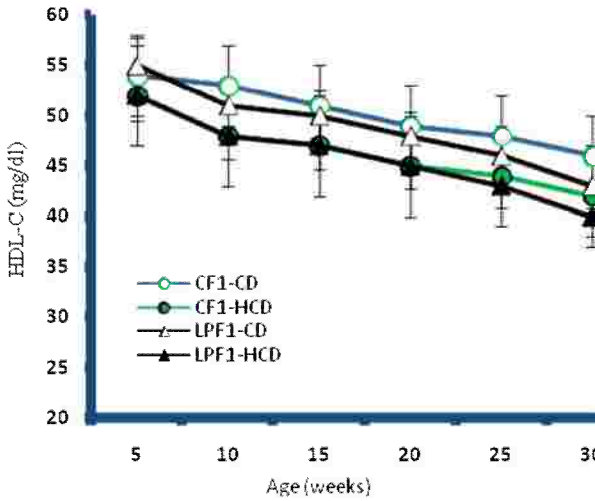


Fig (27B): Age dependent change in serum level of HDL- female F1 offspring under control and HCD-diet

Data represented as mean ± SD

M: Male **F:** Female **CD:** Control diet **HCD:** High-caloric diet

CF1: Offspring of control mothers **LPF1:** Offspring of low-protein nourished mothers

[a] *Signıficandtly dıjferent from CF1-CD group at each age by ANDOVA (p<0.05).*

[b] *Signıficandtly dıjferent from CF1-HCD group at each age by ANDOVA (p<0.05).*

[c] *Signıficandtly dıjferent from LPF1-CD group at each age by ANDOVA (p<0.05).*

[*] *Signıficandt dıjference between male and female in the same group at each age by ANDOVA (p<0.05).*

Table (13) : The LDL-C (mg/dl) of F1 male and female offspring of control and malnourished mothers under control and high caloric diet at different ages.

Age (week)	Sex	CF1-CD	CF1-HCD	LPF1-CD	LPF1-HCD
5	M	76 ± 26	81 ± 17	75 ± 21	85 ± 20
	F	76 ± 12	81 ± 17	73 ± 22	80 ± 19
10	M	80 ± 34	88 ± 20	82 ± 26	94 ± 28
	F	78 ± 21	83 ± 20	81 ± 26	86 ± 18
15	M	68 ± 33	76 ± 16	75 ± 26	91 ± 22
	F	71 ± 18	80 ± 16	76 ± 23	85 ± 24
20	M	80 ± 27	89 ± 17	85 ± 17	95 ± 29
	F	82 ± 22	85 ± 17	84 ± 28	91 ± 27
25	M	95 ± 20	102 ± 17	104 ± 32	101 ± 31
	F	96 ± 20	96 ± 17	101 ± 30	100 ± 30
30	M	96 ± 29	107 ± 20	108 ± 28	112 ± 25
	F	98 ± 23	102 ± 20	104 ± 33	105 ± 26

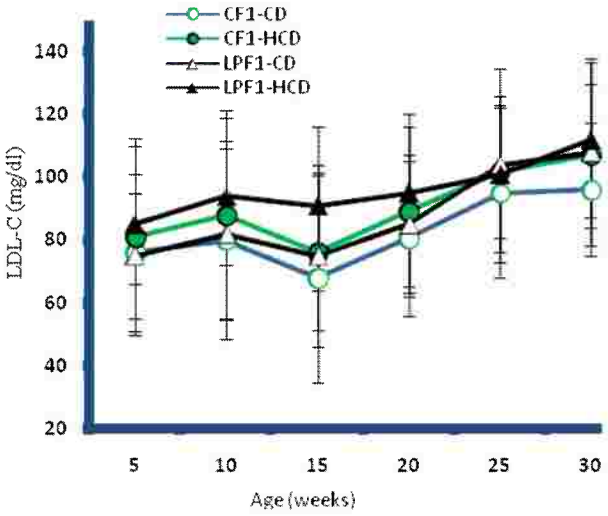


Fig (28 A): Age dependent change in serum level of LDL-C in male F1 offspring under control and HCD-diet

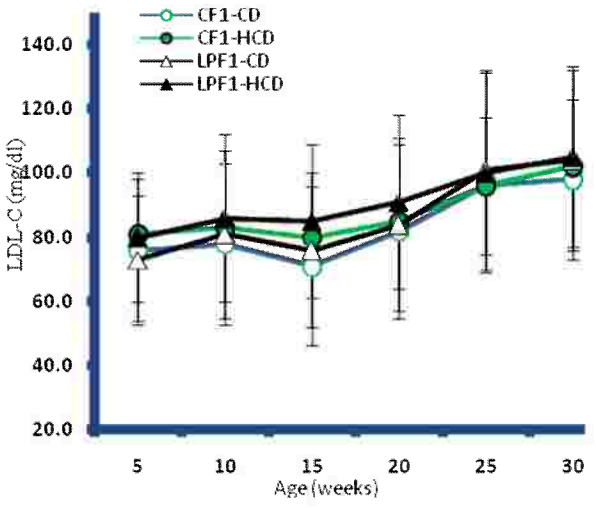


Fig (28 B): Age dependent change in serum level of LDL-C in female F1 offspring under control and HCD-diet

Data represented as mean ± SD

M: Male **F:** Female **CD:** Control diet **HCD:** High-caloric diet

CF1: Offspring of control mothers **LPF1:** Offspring of low-protein nourished mothers

Table (14) : The TG (mg/dl) of F1 male and female offspring of control and malnourished mothers under control and high caloric diet at different ages.

Age (week)	Sex	CF1-CD	CF1-HCD	LPF1-CD	LPF1-HCD
5	M	97 ± 18	102 ± 17	96 ± 13	103 ± 14
	F	89 25	105 ± 18	92 ± 17	102 ± 21
10	M	101 ± 17	104 ± 17	103 ± 16	118 ± 14
	F	105 ± 31	100 ± 18	105 ± 20	110 ± 25
15	M	108 ± 15	116 ± 18	118 ± 17	129 ± 16
	F	113 ± 33	118 ± 20	116 ± 16	120 ± 27
20	M	111 ± 18	124 ± 18	122 ± 13	147^{abc*} ± 15
	F	114 ± 25	128 ± 18	115 ± 17	122 ± 21
25	M	104 ± 17	134^{a} ± 20	132^{a} ± 18	153^{abc*} ± 19
	F	118 ± 27	139 ± 17	122 ± 17	128 ± 21
30	M	111 ± 16	141^{a} ± 19	134^{a} ± 20	169^{abc*} ± 20
	F	121 ± 21	145 ± 20	126 ± 20	135 ± 23

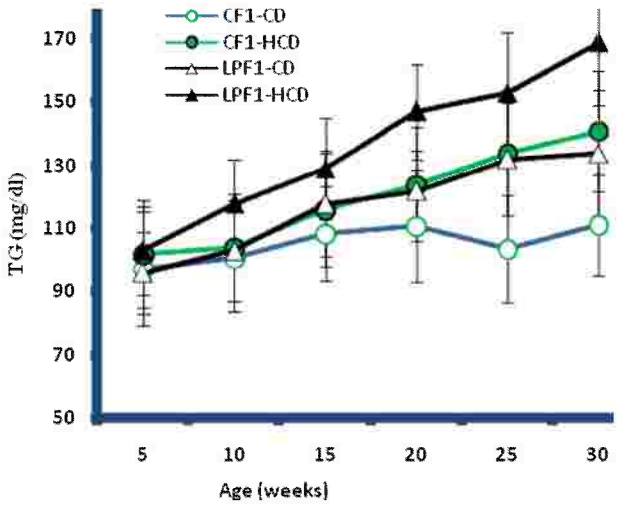


Fig (29 A): Age dependent change in serum level of TG in male F1 offspring under control and HCD-diet

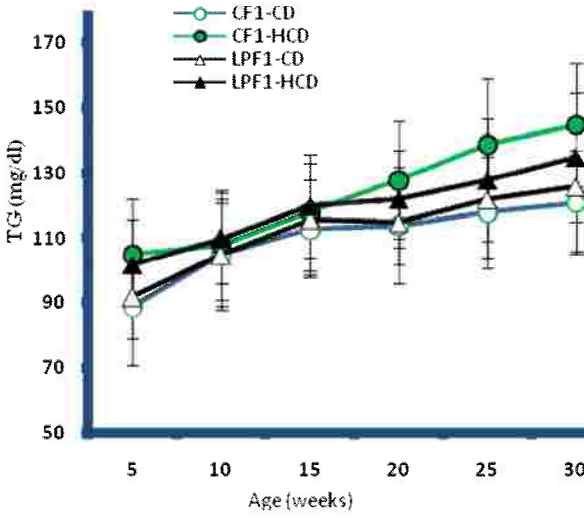


Fig (29 B): Age dependent change in serum level of TG female F1 offspring under control and HCD-diet

Data represented as mean ± SD

M: Male **F:** Female **CD:** Control diet **HCD:** High-caloric diet

CF1: Offspring of control mothers **LPF1:** Offspring of low-protein nourished mothers

[a] *Signıficandtly dıjferent from CF1-CD group at each age by ANDOVA (p<0.05).*

[b] *Signıficandtly dıjferent from CF1-HCD group at each age by ANDOVA (p<0.05).*

[c] *Signıficandtly dıjferent from LPF1-CD group at each age by ANDOVA (p<0.05).*

[*] *Signıficandt dıjference between male and female in the same group at each age by ANDOVA (p<0.05).*

Table (15) : The FFA (mmol/l) of F1 male and female offspring of control and malnourished mothers under control and high caloric diet at different ages.

Age (week)	Sex	CF1-CD	CF1-HCD	LPF1-CD	LPF1-HCD
5	M	1.1 ± 0.11	1.21 ± 0.14	1.1 ± 0.14	1.14* ± 0.13
	F	1.2 ± 0.16	1.28 ± 0.12	1.24 ± 0.14	1.3 ± 0.13
10	M	1.15 ± 0.12	1.32^{a} ± 0.14	1.1^{b*} ± 0.12	1.33^{ac} ± 0.14
	F	1.23 ± 0.12	1.38 ± 0.17	1.28 ± 0.12	1.32 ± 0.14
15	M	1.2 ± 0.15	1.38^{a} ± 0.13	1.16^{b} ± 0.15	1.4^{ac} ± 0.16
	F	1.31 ± 0.18	1.43 ± 0.12	1.32 ± 0.15	1.42 ± 0.16
20	M	1.2* ± 0.12	1.42^{a} ± 0.12	1.18^{b*} ± 0.15	1.47^{ac} ± 0.16
	F	1.33 ± 0.12	1.47 ± 0.13	1.34 ± 0.15	1.41 ± 0.16
25	M	1.18* ± 0.13	1.49^{a} ± 0.14	1.1^{b*} ± 0.14	1.52^{ac} ± 0.15
	F	1.39 ± 0.14	1.5 ± 0.12	1.35 ± 0.14	1.4 ± 0.15
30	M	1.2* ± 0.15	1.52^{a} ± 0.15	1.17^{b*} ± 0.11	1.62^{ac} ± 0.16
	F	1.4 ± 0.2	1.5 ± 0.15	1.36 ± 0.11	1.62^{ac} ± 0.16

