Bull. of Egyp. Soc. Physiol. Sci.

(Official Journal of Egyptian Society for Physiological Sciences)

Exercise and/or Liposomal Curcumin Modulates Sarcolipin/Sarcoplasmic reticulum Ca^{2+}-ATPase pump to Counteract Fibrillin/Asprosin/OLFR734 Pathway in Obese Diabetic Rats

Marwa H. Muhammad[1]*, Sherin M. Elsharkawy[1], Abeer A. Abd Elhameed[2], Esraa H. Khairat[3],Nashwa E. Ahmed[4],Reham M. Ibrahmi[1]

[1]Department of Physiology, Faculty of Medicine, Benha University, Benha 13518, Egypt.
[2]Department of Clinical Pharmacology and Therapeutics, Faculty of Medicine, Benha University, Benha, 13518, Egypt
[3]Department of Histology, Faculty of Medicine, Benha University, Benha 13518, Egypt.
[4]Medical Biochemistry & Molecular Biology Department, Faculty of Medicine, Benha University, Benha 13518, Egypt

Abstract

Background: Predominance of adiposity and diabetes is dramatically expanding. Asprosin, a novel adipokine, serve as cross-organ messenger, linking adipose tissue with skeletal muscle. Sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) pump mediates Ca^{2+} entry from sarcoplasm back to its lumen. Sarcolipin (SLN) is an uncoupler of SERCA pump, playing an important role in muscle-based thermogenesis. **Aim**: We aimed to explore the role of exercise (EX) and/or liposomal curcumin (LP-CUR) to counteract asprosin in the pathogenesis of adiposity by modulating the SLN/SERCA proteins. **Materials and method**: This study was conducted for 8 weeks through 5 Groups of rats. The control group fed normal diet. While, the others were fed high-fat diet (HFD) either alone HFD group, or with performing EX by running on treadmill at 20 m/min for 1 hour/day, 4 days/week, or received LP-CUR 10 mg/kg/day, or combination of both. **Results:** The HFD group exhibited significant dyslipidemia, hyperglycemia, insulin resistance, oxidative and sarcoplasmic reticulum stress, rise in body weight gain%, fat pad mass, and decline in lean body mass with reduced physical endurance *versus* the control group. The asprosin at both mRNA and protein levels were predominantly overexpressed in the HFD group. Fortunately, EX training and/or LP-CUR administration prevented such HFD bad consequences. They caused overexpression of soleus muscle SLN and SERCA proteins, mitochondrial biogenesis, and increased enzymes activities responsible for oxidative metabolism. The core temperature and time to exhaustion were also increased. **Conclusion:** Our study suggests EX and/or LP-CUR enhanced thermogenesis as an effective strategy to counteract obesity and related comorbidities.

Corresponding author: **Marwa Hassan Muhammad,** Physiology Department, Faculty of Medicine, Benha University, Benha, Egypt. E. mail: marwa.moslem@fmed.bu.edu.eg. Mobile: +2 01223249798.

Introduction

Adiposity and its associated negative consequences have become major epidemics worldwide. The anti-adiposity strategies are centered essentially on decreasing energy intake and/or growing energy expenditure [1]. Considering that muscle represents the largest organ (> 40% of body weight) with the limited existence of brown adipose tissue (BAT) in adult mammals, it becomes the major contributor to the energy expenditure [2]. Therefore, great attention is paid to muscle-based thermogenesis and its recruitment via exercise and/or natural products to increase oxidative metabolism as an attractive strategy to control adiposity.

The sarcoplasmic reticulum Ca^{2+}-dependent adenosine triphosphatase (SERCA) pump is a key regulator of myocyte calcium homeostasis from the sarcoplasm back to the endoplasmic reticulum (ER) lumen with adenosine triphosphate (ATP) hydrolysis [3]. Uncoupling proteins have a pivotal role in converting electrochemical energy into thermal energy without ATP generation. Sarcolipin (SLN) is a small regulatory uncoupling protein that binds to SERCA and is exclusively expressed in the skeletal muscle. SLN/SERCA interaction causes ATP hydrolysis of the pump without calcium transport into the ER lumen. Thus, SLN promotes futile cycling of the SERCA pump, augments ATP hydrolysis and heat production that eventually increases muscle thermogenesis and creates an energy-demand state [4]. SLN-based thermogenesis is energetically expensive and, consequently, could be very effective in the metabolic dyshomeostasis related to adiposity.