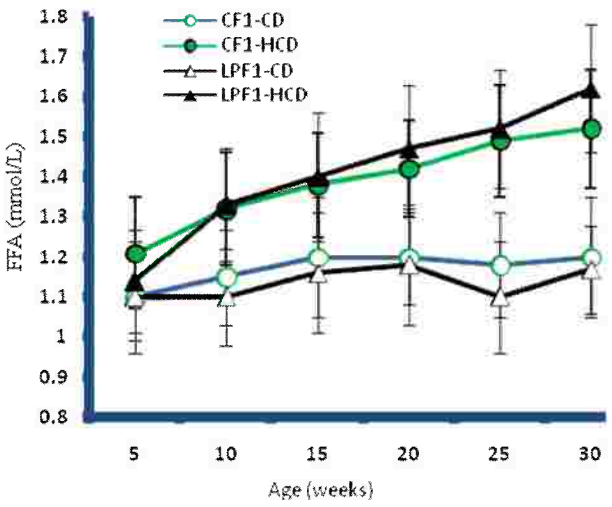


Fig (30 A) Age-dependent change of FFA in male F1 offspring under control or HCD-diet

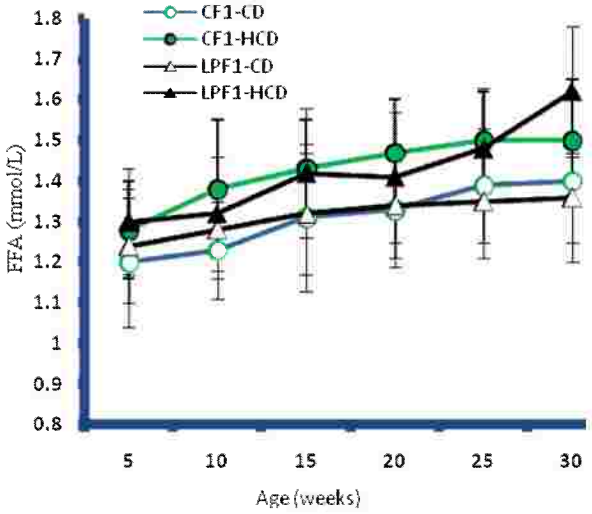


Fig (30 B) Age-dependent change of FFA in female F1 offspring under control or HCD-diet

Data represented as mean ± SD

M: Male **F:** Female **CD:** Control diet **HCD:** High-caloric diet

CF1: Offspring of control mothers **LPF1:** Offspring of low-protein nourished mothers

[a] *Signıficandtly dıfferent from CF1-CD group at each age by ANDOVA (p<0.05).*

[b] *Signıficandtly dıfferent from CF1-HCD group at each age by ANDOVA (p<0.05).*

[c] *Signıficandtly dıfferent from LPF1-CD group at each age by ANDOVA (p<0.05).*

[*] *Signıficandt dıfference between male and female in the same group at each age by ANDOVA (p<0.05).*

4.5 Peripheral glucose sensing parameters

4.5.1 Muscle

4.5.1.1 Content of insulin receptor (IR)

Table (16) and Figure (31 A and B) represents the results of muscle IR in all offspring. It is clear that the change in muscle content of IR with age showed biphasic changes; as during the first 20 week of age in male or 15 week of age in female the IR level in the muscle increased after which its level begin to decline again with aging (Table and Figure A and B). In the both groups of control offspring, the males have significantly higher muscle content of IR than females from 15th week of age. Also, the male offspring of LP mothers under CD (LPF1-CD) showed higher content of IR than females at 15 and 20 week of age.

The control offspring under HCD (CF1-HCD) showed significantly lower muscle content of IR compared to control offspring under CD from 15th week of age for males and females. The offspring of LP mothers under CD showed no significant change compared to CF1-CD except at 25th week for females and 30th for males at which the muscle content of IR become significantly decreased. The offspring of LP mothers under HCD (LPF1-HCD) showed significant lower muscle content of IR compared to CF1-CD as early as 5th week of age, compared to CF1-HCD from the age of 20th week and thereafter, and compared to LPF1-CD from the 10th week of age and thereafter (Table 16 and Figure 31 A and B).

4.5.1.2 Content of Phospho-insulin receptor (Phospho-IR)

The results of muscle content of Phospho-IR were presented in Table 17 and Figure 32 (A and B). The muscle content of Phospho-IR showed no significant change with age or sex. Also, no significant difference was observed between different groups of offspring, however the lowest levels were detected in the offspring of LP mothers under HCD (Table 17 and Figure 32 A and B).

4.5.2 Adipose tissue

4.5.2.1 Content of insulin receptor (IR)

The results of insulin receptor content in adipose tissues were summarized in Table (18) and Figure (33 A and B). It was noted that the adipose tissue content of IR showed an age-dependent increase in all groups of offspring except the LPF1-HCD which showed an age-dependent decline. The male offspring of both control groups (CF1-CD and CF1-HCD) showed higher IR content in adipose tissues than female offspring at nearly all ages (Table 18 and Figure 33 A and B).

The control offspring under HCD have lower adipose tissue content of IR compared to those control offspring under CD at 5th, 15th and 30th weeks of ages for males and at 15th, 20th and 25th week ages for females. The male offspring of LP mothers under CD (LPF1-CD) showed significant decline in the adipose tissue content of IR compared to CF1-CD as early as the 5th week of age until the age of 15 week after which the levels become near normal values then showed decline again at age of 30 week. The male offspring of LPF1-HCD showed lower IR content in the adipose tissues from the 5th week of age and thereafter compared to CF1-CD offspring, and from 10th week of age and thereafter compared to all other groups of offspring. The female offspring of LPF1-HCD showed similar pattern of changes like males but started later, they have lower IR content in the adipose tissues from the 10th week of age and thereafter compared to CF1-CD and

LPF1-CD offspring, and from 25th week of age and thereafter compared to CD-HCD offspring (Table 18 and Figure 33 A and B).

4.5.2.2 Content of Phospho-insulin receptor (Phospho -IR)

The results of adipose tissue content of Phospho-IR were presented in Table 19 and Figure 34 (A and B). The results indicated that the level of Phospho –IR in adipose tissues is nearly constant with age in all groups of offspring except the male offspring of LPF1-HCD which showed age – dependent decline. There is no sex difference observed in the level of Phospho –IR in adipose tissues.

There is no significant difference observed between the different groups of offspring regarding Phospho –IR content in adipose tissues except the male offspring of LPF1-HCD which showed significant decline at late ages; 25th and 30th week of age (Table 19 and Figure 34 A and B).

4.5.3. In Hepatic Tissue

4.5.3.1 Content of insulin receptor (IR)

The results of hepatic content of IR were summarized in Table (20) and Figure 35 (A and B). The hepatic IR content show little change during the study period with no specific pattern of change with age in all studied groups of offspring and fluctuate between 0.2 and 0.27 μg/mg protein. In the control groups of offspring (CF1-CD and CF1-HCD) the males have significantly lower hepatic content of IR during early age (from 5th to 15th week of age), while the male offspring of LPF1-CD showed higher hepatic content of IR than females during later age (20th and 30th week of age).

The offspring of LP mothers under control diet (LPF1-CD) showed lower hepatic content of IR compared to CF1-CD at age of 5 and 15 weeks in females while in males the hepatic IR showed higher values at ages of 10 and 30 week. In the offspring of LP mothers under HCD (LPF1-HCD), the male offspring showed lower hepatic IR at early age (5th week) compared to control while at later ages (10th , 15th and 30th week) the levels become significantly higher than offspring of control mothers. The female offspring of LPF1-HCD showed higher hepatic IR than CF1-CD and LPF1-CD at 10th, 25th and 30th week of ages (Table 20 and Figure 35 A and B).

4.5.3.2 Content of Phospho-insulin receptor (Phospho -IR)

The results of Hepatic Phospho-IR were represented in Table (21) and Figure 36 (A and B). The change in hepatic content of Phospho-IR with age showed biphasic changes especially in males; as during the first 20 week of age the Phospho-IR level in the liver increased after which its level begin to decline again with aging (Table 21 and Figure 36 A and B). In nearly all groups of offspring, the males have higher hepatic content of Phospho-IR than females which significant at different ages.

The only male offspring of control mother under HCD (CF1-HCD) showed significantly higher hepatic content of Phospho-IR compared to control offspring under CD at ages of 5 and 25 weeks. The offspring of LP mothers under CD showed higher hepatic Phospho-IR content compared to CF1-CD from the 10th to 20th week of age for females and from 15th to 25th week of age for males. The offspring of LP mothers under HCD (LPF1-HCD) showed significant lower hepatic content of Phospho-IR compared to LPF1-CD at all ages irrespective to sex. Those offspring showed lower hepatic Phospho-IR compared to the control groups of offspring (CF1-CD and CF1-HCD) from the 15th week of age and thereafter (Table 21 and Figure 36 A and B).

Table (16) : The Muscle-IR (ug/mg protein) of F1 male and female offspring of control and malnourished mothers under control and high caloric diet at different ages.

Age (week)	Sex	CF1-CD	CF1-HCD	LPF1-CD	LPF1-HCD
5	M	0.26 ± 0.02	0.25 ± 0.02	0.25 ± 0.022	0.23^{a} ± 0.02
	F	0.25 ± 0.02	0.24 ± 0.02	0.24 ± 0.022	0.22^{a} ± 0.02
10	M	0.27 ± 0.02	0.26 ± 0.02	0.28 ± 0.021	0.24^{ac} ± 0.02
	F	0.27 ± 0.02	0.25 ± 0.02	0.27 ± 0.021	0.23^{ac} ± 0.02
15	M	0.33^{*} ± 0.01	0.3^{a*} ± 0.016	0.32^{*} ± 0.024	0.25^{abc} ± 0.02
	F	0.3 ± 0.01	0.26^{a} ± 0.016	0.29^{b} ± 0.024	0.25^{ac} ± 0.02
20	M	0.34^{*} ± 0.02	0.31^{a*} ± 0.021	0.35^{b*} ± 0.02	0.26^{abc*} ± 0.019
	F	0.3 ± 0.02	0.25^{a} ± 0.021	0.3^{b} ± 0.02	0.23^{ac} ± 0.019
25	M	0.3^{*} ± 0.02	0.27^{a*} ± 0.024	0.31^{b} ± 0.018	0.22^{abc} ± 0.02
	F	0.27 ± 0.02	0.24 ± 0.024	0.3^{ab} ± 0.018	0.23^{abc} ± 0.02
30	M	0.3^{*} ± 0.02	0.27^{a*} ± 0.02	0.27^{a} ± 0.024	0.22^{abc} ± 0.022
	F	0.27 ± 0.02	0.22^{a} ± 0.02	0.28^{b} ± 0.024	0.21^{ac} ± 0.022

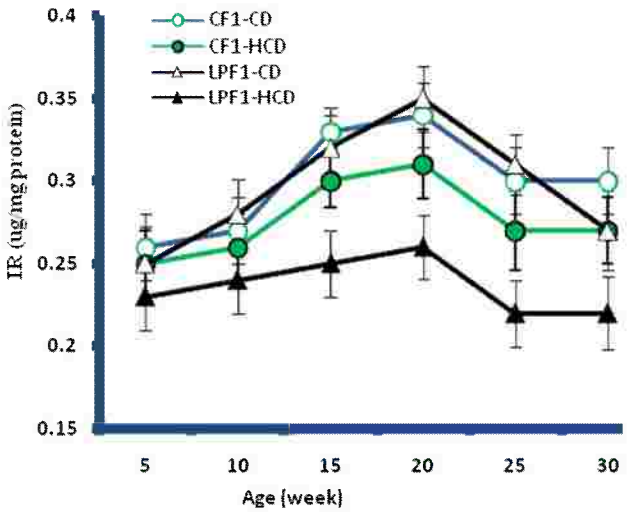


Fig (31 A) Age-dependent change of muscle IR of male F1 offspring under control or HCD-diet

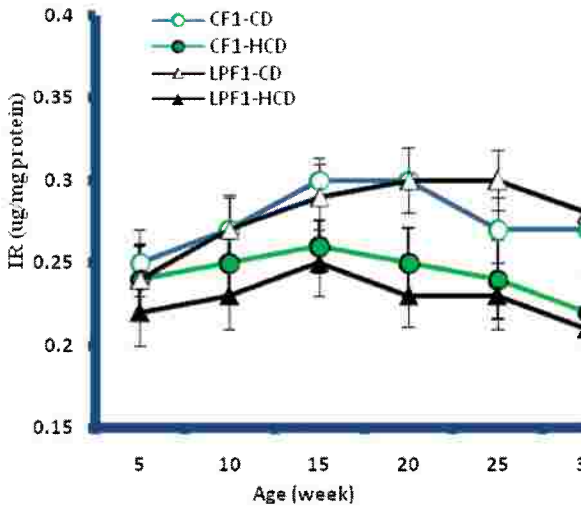


Fig (31 B) Age-dependent change of muscle IR female F1 offspring under control or HCD-diet

Data represented as mean ± SD

M: Male **F:** Female **CD:** Control diet **HCD:** High-caloric diet

CF1: Offspring of control mothers **LPF1:** Offspring of low-protein nourished mothers

[a] *Significandtly different from CF1-CD group at each age by ANDOVA (p<0.05).*

[b] *Significandtly different from CF1-HCD group at each age by ANDOVA (p<0.05).*

[c] *Significandtly different from LPF1-CD group at each age by ANDOVA (p<0.05).*

[*] *Significandt difference between male and female in the same group at each age by ANDOVA (p<0.05).*

Table (17) : The Muscle Phospho-IR (U/mg protein) of F1 male and female offspring of control and malnourished mothers under control and high caloric diet at different ages.

Age (week)	Sex	CF1-CD	CF1-HCD	LPF1-CD	LPF1-HCD
5	M	17.1 ± 2.1	17.3 ± 2.4	16.9 ± 2.0	17 ± 2.6
	F	18 ± 2.0	17.1 ± 2.3	17.2 ± 1.8	17 ± 2.4
10	M	17.3 ± 2.3	17.1 ± 2.3	16.8 ± 2.2	16.4 ± 1.2
	F	17.5 ± 2.1	17.1 ± 2.3	17 ± 2.4	16.6 ± 1.2
15	M	17.1 ± 2.0	16.9 ± 2.4	16.7 ± 2.0	16 ± 2.3
	F	17.3 ± 2.0	17 ± 2.6	17 ± 2.1	16.4 ± 2.3
20	M	16.9 ± 2.3	16.6 ± 1.3	16.5 ± 1.6	15.8 ± 1.9
	F	17.1 ± 2.4	16.4 ± 1.2	17.3 ± 1.8	15.8 ± 2.1
25	M	16.8 ± 2.1	16.5 ± 2.3	16.4 ± 2.1	15.5 ± 1.9
	F	17.1 ± 2.1	16.3 ± 2.2	17.2 ± 2.1	15.6 ± 1.8
30	M	16.6 ± 1.8	16.5 ± 2.1	16.4 ± 2.2	15.4 ± 2.1
	F	17 ± 1.8	16.2 ± 2.2	16.9 ± 2.1	15.4 ± 2.1

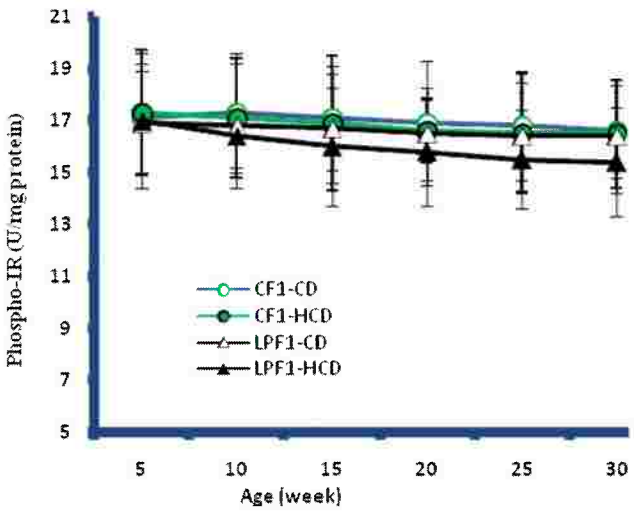


Fig (32 A) Age-dependent change of muscle Phospho-IR of male F1 offspring under control or HCD-diet

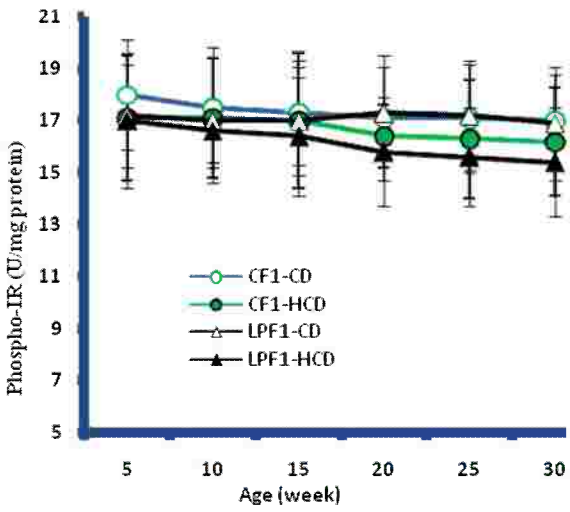


Fig (32 B) Age-dependent change of muscle Phospho-IR of female F1 offspring under control or HCD-diet

Data represented as mean ± SD

M: Male **F:** Female **CD:** Control diet **HCD:** High-caloric diet

CF1: Offspring of control mothers **LPF1:** Offspring of low-protein nourished mothers

Table (18) : The Adipose tissue IR (ug/mg protein) of F1 male and female offspring of control and malnourished mothers under control and high caloric diet at different ages.

Age (week)	Sex	CF1-CD	CF1-HCD	LPF1-CD	LPF1-HCD
5	M	$0.26^{*} \pm 0.02$	$0.22^{a} \pm 0.02$	$0.22^{a} \pm 0.02$	$0.21^{a} \pm 0.02$
	F	0.22 ± 0.021	0.21 ± 0.02	0.22 ± 0.022	0.2 ± 0.02
10	M	$0.28^{*} \pm 0.02$	$0.26^{*} \pm 0.02$	$0.24^{ab} \pm 0.01$	$0.2^{abc} \pm 0.016$
	F	0.24 ± 0.02	0.22 ± 0.018	$0.24^{b} \pm 0.01$	$0.21^{ac} \pm 0.018$
15	M	$0.3^{*} \pm 0.01$	$0.28^{a*} \pm 0.016$	$0.26^{ab} \pm 0.02$	$0.19^{abc*} \pm 0.021$
	F	0.26 ± 0.014	$0.22^{a} \pm 0.016$	$0.25^{b} \pm 0.023$	$0.22^{ac} \pm 0.021$
20	M	$0.31^{*} \pm 0.02$	$0.3^{*} \pm 0.021$	$0.29^{*} \pm 0.016$	$0.19^{abc*} \pm 0.02$
	F	0.27 ± 0.018	$0.24^{a} \pm 0.021$	$0.27^{b} \pm 0.019$	$0.22^{ac} \pm 0.022$
25	M	$0.32^{*} \pm 0.02$	$0.31^{*} \pm 0.024$	0.31 ± 0.021	$0.18^{abc} \pm 0.02$
	F	0.29 ± 0.021	$0.26^{a} \pm 0.022$	$0.3^{b} \pm 0.021$	$0.2^{abc} \pm 0.022$
30	M	$0.33^{*} \pm 0.02$	$0.29^{a} \pm 0.02$	$0.3^{a} \pm 0.024$	$0.18^{abc} \pm 0.02$
	F	0.3 ± 0.019	0.28 ± 0.023	0.29 ± 0.024	$0.2^{abc} \pm 0.018$

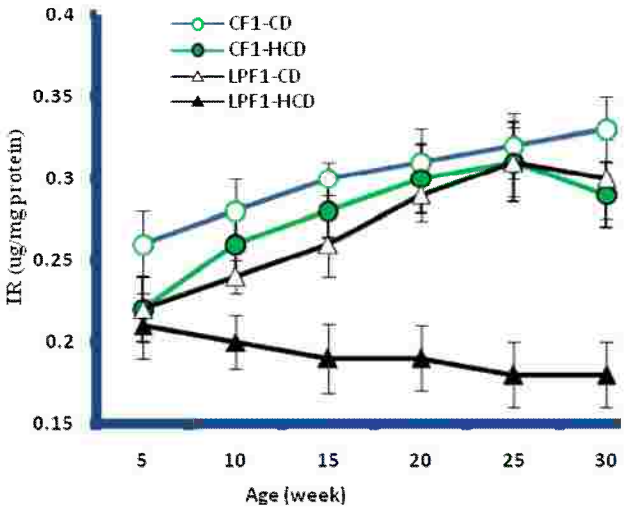


Fig (33 A) Age-dependent change of adipose tissue IR of male F1 offspring under control or HCD-diet

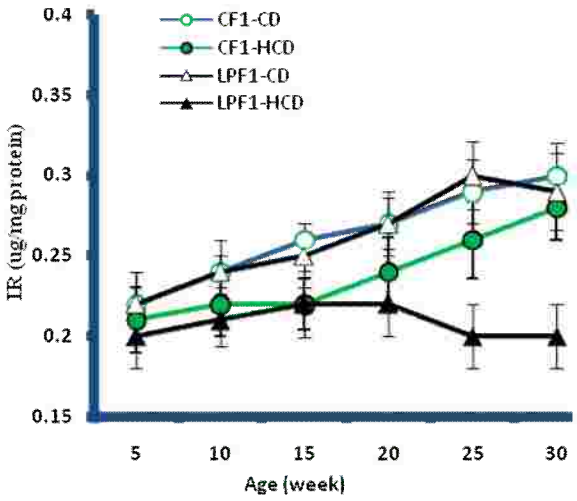


Fig (33 B) Age-dependent change of adipose tissue IR of female F1 offspring under control or HCD-diet

Data represented as mean ± SD

M: Male **F:** Female **CD:** Control diet **HCD:** High-caloric diet

CF1: Offspring of control mothers **LPF1:** Offspring of low-protein nourished mothers

[a] *Significandtly different from CF1-CD group at each age by ANDOVA (p<0.05).*

[b] *Significandtly different from CF1-HCD group at each age by ANDOVA (p<0.05).*

[c] *Significandtly different from LPF1-CD group at each age by ANDOVA (p<0.05).*

[*] *Significandt difference between male and female in the same group at each age by ANDOVA (p<0.05).*

Table (19) : The Adipose tissue Phospho-IR (U/mg protein) of F1 male and female offspring of control and malnourished mothers under control and high caloric diet at different ages.