Since the white adipose tissue (WAT) is extremely expanded in adiposity, its endocrinal function has gained much interest. Asprosin is a novel adipokine that is dominantly expressed in WAT. It exhibits a potent role in glucose homeostasis, obesity development, and insulin resistance (IR) [5]. However, whether it can affect muscle thermogenesis is not clear. Asprosin's biological effects are mediated through interaction with the olfactory receptor 734 (OLFR734) that is expressed in olfactory epithelium, liver, and skeletal muscle [6]. Asprosin is produced by a C-terminal cleavage of the pro-fibrillin protein encoded by the Fibrillin-1 (FBN1) gene that is plentifully expressed in WAT and in mesangioblasts derived from myocytes, osteoblast-like cells, and mesenchymal stem cells, signifying the possible role of the musculoskeletal system in the regulation of asprosin [7, 8].

The sarco/endoplasmic reticulum (ER) is the principal organelle accountable for calcium homeostasis and protein synthesis and folding. Adiposity-associated free radicals and dyslipidemia, particularly the saturated free fatty acids (FFAs), are major triggers for ER stress and disruption of ER homeostasis [9]. Within the ER, glucose-regulated protein 78 (GRP78) is an important chaperone that blocks signals mediating ER stress and can maintain its functional integrity. On the contrary, the transcription factor CCAAT-enhancer-binding protein homologous protein (CHOP) is a major contributor to ER stress and dysfunctions and its surge shares in the pathogenesis of obesity and diabetes [10].

Approaches for obesity management and avoidance involve exercise (EX) training and use of naturally occurring polyphenols. Exercise

stands as the best and safest non-pharmacological strategy. Curcumin (CUR), as a safe naturally occurring polyphenol, has gained great attention. It can regulate lipid metabolism, which is the cornerstone of adiposity and its comorbidities [11], but its effect on the energy metabolism in muscle is still scarce. Fortunately, liposomes, small artificial spherical vesicles with phospholipid membrane bilayers, have been used as carrier molecules for CUR yielding into Liposomal CUR (LP-CUR). LP-CUR has been proven to be more powerful and effective with increased stability, solubility, and tissue distribution [12].

In the current work, EX was selected alone or in conjunction with LP-CUR to test their efficacy in rats fed a high-fat diet. Also, this study was intended to illustrate the role of the adipokine asprosin pathway and the sarcoplasmic reticulum functions in the pathogenesis and protection against obesity and insulin resistance. SLN-mediated uncoupling of the SERCA pump was investigated as a potential mechanism.

Materials and Method

Animals used

Male Wistar rats were used in this study. They were acquired from Veterinary Medicine Animal House, Benha University (Egypt). They were housed in metallic cages at the prevailing atmospheric conditions and room temperature of about 25°C.The experimental diets and drinking water were ad libitum. Rats were acclimatized to the environmental conditions for one week before the experiment. The experimental procedures, animal handling, sampling, and scarification were done in accordance with the Guide for the Care and Use of Laboratory Animals, Eighth Edition. The protocol of this study was revised and

approved by the Ethical Committee of the Care and Use of Experimental Animals, Faculty of Medicine, Benha University, Egypt (REC- No. 11-9-2023).

The experimental diets, normal diet (ND) and High-fat diet (HFD) composition and ingredients were as constructed by Gheit and coauthors [13] and shown in Table 1

LP-CUR solution was prepared at the National Research Institute of Egypt in by the procedure of Khater and coauthors [12].

Aerobic exercise training protocol

A rat's treadmill (Panlab/Letica LE8706, Barcelona, Spain) was used. The rats were made to run at 20 m/min for 1 hour/day, 4 days/week, for 8 weeks on the treadmill without incline [14].