Age (week)	Sex	CF1-CD	CF1-HCD	LPF1-CD	LPF1-HCD
5	M	12.3 ± 0.7	12.4 ± 0.8	12.2 ± 0.6	12.3 ± 0.7
	F	12.5 ± 0.8	12.3 ± 0.7	12.5 ± 0.6	12.3 ± 0.7
10	M	12.8 ± 0.8	12.9 ± 0.8	12.6 ± 0.7	12.5 ± 0.7
	F	13 ± 0.6	12.7 ± 0.7	13.1 ± 0.7	12.5 ± 0.6
15	M	12.8 ± 0.7	12.7 ± 0.6	12.7 ± 0.6	12.3 ± 0.6
	F	13 ± 0.7	12.8 ± 0.8	13.2 ± 0.8	12.6 ± 0.6
20	M	13 ± 0.8	12.8 ± 0.8	12.9 ± 0.8	12^{*} ± 0.7
	F	13.4 ± 0.8	12.8 ± 0.6	13.3 ± 0.8	12.8 ± 0.7
25	M	12.8 ± 0.7	12.6 ± 0.7	12.9 ± 0.6	11.8^{abc} ± 0.6
	F	13 ± 0.6	12.5 ± 0.7	12.9 ± 0.6	12.4 ± 0.7
30	M	12.7 ± 0.7	12.5 ± 0.8	12.8 ± 0.6	11.7^{ac} ± 0.7
	F	12.9 ± 0.6	12.5 ± 0.7	13 ± 0.7	12.3 ± 0.7

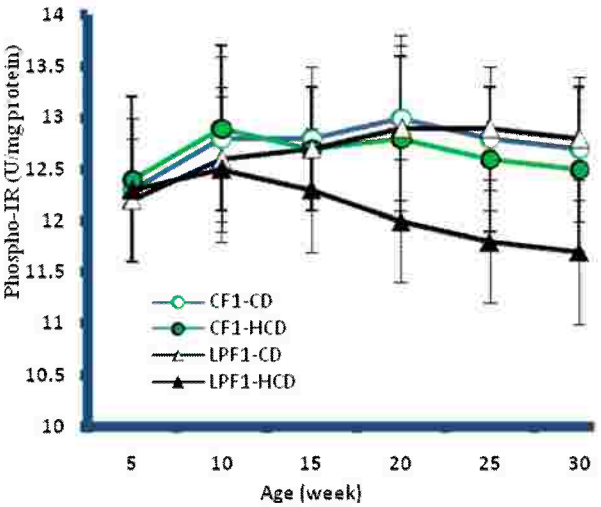


Fig (34 A) Age-dependent change of adipose tissue phospho-IR of male F1 offspring under control or HCD-diet

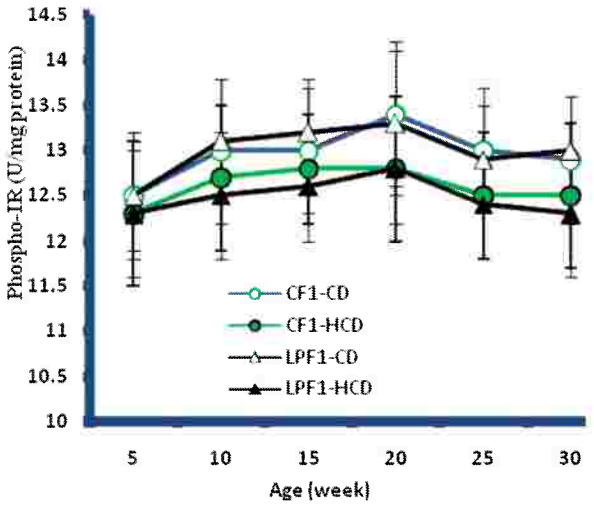


Fig (34 A)Age-dependent change of adipose tissue phospho-IR of female F1 offspring under control or HCD-diet

Data represented as mean ± SD

M: Male **F:** Female **CD:** Control diet **HCD:** High-caloric diet

CF1: Offspring of control mothers **LPF1:** Offspring of low-protein nourished mothers

[a] *Significandtly different from CF1-CD group at each age by ANDOVA (p<0.05).*

[b] *Significandtly different from CF1-HCD group at each age by ANDOVA (p<0.05).*

[c] *Significandtly different from LPF1-CD group at each age by ANDOVA (p<0.05).*

[*] *Significandt difference between male and female in the same group at each age by ANDOVA (p<0.05).*

Table (20) : The Hepatic IR (ug/mg protein) of F1 male and female offspring of control and malnourished mothers under control and high caloric diet at different ages.

Age (week)	Sex	CF1-CD	CF1-HCD	LPF1-CD	LPF1-HCD
5	M	$0.2^{*} \pm 0.02$	$0.21^{*} \pm 0.02$	0.21 ± 0.031	$0.24^{abc} \pm 0.014$
	F	0.23 ± 0.017	0.24 ± 0.021	$0.2^{ab} \pm 0.017$	$0.24^{c} \pm 0.019$
10	M	$0.21^{*} \pm 0.02$	$0.22^{*} \pm 0.02$	$0.24^{a} \pm 0.028$	$0.24^{a*} \pm 0.018$
	F	0.24 ± 0.02	$0.26^{a} \pm 0.017$	0.24 ± 0.021	$0.27^{ac} \pm 0.02$
15	M	$0.22^{*} \pm 0.01$	$0.22^{*} \pm 0.016$	0.23 ± 0.02	$0.25^{ab} \pm 0.02$
	F	0.26 ± 0.022	0.27 ± 0.023	$0.23^{ab} \pm 0.018$	$0.26^{c} \pm 0.021$
20	M	0.24 ± 0.02	0.25 ± 0.021	$0.25^{*} \pm 0.024$	$0.23^{*} \pm 0.017$
	F	0.23 ± 0.02	0.24 ± 0.022	0.22 ± 0.02	$0.25^{c} \pm 0.018$
25	M	0.22 ± 0.02	0.23 ± 0.024	$0.24^{*} \pm 0.02$	0.24 ± 0.02
	F	0.21 ± 0.019	0.22 ± 0.021	0.2 ± 0.021	$0.24^{ac} \pm 0.02$
30	M	0.21 ± 0.02	0.22 ± 0.02	$0.24^{a*} \pm 0.019$	$0.25^{ab} \pm 0.018$
	F	0.2 ± 0.022	0.22 ± 0.02	0.2 ± 0.019	$0.24^{ac} \pm 0.021$

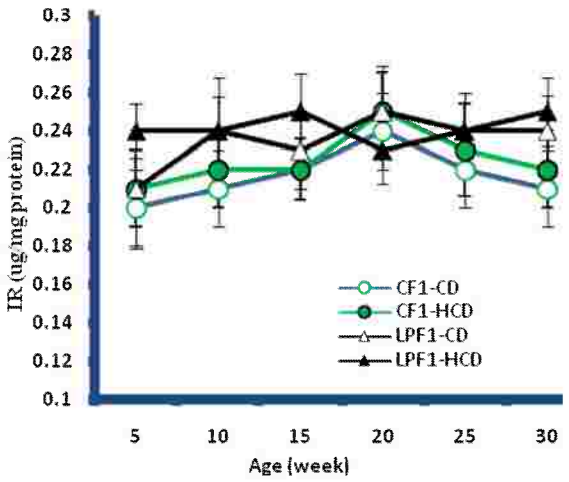


Fig (35 A) Age-dependent change of hepatic IR of male F1 offspring under control or HCD-diet

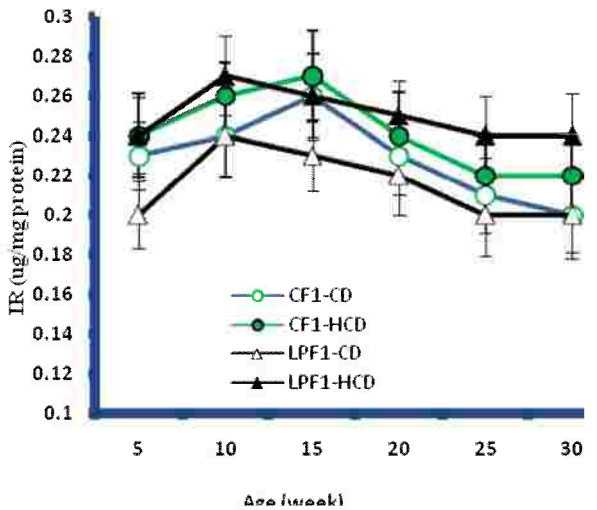


Fig (35 A) Age-dependent change of hepatic IR of female F1 offspring under control or HCD-diet

Data represented as mean ± SD

M: Male **F:** Female **CD:** Control diet **HCD:** High-caloric diet

CF1: Offspring of control mothers **LPF1:** Offspring of low-protein nourished mothers

[a] *Significandtly different from CF1-CD group at each age by ANDOVA (p<0.05).*

[b] *Significandtly different from CF1-HCD group at each age by ANDOVA (p<0.05).*

[c] *Significandtly different from LPF1-CD group at each age by ANDOVA (p<0.05).*

[*] *Significandt difference between male and female in the same group at each age by ANDOVA (p<0.05).*

Table (21) : The Hepatic Phospho-IR (U/mg protein) of F1 male and female offspring of control and malnourished mothers under control and high caloric diet at different ages.

Age (week)	Sex	CF1-CD	CF1-HCD	LPF1-CD	LPF1-HCD
5	M	13.2 ± 0.84	14.1^{a*} ± 0.7	13.6^{b} ± 0.65	12.7^{bc} ± 0.6
	F	12.8 ± 0.67	12.5 ± 0.71	13.1 ± 0.77	12.1^{ac} ± 0.59
10	M	14.6 ± 0.74	14.9 ± 0.67	15.3 ± 0.71	14.2^{c*} ± 0.71
	F	14 ± 0.58	14.5 ± 0.68	14.7^{a} ± 0.6	13.4^{bc} ± 0.65
15	M	15.4^{*} ± 0.65	15.8^{*} ± 0.82	16.2^{a*} ± 0.6	15.1^{c*} ± 0.8
	F	14.2 ± 64	14.7 ± 0.8	15.1^{a} ± 0.57	13.2^{abc} ± 0.67
20	M	16.7^{*} ± 0.71	17.1^{*} ± 0.64	17.8^{a*} ± 0.72	14.2^{abc*} ± 0.59
	F	14.8 ± 0.7	15.4 ± 0.7	16.1^{ab} ± 0.54	13.4^{abc} ± 0.8
25	M	15.3 ± 0.55	16.4^{a*} ± 0.58	16.8^{a*} ± 0.8	14.4^{abc*} ± 0.57
	F	15.1 ± 0.75	15.2 ± 0.66	15.6 ± 0.69	13.1^{abc} ± 0.71
30	M	15.1 ± 0.6	15.9^{*} ± 0.7	15.4 ± 0.77	13.2^{abc} ± 0.62
	F	14.6 ± 0.654	15 ± 0.74	15.5 ± 0.81	12.6^{abc} ± 0.63

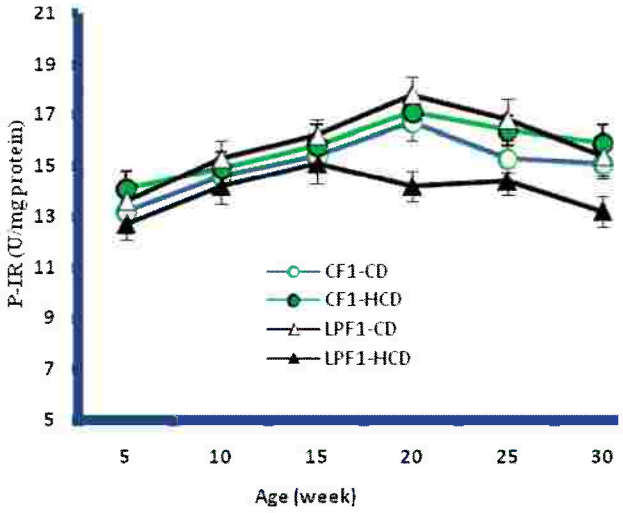


Fig (36 A)Age-dependent change of hepatic phospho-IR of male F1 offspring under control or HCD-diet

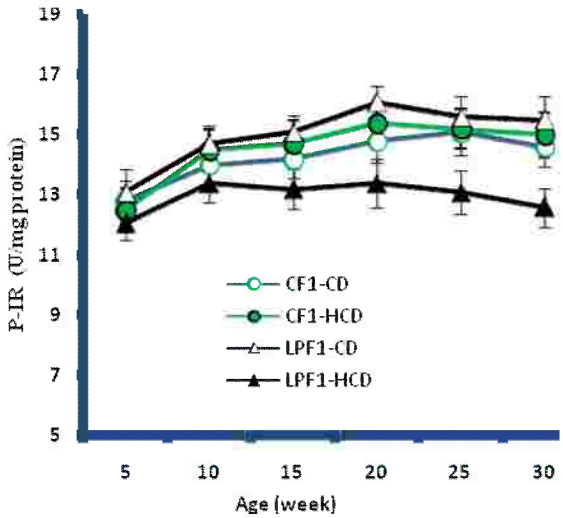


Fig (36 B)Age-dependent change hepatic phospho-IR of female F1 offspring under control or HCD-diet