Experimental groups and procedure

Thirty-five male Wistar rats were used. They were divided into 5 groups ($n = 7$) as follows:

Group 1: The control group was fed a ND and was given distilled water by oral gavage for 8 weeks. Group 2: The HFD group was fed a HFD for 8 weeks [15]. Group 3: HFD + EX group; HFD-fed rats were made to run at 20 m/min for 1 hour/day, 4 days/week, for 8 weeks [14]. Group 4: HFD + LP-CUR group, HFD-fed rats were given LP-CUR, 10 mg/kg once daily by oral gavage for 8 weeks [12].Group 5: HFD + LP-CUR + EX group, HFD-fed rats that were given LP-CUR, 10 mg/kg once daily and performed aerobic EX training by running at 20 m/min for 1 hour/day, 4 days/week for 8 weeks.

Throughout the experimental period, food consumption and rectal temperature were assessed. **At the end of the 8th week,** a **swimming-until-exhaustion exercise test** was performed to evaluate skeletal muscle performance. A

cylindrical swimming pool (60 x 20 cm) with water 40 cm deep maintained at 27 ^{o}C was used. The rat was forced to swim to exhaustion with steel rings load attached to the tail root matching to 5% of its body weight. Each rat was considered exhausted when it failed to raise its face to the water surface within 5 seconds. The time from beginning to exhaustion was recorded. The longer the time to exhaustion, the better the endurance performance of each rat was [16].Just after the exhaustive exercise, a blood sample from the tail vein was taken to measure **the blood lactate** level using Lactate Pro 2 (Arkray, Japan).

- The animals were then subjected to overnight fasting and 24 hours rest to avoid the acute exercise effects on the assessed parameters. The rats were weighed and then anesthetized by pentobarbital sodium (40 mg/kg/i.p) followed by measurement of the animal length from the base of the lower incisors to the anus by a measuring tape. The rats were then decapitated. An anterior longitudinal skin incision from neck to tail was performed. Blood samples were collected by cardiac puncture. Then the entire skin of fur and subcutaneous fat was detached and weighed. The heart, kidneys, and liver were carefully removed and weighed. Then all fat pad compartments such as inguinal and retroperitoneal were removed and weighed [17].

Blood and tissue sampling

The collected blood samples were left to be clotted and centrifuged (3000 revolutions per minute (rpm) for 15 min) then the serum was separated and stored at − 80 ^{o}C to be analyzed for serum asprosin, insulin, glucose, and lipid profile. After tissue weighting, the soleus muscle was removed from the right hind limb together withthe inguinal

WAT. They were immediately frozen in liquid Nitrogen, and stored at − 80 °C for genes, proteins, and enzyme activities analysis. The soleus from the left hind limb was kept in 10 % formaldehyde for histopathological evaluation.

I- Anthropometric parameters

Rectal temperature assay

The rectal body temperature (^{o}C) was measured once/a week with a rodent thermometer (BIO-TK8851, Bioseb Lab, Vitrolles, France), to assess the core body temperature. The probe was inserted approximately 3 cm from the anus into the rectum [17]

Calculation of food intake

The consumed food was calculated the difference between the weights of food supplied and remained (g) divided by the number of rats/cage to obtain the average consumption value for each rat [17].

Calculation of body weight gain percent (BWG %)

The initial and final body weight (BW) of each rat was recorded, then the % of BW gained was calculated as follow; Final BW – Initial BW x 100 divided by the initial BW [18]

Calculation of body mass index (BMI)

Body mass index (BMI) for each rat was calculated from this equation: BMI $(gm/cm^2) =$ Final BW (g)/ (length)^2(the measure from the base of the lower incisors to the anus)^2. It is a marker of adiposity [19].

Lean (fat-free) body mass (LBM)

LBM is the best interpreter of resting energy expenditure. It equals the mass of the residual cadaver consisting of a musculoskeletal portion (skeletal muscle, bones, and the skinned head)

added to the weight of the liver, heart, and kidneys [19].