Data represented as mean ± SD

M: Male **F:** Female **CD:** Control diet **HCD:** High-caloric diet

CF1: Offspring of control mothers **LPF1:** Offspring of low-protein nourished mothers

[a] *Significandtly different from CF1-CD group at each age by ANDOVA (p<0.05).*

[b] *Significandtly different from CF1-HCD group at each age by ANDOVA (p<0.05).*

[c] *Significandtly different from LPF1-CD group at each age by ANDOVA (p<0.05).*

[*] *Significandt difference between male and female in the same group at each age by ANDOVA (p<0.05).*

4.6 Gene Expression of mitochondrial transcription factor A (mTFA)

4.6.1 Muscle expression of mTFA gene

Table (22) and Figure 37 (A and B) represent the analysis of gene expression of muscle mTFA. The expression of mTFA gene appears to be relatively constant with age. No significant difference observed between males and females in all offspring with the exception of the offspring of LPF1-HCD which showed higher expression level in males than females (Table 22 and Figure 37 A and B)

The offspring of CF1-HCD and LPF1-CD showed significantly higher gene expression level than offspring of CF1-CD from the 15th week of age. In the offspring of LP mothers under HCD (LPF1-HCD), the male offspring showed enhanced gene expression of muscle mTFA compared to CF1-CD at ages of 10, 15, and 30th week, while the female offspring of the same group showed lower gene expression compared to all other groups of offspring at ages of 25th and 30th week (Table 22 and Figure 37 A and B).

4.6.2 Adipose expression of mTFA gene

The results of adipose tissue mTFA expression were presented in Table (23) and Figure 38 (A and B). The expression of mTFA gene in adipose tissues appears to be relatively constant with age in all groups of offspring except male offspring of LPF1-HCD and female offspring of CF1-HCD which showed large variation with age. No significant difference observed between males and females in offspring under CD (CF1-CD and LPF1-CD) while in the offspring under HCD (CF1-HCD and LPF1-HCD) at age of 25 and 30weeks the males showed higher expression level than females (Table 23 and Figure 38 A and B).

The HCD causes increased expression of adipose tissues mTFA in the control offspring compared to CD. Female offspring of LP mothers under CD showed significant down regulation of mTFA gene expression compared to CF1-CD in the first 15 weeks of age, however, the male and female offspring showed significant down regulation compared with CF1-HCD as early as 5 weeks and thereafter. Also, it was noted that the female offspring of LPF1-HCD showed a significant down regulation compared with CF1-CD and CF1-HCD as early as 5 weeks and thereafter while males show significant down regulation of mTFA only at ages of 10, 20 and 30 weeks compared to CF1-HCD (Table 23 and Figure 38 A and B).

4.6.3 Hepatic expression of mTFA gene

Table (24) and Figure 39 (A and B) represent the results of hepatic mTFA expression. It was noted that the expression of hepatic mTFA gene in females is relatively constant in nearly all groups of offspring, while in males with the exception of CF1-CD offspring, other offspring showed age-dependent decline in the expression. The male offspring of CF1-HCD and LPF1-CD showed lower expression level of hepatic mTFA than females at many ages (Table 24 and Figure 39 A and B).

The male offspring of CF1-HCD and LPF1-CD showed significant down regulation of hepatic mTFA compared to offspring of CF1-CD from the age of 20 week and thereafter. Also, the male and female offspring of LPF1-HCD showed significant down regulation of hepatic mTFA compared to offspring of CF1-CD from the age of 20 week and thereafter. The lowest levels of gene expression were detected in the male offspring of LPF1-HCD at age of 30 week (Table 24 and Figure 39 A and B).

Table (22) : The Muscle mTFA (AU) of F1 male and female offspring of control and malnourished mothers under control and high caloric diet at different ages.

Age (week)	Sex	CF1-CD	CF1-HCD	LPF1-CD	LPF1-HCD
5	M	1.0 ± 0.08	1.05 ± 0.1	1.03 ± 0.08	1.08 ± 0.08
	F	1.0 ± 0.04	1.05 ± 0.05	0.98 ± 0.04	1.05 ± 0.04
10	M	1.0 ± 0.06	1.05 ± 0.08	1.05 ± 0.08	1.07 ± 0.11
	F	1.0 ± 0.03	1.07 ± 0.04	0.98 ± 0.04	1.05 ± 0.06
15	M	1.0 ± 0.06	1.07^{a} ± 0.08	1.14^{a} ± 0.1	1.12^{a*} ± 0.1
	F	1.0 ± 0.03	1.07^{a} ± 0.04	1.07^{a} ± 0.05	1.04 ± 0.05
20	M	1.0 ± 0.08	1.02 ± 0.08	1.06 ± 0.12	1.06 ± 0.12
	F	1.0 ± 0.04	1.08^{a} ± 0.04	1.08^{a} ± 0.06	1.02 ± 0.06
25	M	1.0 ± 0.1	1.05 ± 0.1	1.09 ± 0.08	1.04^{*} ± 0.11
	F	1.0 ± 0.05	1.05 ± 0.05	1.07^{a} ± 0.04	0.95^{bc} ± 0.05
30	M	1.0 ± 0.12	1.15^{a} ± 0.12	1.11 ± 0.06	1.09^{*} ± 0.08
	F	1.0 ± 0.06	1.05 ± 0.06	1.03 ± 0.03	0.93^{abc} ± 0.04

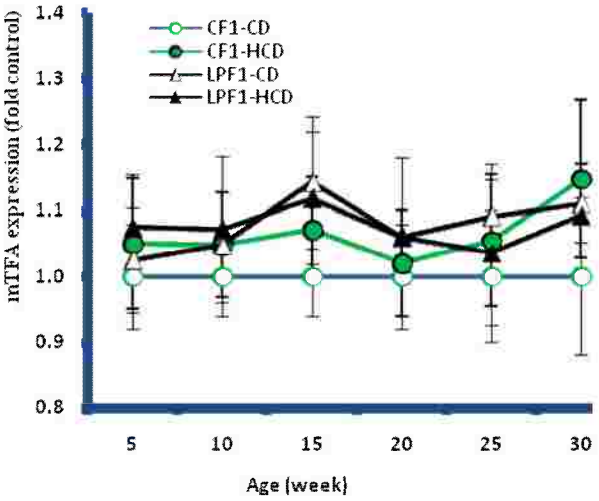


Fig (37 A)Age-dependent change of muscle mTFA of male F1 offspring under control or HCD-diet

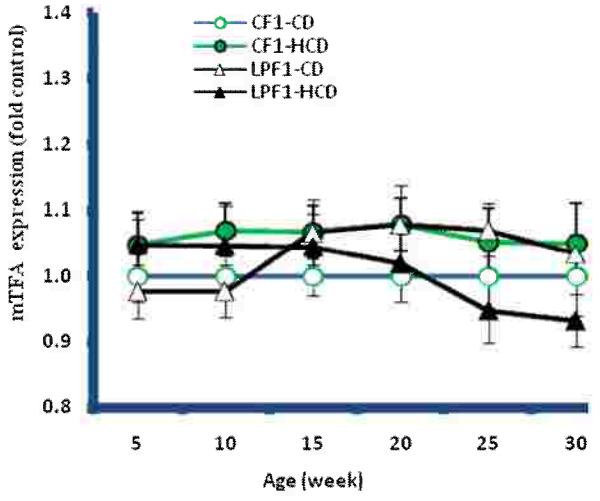


Fig (37 B)Age-dependent change of muscle mTFA of female F1 offspring under control or HCD-diet

Data represented as mean ± SD

M: Male **F:** Female **CD:** Control diet **HCD:** High-caloric diet

CF1: Offspring of control mothers **LPF1:** Offspring of low-protein nourished mothers

[a] *Significandtly different from CF1-CD group at each age by ANDOVA (p<0.05).*

[b] *Significandtly different from CF1-HCD group at each age by ANDOVA (p<0.05).*

[c] *Significandtly different from LPF1-CD group at each age by ANDOVA (p<0.05).*

[*] *Significandt difference between male and female in the same group at each age by ANDOVA (p<0.05).*

Table (23) : The Adipose tissue mTFA (AU) of F1 male and female offspring of control and malnourished mothers under control and high caloric diet at different ages.

Age (week)	Sex	CF1-CD	CF1-HCD	LPF1-CD	LPF1-HCD
5	M	1.0 ± 0.06	1.06 ± 0.08	0.97 ± 0.06	0.97 ± 0.08
	F	1.0 ± 0.03	1.0 ± 0.04	0.92^{ab} ± 0.03	0.92^{ab} ± 0.04
10	M	1.0 ± 0.06	1.08 ± 0.09	0.95^{b} ± 0.07	0.97^{b} ± 0.08
	F	1.0 ± 0.03	1.03 ± 0.04	0.92^{ab} ± 0.03	0.95^{ab} ± 0.04
15	M	1.0 ± 0.08	1.05 ± 0.06	0.9^{b} ± 0.1	0.98 ± 0.11
	F	1.0 ± 0.04	1.0 ± 0.03	0.93^{ab} ± 0.05	0.93^{ab} ± 0.06
20	M	1.0 ± 0.11	1.07 ± 0.06	0.93^{b} ± 0.07	0.91^{b} ± 0.08
	F	1.0 ± 0.06	1.02 ± 0.03	0.96^{b} ± 0.03	0.89^{abc} ± 0.04
25	M	1.0 ± 0.1	1.14^{a*} ± 0.1	0.95^{b} ± 0.06	0.91^{b} ± 0.09
	F	1.0 ± 0.05	1.04 ± 0.05	0.96^{b} ± 0.03	0.89^{abc} ± 0.04
30	M	1.0 ± 0.12	1.14^{a*} ± 0.12	0.95^{b} ± 0.08	1.02^{*} ± 0.07
	F	1.0 ± 0.06	1.02 ± 0.06	0.96 ± 0.04	0.91^{ab} ± 0.03

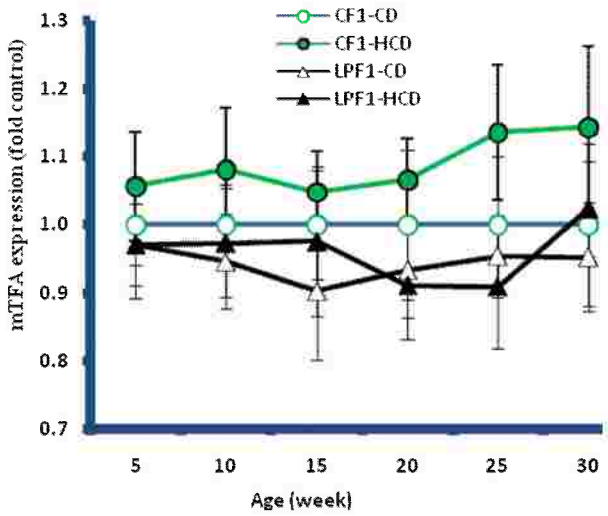


Fig (38 A)Age-dependent change of Adipode tissue mTFA of male F1 offspring under control or HCD-diet

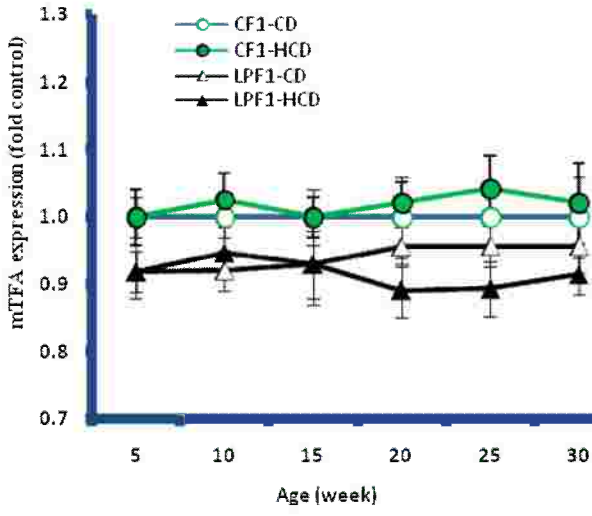


Fig (38 B)Age-dependent change of Adipose tissu mTFA of female F1 offspring under control or HC diet

Data represented as mean ± SD

M: Male **F:** Female **CD:** Control diet **HCD:** High-caloric diet

CF1: Offspring of control mothers **LPF1:** Offspring of low-protein nourished mothers

[a] *Signıficandtly dıjferent from CF1-CD group at each age by ANDOVA (p<0.05).*

[b] *Signıficandtly dıjferent from CF1-HCD group at each age by ANDOVA (p<0.05).*

[c] *Signıficandtly dıjferent from LPF1-CD group at each age by ANDOVA (p<0.05).*

[*] *Signıficandt dıjference between male and female in the same group at each age by ANDOVA (p<0.05).*

Table (24) : The Hepatic mTFA (AU) of F1 male and female offspring of control and malnourished mothers under control and high caloric diet at different ages.

Age (week)	Sex	CF1-CD	CF1-HCD	LPF1-CD	LPF1-HCD
5	M	1.00 ± 0.012	1.00 ± 0.1	1.11^{*} ± 0.12	1.00 ± 0.12
	F	1.00 ± 0.14	1.05 ± 0.11	0.96 ± 0.11	0.99 ± 0.13
10	M	1.00 ± 0.1	1.00 ± 0.14	1.10 ± 0.11	1.00 ± 0.1
	F	1.00 ± 0.12	1.06 ± 0.15	1.03 ± 0.14	1.01 ± 0.11
15	M	1.00 ± 0.12	0.90^{*} ± 0.12	0.99 ± 0.12	0.99 ± 0.1
	F	1.00 ± 0.11	1.03 ± 0.11	1.04 ± 0.1	0.97 ± 0.11
20	M	1.00 ± 0.11	0.85^{a*} ± 0.1	0.94^{*} ± 0.12	0.85^{a*} ± 0.1
	F	1.00 ± 0.12	1.03 ± 0.12	1.16^{a} ± 0.14	0.99^{a} ± 0.12
25	M	1.00 ± 0.12	0.76^{a*} ± 0.1	0.85^{a*} ± 0.1	0.83^{a} ± 0.14
	F	1.00 ± 0.12	1.06 ± 0.14	1.12 ± 0.15	0.94^{c} ± 0.14
30	M	1.00 ± 0.1	0.82^{a*} ± 0.14	0.82^{a*} ± 0.12	0.75^{a} ± 0.12
	F	1.00 ± 0.14	1.05 ± 0.14	1.07 ± 0.12	0.87^{bc} ± 0.11

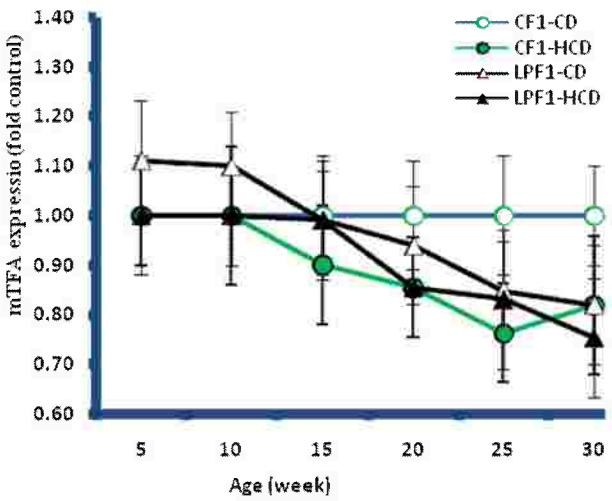


Fig (39 A) Age-dependent change of hepatic mTFA of male F1 offspring under control or HCD-diet

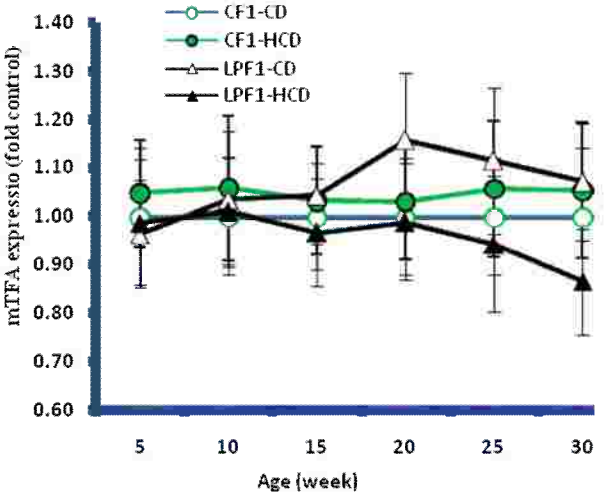


Fig (39 B) Age-dependent change of hepatic mTFA of female F1 offspring under control or HCD-diet

Data represented as mean ± SD

M: Male **F:** Female **CD:** Control diet **HCD:** High-caloric diet

CF1: Offspring of control mothers **LPF1:** Offspring of low-protein nourished mothers

[a] *Significandtly different from CF1-CD group at each age by ANDOVA (p<0.05).*

[b] *Significandtly different from CF1-HCD group at each age by ANDOVA (p<0.05).*

[c] *Significandtly different from LPF1-CD group at each age by ANDOVA (p<0.05).*

[*] *Significandt difference between male and female in the same group at each age by ANDOVA (p<0.05).*

4.7 Gene Expression of uncoupling protein 2 (UCP2)

4.7.1 Muscle expression of UCP2 gene

The results of muscle UCP2 are presented in Table (25) and Figure 40 (A and B). The expression of UCP2 appears to be relatively constant with age in all groups of offspring, however the expression increased slightly with age in the offspring of maintained under HCD (CF1-HCD and LPF1-HCD). There was no sex difference in the expression of UCP2 in the muscles (Table 25 and Figure 40 A and B).

It is clear that the muscle UCP2 expression in all groups showed no significant change compared to control group in spite of the slight increase with the age in both male and female offspring of CF1-HCD and LPF1-HCD.

4.7.2 Adipose tissues expression of UCP2 gene

Table (26) and Figure 41 (A and B) summarize the results of adipose tissue UCP2 expression. The expression of UCP2 gene appears to be relatively constant with age with the exception of the offspring of CF1-HCD which showed an age-dependent up regulation of the gene expression. No significant difference between males and females in the expression of UCP2 in adipose tissues (Table 26 and Figure 41 A and B).

Only the offspring of control mothers under HCD showed significant up regulation of the gene expression of UCP2 in adipose tissues compared to those control offspring under CD at age of 25^{th} and 30^{th} week in males and 30^{th} week in females. All other offspring showed no significant difference in the expression of UCP2 compared to CF1-CD. The females offspring of LP mothers under CD showed significant down regulation of mTFA compared to CF1-HCD at ages of 20 and 30 weeks. Also, the offspring of LP mothers under HCD show significant down regulation of UCP2 gene expression compared to control offspring under HCD at age of 20 week and thereafter (Table 26 and Figure 41 A and B).

4.7.3 Hepatic expression of UCP2 gene

The results of hepatic UCP2 expression are presented in Table (27) and Figure (42 A and B). The hepatic expression of UCP2 gene appears to be relatively constant with age in each group of offspring with the exception of the offspring of LPF1-CD which showed a slight age-dependent down regulation of the gene expression. No significant difference observed between males and females offspring (Table 27 and Figure 42 A and B).

The male and female offspring of LP mothers under CD showed significant down regulation of the UCP2 gene expression compared to the control offspring (CF1-CD and CF1-HCD) from the 10^{th} week of age and thereafter. In contrast, the male and female offspring of LP mothers under HCD showed up regulation of hepatic UCP2 gene expression compared to CF1-CD and LPF1-CD (Table 27 and Figure 42 A and B).

Table (25) : The Muscle UCP2 expression (AU) of F1 male and female offspring of control and malnourished mothers under control and high caloric diet at different ages.

Age (week)	Sex	CF1-CD	CF1-HCD	LPF1-CD	LPF1-HCD
5	M	1.0 ± 0.11	1.03 ± 0.12	0.98 ± 0.14	1.0 ± 0.13
	F	1.0 ± 0.12	1.02 ± 0.12	1.0 ± 0.11	1.02 ± 0.13
10	M	1.0 ± 0.11	1.05 ± 0.13	1.02 ± 0.12	1.03 ± 0.13
	F	1.0 ± 0.1	1.03 ± 0.14	1.0 ± 0.12	1.03 ± 0.14
15	M	1.0 ± 0.11	1.06 ± 0.11	1.02 ± 0.12	1.05 ± 0.14
	F	1.0 ± 0.11	1.06 ± 0.13	1.02 ± 0.12	1.06 ± 0.15
20	M	1.0 ± 0.11	1.11 ± 0.14	1.03 ± 0.14	1.14 ± 0.15
	F	1.0 ± 0.12	1.1 ± 0.14	1.0 ± 0.14	1.1 ± 0.14
25	M	1.0 ± 0.12	1.13 ± 0.15	1.02 ± 0.14	1.08 ± 0.12
	F	1.0 ± 0.12	1.13 ± 0.15	1.0 ± 0.14	1.1 ± 0.1
30	M	1.0 ± 0.12	1.13 ± 0.13	0.98 ± 0.13	1.05 ± 0.11
	F	1.0 ± 0.14	1.1 ± 0.14	0.97 ± 0.13	1.05 ± 0.11

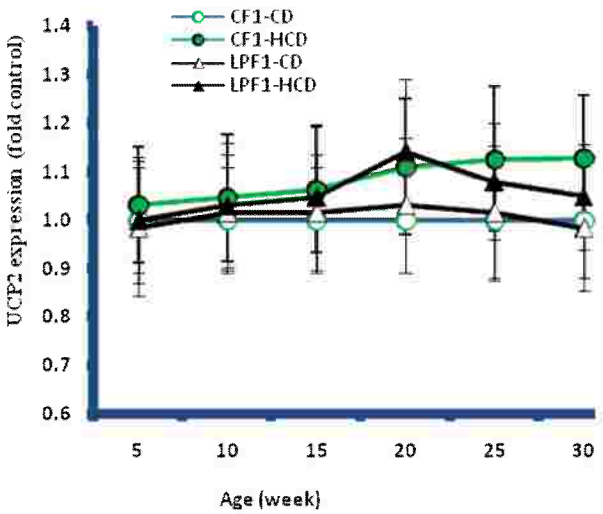


Fig (40 A) Age-dependent change of muscle UCP2 of male F1 offspring under control or HCD-diet

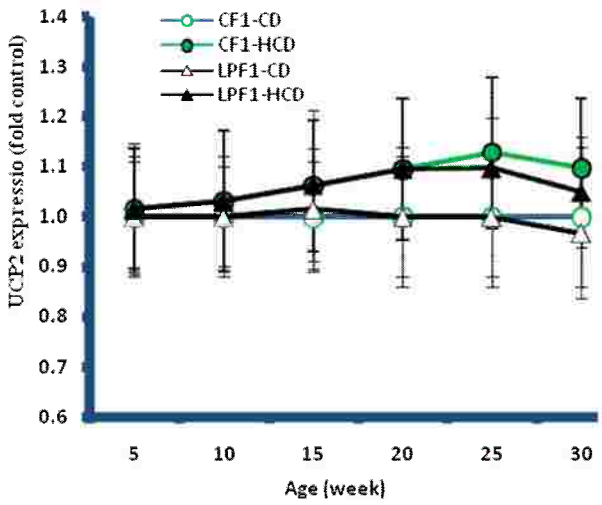


Fig (40 B) Age-dependent change of muscle UCP2 of female F1 offspring under control or HCD-diet

Data represented as mean ± SD

M: Male **F:** Female **CD:** Control diet **HCD:** High-caloric diet

CF1: Offspring of control mothers **LPF1:** Offspring of low-protein nourished mothers

Table (26) : The Adipose Tissue UCP2 (AU) of F1 male and female offspring of control and malnourished mothers under control and high caloric diet at different ages.