II- Biochemical analysis

Measurement of serum asprosin protein

A rat asprosin ELISA kit obtained from Mybiosourse (Cat NO. MBS1600686, CA, USA) was used according to the manufacturer's instructions. The absorbance was determined spectrophotometrically at 450 nm, after adding the stop solution, in a microplate reader. Quantitative results were calculated by standard curves and expressed as ng/mL.

Measurement of soleus muscle SLN and SERCA proteins

A portion (300-500 mg) of soleus muscle was homogenized in 500 ul PBS on ice. The homogenates were then centrifuged for 15 minutes at 5000 rpm. The supernatant was removed. Rat SLN ELISA Kits (Cat No abx156065, abbexa company, Cambridge, UK) and Rat SERCA EISA Kits purchased from MYBIOSOURSE (Cat NO. MBS72555556, CA, USA) were utilized. The assessment protocol was done according to the manufacturer instructions.

Measurement of serum glucose and insulin levels

Serum glucose was measured using diagnostic kits obtained from (Biodiagnostics Co, Cairo, Egypt)[20], while serum insulin was assessed using ELISA kits obtained from (BioVendo Brno, Czech) [21]. Theinsulin resistance (IR) and insulin sensitivity indices were calculated as follow:

Homeostasis model assessment of insulin resistance (HOMA-IR) is a well-documented marker for IR. It was calculated according to the following formulaHOMA-IR = fasting glucose (mg/dl) × fasting insulin (μU/mL) / 405)[22].

Quantitative insulin-sensitivity check index (QUICKI) is taken as a measure for insulin sensitivity. It was indicated by the inverse log sum of fasting insulin (μU/ml) and fasting glucose (mg/dl)[23].

Serum lipids profile assessment

Triglycerides (TGs), total cholesterol (TC), and high-density lipoprotein-cholesterol (HDL) enzymatic colorimetric assay kits (Diamond Diagnostic, Cairo, Egypt) were used[24]. Very low-density lipoprotein-cholesterol (VLDL) was calculated as follows: VLDL = TGs/5 while, LDL was ascertained according to the equation of **Friedewald and coauthors [25]:** LDL (mg/ dL) =TC - HDL - (TGs/5).Free fatty acids (FFAs) were measured by enzymatic colorimetric methods, as described by **Foster and Dunn [26]**, using commercially available kits (Diamond Diagnostics Company, Egypt).

Evaluation of serum oxidative stress-related parameters

The serum colorimetric enzyme activity of catalase [27] and superoxide dismutase (SOD)[28] was done using kits from (Cayman Chemical Co., Ann Arbor, MI, USA). In addition, Lipid peroxidation malondialdehyde (MDA) assay kits (BML-AK171, Enzo Life Science, NY, USA) were utilized as mentioned by Gérard-Monnier and collaborators [29].

Assessment of soleus Citrate synthase (CS) and Succinate dehydrogenase (SDH) enzyme activity

A portion of soleus muscle removed from the right hind limbs was homogenized (using Precellys24Tissue Homogenizer, Bertin Technologies, France) in a solution of, 50 mM Tris–HCl, at pH 7.4 containing 0.01 mM

phenylmethylsulphonyl fluoride and 1 mM EDTA. The homogenate was then used.

CS, as a marker of mitochondrial function, was assayed by spectrophotometric detection of reduced thionitrobenzoic acid, from the soleus muscle, using (BTS 350 semiautomatic analyzer, Spain). CS activity was reported as nmol/min/mg protein. SDH, as an indicator of the oxidative metabolic capacity of the skeletal muscle was also determined. The procedure was as previously described by Gheit and coauthors [13].

III- Quantitative estimation of relative gene expressions by real-time reverse transcription PCR (RT-PCR)

A total RNA isolation kit (Qiagen, Hiden, Germany) was utilized for extraction of total RNA from the frozen portion of inguinal WAT for FBN1 gene and the soleus skeletal muscle for the OLFR734, peroxisome proliferator activated receptor gamma coactivator-1 alpha (PGC1α) as a marker for mitochondrial biogenesis, and the uncoupling protein 1 (UCP1) genes according to the manufacturer's protocols. SuperScript ® III First-Strand Synthesis System for RT-PCR kit (Life Technologies) was used after the first step to form the first strand. Power SYBR Green PCR Master Mix (Life Technologies) was used to carry out PCR reactions. Target genes mRNA expression was assayed relative to the housekeeping gene, Glyceraldehyde 3-phosphate dehydrogenase (GAPDH).Relative gene expression was automatically calculated using the comparative threshold (Ct) method for the values of the target and the reference genes using the $2^{-\Delta\Delta CT}$ formula by using specific software (Qiagen, Valencia, CA, USA).The primer sequences of the assessed genes are as follows:FBN1F:GAGTGTGAACTGAGCGCGA

and R:AGGCACACTCGTACTTCCCA, OLFR 734F: ACCTGCAAATGATACCACCGT and R: TCCGGACAACCTGAGTGATG, PGC-1αF: GAGAACAAGACTATTGAGCGAAC and R: GTGGAGTGGCTGCCTTGGGT, UCP1 F:ACTGCCACACCTCCAGTCATTand R: CTTTGCCTCACTCAGGATTGG,and GAPDH F:GGTGAAGTTCGGAGTCAACGGAand R: GAGGGATCTCGCTCCTGGAAGA[6, 30].

IV- Western blotting analysis for ER stress-related markers CHOP and GRP78 proteins

Proteins expression of CHOP and GRP78 were determined by using Western blotting analysis. The antibody used were anti-CHOP (#D46F1, Cell signaling, Baverly, MA, USA),anti-GRP78 (#11587-1-AP, Proteintech, San Diego, CA, USA), and anti-β actin (housekeeping protein) (#sc-47778, Santa Cluz Biotechnology, Texas, USA).The Bio-Rad Inc. ReadyPrepTM protein extraction kit (Catalogue #163-2086) was used for soleus muscle tissue proteins extraction. Proteins were then electrophoretically transferred topolyvinylidenedifluoride membranes and probed with CHOP and GRP78 antibodies overnight at 4 °C after blocking in 5% non-fat milk in Tris-buffered saline with Tween-20. Secondary peroxidase-coupled antibodies were conjugated and a chemiluminescent substrate system was used to detect signals. Image analysis software was used to read the band intensity of the target proteins against control sample β -actin by protein normalization on the ChemiDoc MP imager (Biorad, Hercules, CA, USA).

V- Histopathological evaluation of the soleus muscle

The excised left soleus muscles were fixed in a 10% neutral buffered formalin solution for 24 hours, embedded in paraffin, sectioned at 5 μm thickness, and stained with hematoxylin and eosin

(H&E).Slides were digitized using Olympus® digital camera installed on Olympus® microscope with 1/2 X photo adaptor, using 20 X objective. The result images were analyzed on Intel® Core I7® based computer using Video Test Morphology® software (Russia) with a specific built-in routine for area measurement and object counting and geometrical analysis. Fiber area and number of nuclei per fiber were assessed.

VI- Statistical analysis

For statistical analysis, SPSS for Windows (Version 20.0; SPSS Inc., Chicago, Illinois) was employed. In order to assess the significance of differences between more than two groups, One-way analysis of variance (ANOVA) was utilized. If it showed a significant difference, LDS post hoc test was used to analyze the differences within each group. Results are given as the mean ± standard deviation (SD) of the mean. p value of 0.05 or less was regarded as significant. Pearson's correlation coefficient (r) 2-tailed test was used to examine the correlations between the parameters. A p-value ≤ 0.05 was considered statistically significant.

Results

The effect of HFD, EX, and LP-CUR on anthropometric measurements (Table 2)

BWG% reported in the HFD group was significantly raised to 112.6% compared to 33.3% in the control group. It was linked with a significant increase in the BMI in contrast to the BMI in control group ($P < 0.05$). Also, the HFD-fed rats group exhibited a significant increase in the food intake with respect to the control group ($P < 0.05$). Similarly, a rise in the fat pad mass (42%) at the expense of reduced LBM (56%) was recorded in the HFD group to a level that is comparable to that of the control group (15% and 82.5%, respectively) ($P < 0.05$).