Age (week)	Sex	CF1-CD	CF1-HCD	LPF1-CD	LPF1-HCD
5	M	1.0 ± 0.1	1.02 ± 0.13	0.98 ± 0.14	0.96 ± 0.1
	F	1.0 ± 0.12	1.02 ± 0.13	0.98 ± 0.15	0.96 ± 0.11
10	M	1.0 ± 0.1	1.08 ± 0.14	1.04 ± 0.15	1.04 ± 0.11
	F	1.0 ± 0.11	1.02 ± 0.14	1.0 ± 0.15	0.98 ± 0.13
15	M	1.0 ± 0.11	1.1 ± 0.15	0.98 ± 0.14	1.0 ± 0.12
	F	1.0 ± 0.11	1.1 ± 0.12	0.96 ± 0.13	1.0 ± 0.12
20	M	1.0 ± 0.1	1.15 ± 0.14	0.98[b] ± 0.12	0.96[b] ± 0.12
	F	1.0 ± 0.13	1.14 ± 0.14	0.96 ± 0.12	0.98 ± 0.14
25	M	1.0 ± 0.12	1.17[a] ± 0.12	1.02 ± 0.1	0.96[b] ± 0.14
	F	1.0 ± 0.12	1.15 ± 0.12	0.98 ± 0.13	0.96[b] ± 0.14
30	M	1.0 ± 0.1	1.22[a] ± 0.1	0.98[b] ± 0.11	0.93[b] ± 0.13
	F	1.0 ± 0.12	1.22[a] ± 0.12	0.98[b] ± 0.11	0.96[b] ± 0.13

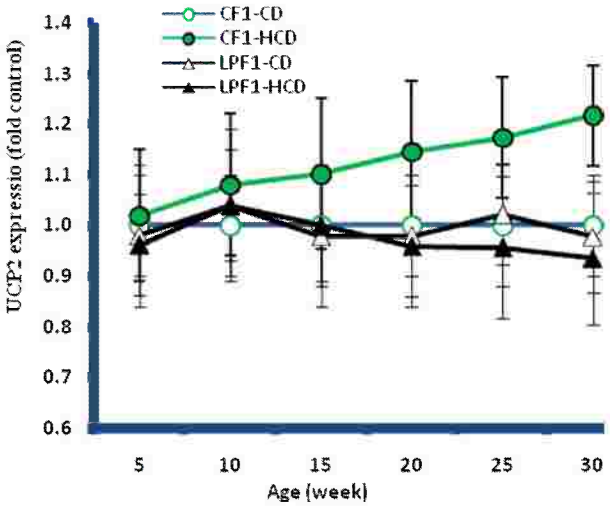


Fig (41 A)Age-dependent change of adipose tissue UCP2 of male F1 offspring under control or HCD-diet

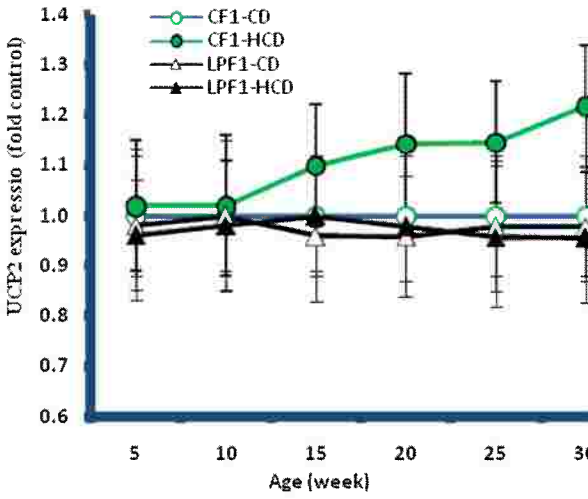


Fig (41 B) Age-dependent change of adipose tissu UCP2 of female F1 offspring under control or HC diet

Data represented as mean ± SD

M: Male **F:** Female **CD:** Control diet **HCD:** High-caloric diet

CF1: Offspring of control mothers **LPF1:** Offspring of low-protein nourished mothers

[a] *Signıficandtly dıjferent from CF1-CD group at each age by ANDOVA (p<0.05).*

[b] *Signıficandtly dıjferent from CF1-HCD group at each age by ANDOVA (p<0.05).*

Table (27) : The Hepatic UCP2 (AU) of F1 male and female offspring of control and malnourished mothers under control and high caloric diet at different ages.

Age (week)	Sex	CF1-CD	CF1-HCD	LPF1-CD	LPF1-HCD
5	M	1.00 ± 0.11	1.05 ± 0.11	0.90^{b} ± 0.11	1.08^{c} ± 0.12
	F	1.00 ± 0.11	1.06 ± 0.1	0.79^{ab} ± 0.12	0.98^{c} ± 0.1
10	M	1.00 ± 0.1	1.05 ± 0.12	0.88^{b} ± 0.13	1.10^{c} ± 0.09
	F	1.00 ± 0.12	1.06 ± 0.1	0.76^{ab} ± 0.1	1.07^{c} ± 0.14
15	M	1.00 ± 0.12	1.07 ± 0.09	0.83^{ab} ± 0.11	1.24^{abc} ± 0.11
	F	1.00 ± 0.1	1.13 ± 0.14	0.79^{ab} ± 0.12	1.14^{ac} ± 0.12
20	M	1.00 ± 0.1	1.02 ± 0.09	0.72^{ab} ± 0.1	1.10^{c} ± 0.11
	F	1.00 ± 0.12	1.07 ± 0.12	0.77^{ab} ± 0.11	1.13^{ac} ± 0.1
25	M	1.00 ± 0.09	1.05 ± 0.1	0.75^{ab} ± 0.09	1.11^{c} ± 0.12
	F	1.00 ± 0.1	1.05 ± 0.14	0.79^{ab} ± 0.12	1.17^{ac} ± 0.1
30	M	1.00 ± 0.11	1.15^{a} ± 0.12	0.80^{ab} ± 0.11	1.20^{ac} ± 0.11
	F	1.00 ± 0.15	1.06 ± 0.15	0.78^{ab} ± 0.1	1.13^{c} ± 0.14

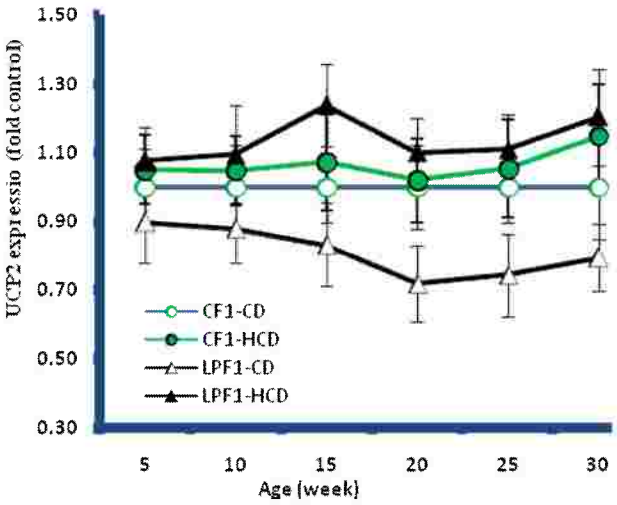


Fig (42 A)Age-dependent change of hepatic UCP2 of male F1 offspring under control or HCD-diet

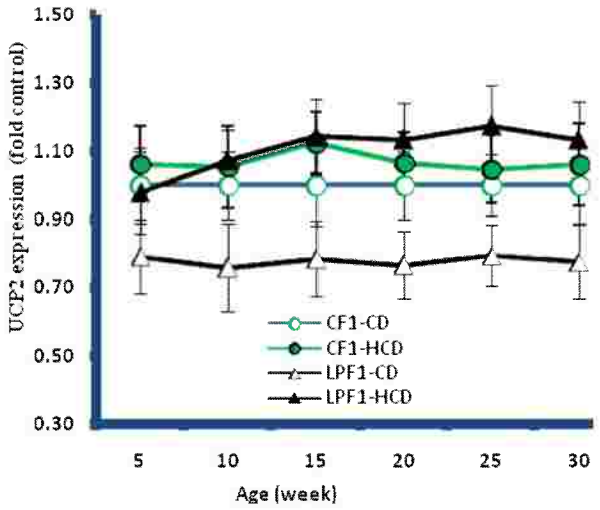


Fig (42 B)Age-dependent change of hepatic UCP2 of female F1 offspring under control or HCD-diet

Data represented as mean ± SD

M: Male **F:** Female **CD:** Control diet **HCD:** High-caloric diet

CF1: Offspring of control mothers **LPF1:** Offspring of low-protein nourished mothers

[a] *Significandtly different from CF1-CD group at each age by ANDOVA (p<0.05).*

[b] *Significandtly different from CF1-HCD group at each age by ANDOVA (p<0.05).*

[c] *Significandtly different from LPF1-CD group at each age by ANDOVA (p<0.05).*

4.8 Histopathological study

The histpathological finding for pancreatic tissues of offspring of animals groups at 30th week of age showed the islet cells are seen embedded within the acinar cells and surrounded by a fine capsule in the control offspring (CF1-CD and CF1-HCD). Also, the pancreas of offspring of LP mothers showed normal architectures with mild increased of islets volume. The pancreas of LP mothers under HCD (LPF1-HCD) showed mid dilatation of acinar cells and islets increase with a large proportion of islet cells volume as compared with control. There is very scanty proliferating B cells with basophilic cytoplasm cell and crowded left the other islet cells at one side of islet were seen (Fig. 43 A-D)

4.9 Immunohistochemical and morphometrical studies

The paraffin sections of pancreatic tissue were examined by immunohistochemical staining of insulin antibody using the avidine biotin complex (ABC) and DAB as a chromogen. The positive reaction of insulin expression appeared as brown granules scattered in the cytoplasm of β-cell in pancreatic islet and absent in nuclei of β-cell and other pancreatic cells were appeared as a blue color Fig. (45 A) .The results illustrated the mild to moderate insulin expression in offspring under CD Fig. (45 B). Moderate reaction of insulin expression in most crowded β-cell and area of blue absent reaction of pancreatic islet and acinar cells of CF1-HCD offspring. Also, strong reaction of insulin expression in all β-cell of male LPF1-HCD offspring was seen Fig. (45C)

The main core of our study was to evaluate the β-cell mass. Quantitative evaluation of positive β-cell count as well as individual(Inv) and total(T) β-cell mass were evaluated on the image of immunostaining sections, through measuring areas of insulin-positive cells, as well as those of total pancreatic cells by image J software and total pancreatic weight. Table (28) illustrated the morphometric parameters of pancreas and β-cells including total and individual β-cell areas and masses.

The present results indicated that, the weight of the pancreas has no significant difference between the studied groups at 30 weeks of age in males and female offspring. In spite of LPF1-CD and LPF1-HCD offspring have lower pancreatic weights; the differences from control offspring were not significant.

It is clear that the male offspring of control mother under control diet showed significantly higher total and total β-cell mass than female offspring but lower individual β-cell area. Also, the male offspring of LP mothers under HCD showed higher individual β-cell area and mass than female offspring.

The male offspring of all groups (CF1-HCD, LPF1-CD and LPF1-HCD) and female offspring of LPF1-HCD only showed lower total β-cell area compared to control offspring (CF1-CD). Total β-cell mass in all groups showed significant decrease compared with control irrespective to the sex. Also it is noted that the LP offspring under HCD showed significantly low total β-cell mass compared with LP under CD. Also, male offspring of LP mothers under HCD showed higher individual β-cell area and mass and lower total β-cell mass compared to control while the females showed lower individual β-cell area and total β-cell mass. (Table 28)

Table (28): Morphometeric parameters of pancreas and β-cell islets in the offspring of control and malnourished mothers under control and high caloric diet at 30 weeks of age.

	Sex	CF1-CD	CF1-HCD	LPF1-CD	LPF1-HCD
Pancreatic weight (mg)	**M**	728 ± 115	758 ± 54	710 ±61	742 ± 68
	F	710 ± 114	726 ± 111	689 ± 97	704 ± 80
Total β-cell area (μm^2)	**M**	9.62* ± 1.2	5.26^{a} ± 1.4	6.56^{a} ± 1.2	5.15^{a} ± 1.5
	F	7.65 ± 1.8	5.2± 1.8	6.28 ± 1.7	5.01^{a} ± 2.2
Individual β-cell area (μm^2)	**M**	0.282* ±0.027	0.248±0.021	0.315^{b} ±0.034	0.411^{ab*} ±0.051
	F	0.332±0.029	0.255^{a} ±0.032	0.321±0.024	0.287^{a} ±0.031
Total β-cell mass (mg)	**M**	6.17* ±0.53	3.51^{a} ±0.40	4.09^{a} ±0.45	3.35^{ac} ±0.42
	F	4.79±0.49	3.30^{a} ±0.38	3.81^{a} ±0.48	3.10^{ac} ±0.35
Individual β-cell mass (mg)	**M**	0.17±0.017	0.16±0.014	0.21^{a} ±0.032	0.21^{a*} ±0.021
	F	0.18±0.019	0.16±0.020	0.19^{b} ±0.014	0.16^{c} ±0.019

Data represented as mean ± SD

M: Male **F:** Female **CD:** Control diet **HCD:** High-caloric diet

CF1: Offspring of control mothers **LPF1:** Offspring of low-protein nourished mothers

[a] *Significandtly different from CF1-CD group at each age by ANDOVA (p<0.05).*

[b] *Significandtly different from CF1-HCD group at each age by ANDOVA (p<0.05).*

[c] *Significandtly different from LPF1-CD group at each age by ANDOVA (p<0.05).*

[*] *Significandt difference between male and female in the same group at each age by ANDOVA (p<0.05).*

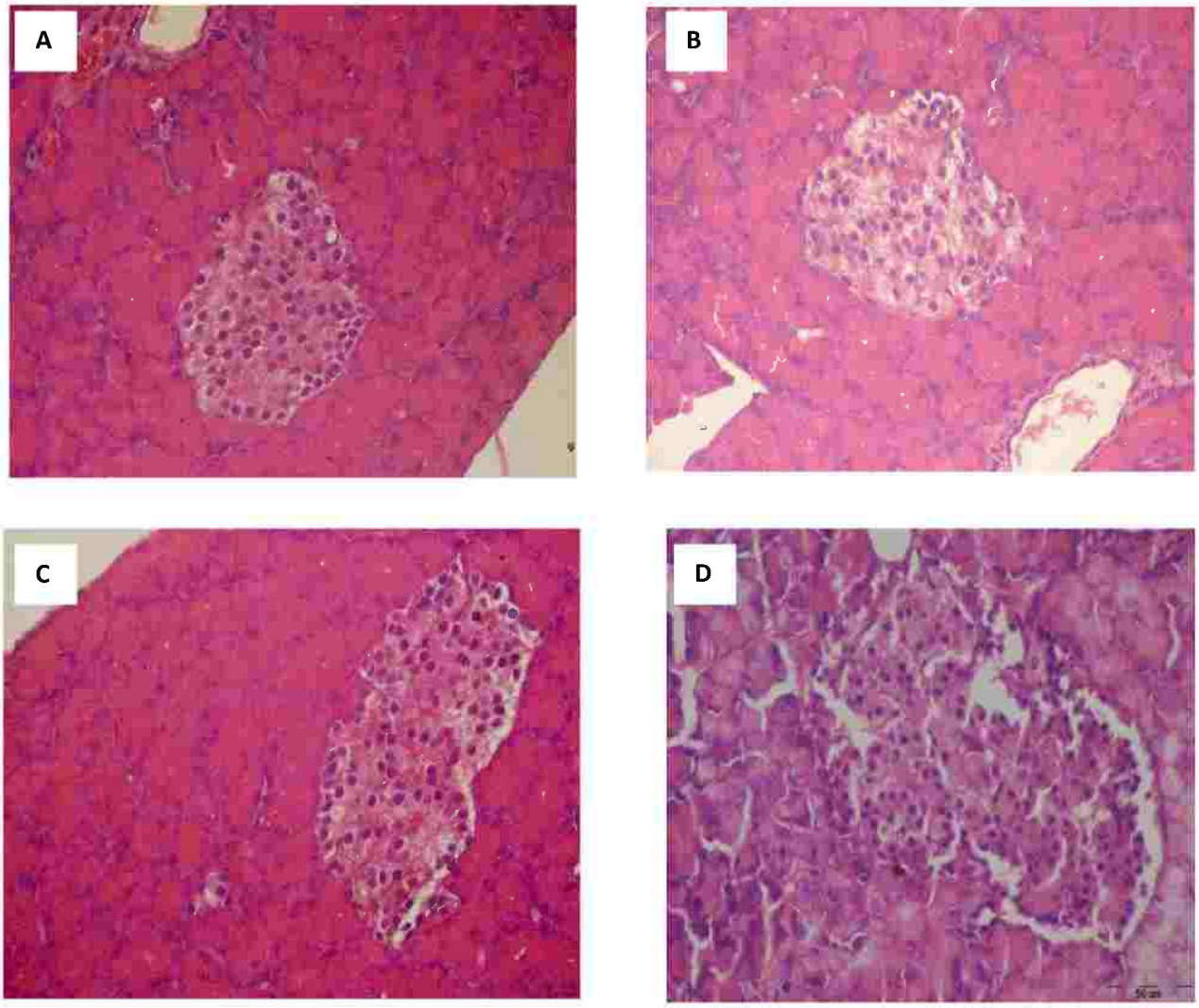


Figure (43): Paraffin sections of pancreatic rat offspring Stain by H & E (bar=50).

Notes; A: (CF1-CD) normal embedded pancreatic islets surrounded by acinar cells. **B:** (CF1-HCD) dilatation of acinar cells with undifferentiated islet cells. **C:** (LPF1-CD) pancreatic islets increase with well differentiated cells with crowded activated β-cells. **D:** (LPF1-HCD) dilatation of blood vessels and inflammatory cells in pancreatic islets with crowded activated b-cells (basophilic b-cells)

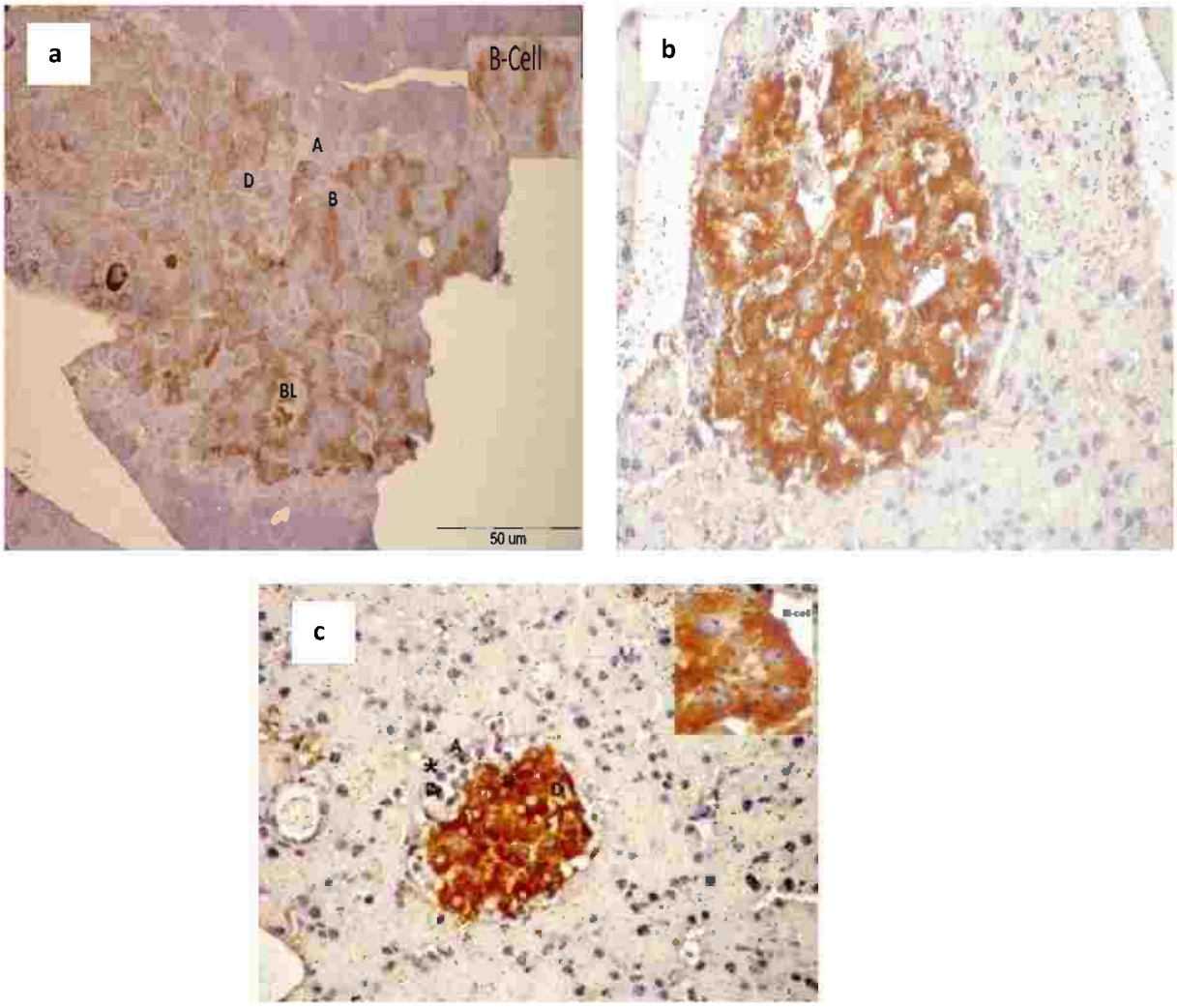


Figure (44) Paraffin sections of pancreatic rat offspring stain by Insulin &DAB(Bar=50).

Notes: **a)** normal CF1-CD animals, note the moderate expression (2+) of insulin in most B-cell of pancreatic islet. **b)** CF1-HCD note, the large islet with crowded beta cell with moderate brown granules(2+) of insulin expression in the β-cell cytoplasm and blue color area or absent insulin in area of alpha and delta cell(*) **c)** of LPF1-HCD group, increased of brown granules of insulin expression (strong activity 3+) in all β-cells (B) and absent in both alpha(A) and delta cells(D) as well as acinar cells surrounded the islet (blue color)