EX training and/or LP-CUR administration along with HFD ingestion had a conservative impact against such HFD-associated changes; there were observable declines in the BWG%, BMI, food intake, and fat pad mass with an evident rise in LBM in comparison to the HFD group ($P < 0.05$). The combined EX and LP-CUR regimen presented better findings, with an obvious increase in the LBM of 85% at the expense of fat pad mass of 13% that was significant to their corresponding (82.5% and 15%, respectively) in the control group ($P < 0.05$).

The effect of HFD, EX, and LP-CUR on glucose homeostasis (Table 3)

The HFD-fed group exhibited significant hyperglycemia and hyperinsulinemia, as well as an increase in the insulin resistance index HOMA-IR *versus* the normal diet-fed control group ($P < 0.05$). Furthermore, there was a statistically significant reduction in peripheral insulin sensitivity as indicated by the decline in QUICKI index in the HFD group *versus* the control ($P < 0.05$).

The EX training and/or LP-CUR beside HFD ingestion preserved the glucose homeostasis state. There were significant decreases in the serum glucose, insulin, and HOMA-IR when compared to the HFD group ($P < 0.05$), with much lower values in the combined management. The insulin sensitivity state was substantially improved, and the QUICKI index increased and became closer to the normal value in the HFD + EX, HFD + LP-CUR, and HFD + LP-CU+ EX groups ($P < 0.05$).

Effect of HFD, EX, and LP-CUR on serum lipid profile (Table 4)

The lipid profile in the HFD group revealed dyslipidemia compared to the control group. There were observable increases in serum TGs, TC, LDL, and VLDL, but significant decrease in the

protective HDL levels ($P < 0.05$). On the other hand, intervention by EX training and/or LP-CUR administration significantly prevented such HFD-associated dyslipidemia ($P < 0.05$) since the previous parameters were reversed in comparison to the HFD group.

The effect of HFD, EX, and LP-CUR on oxidative stress-related markers (Table 5)

In the HFD group, there was a significant increase in the lipid peroxidation as indicated by MDA levels when compared to control group ($P < 0.05$). Furthermore, EX training and/or LP-CUR administration to HFD-fed rats tended to decrease lipid peroxidation levels compared to the HFD group ($P < 0.05$). Moreover, serum SOD and catalase antioxidant activities were significantly reduced in the HFD group *versus* the control one ($P < 0.05$). Fortunately, EX training or LP-CUR along with HFD ingestion preserved the antioxidant status and caused an observable rise in the SOD and catalase activities compared to HFD group ($P < 0.05$). Combined regimen with HFD recorded higher antioxidant vales compared to either EX or LP-CUR alone regimen with HFD ($P < 0.05$).

Table 1 Composition of the experimental diets

Nutrient composition and ingredients (grams per kilogram) of the experimental diets		
Contents(g/kg diet)	**ND**	**HFD**
Cornstarch	620.7	250.7
Casein (≥85% protein))	140.0	190.0
Sucrose	100.0	100.0
Soybean oil	40.0	40.0
Fiber	50.0	50.0
Lard	-------	320.0
AIN- 93 Mineral mix	35.0	35.0
AIN- 93 Vitamin mix	10.0	10.0
Choline	2.5	2.5
Antioxidant	0.008	0.008
L-Cystin	1.8	1.8
Energy intake/gm of diet	3.573	5.404

ND; normal diet, HFD; high-fat diet [13]

Table 2 Anthropometric parameters in the experimental groups

Parameters	Experimental groups				
	Group 1 Control	Group 2 HFD	Group 3 HFD+EX	Group 4 HFD+LP-CUR	Group 5 HFD+LP-CUR+EX
Initial BW (g)	198.29 ± 5.4	198.0 ± 2.4	198.1 ± 4.5	198.2 ± 4.6	197.9 ± 3.6
Final BW (g)	264.1 ± 10.1	425.1 ± 10.6 [a]	294.3 ± 9.7 [a, b]	298.7 ± 6.8 [a, b]	275.1 ± 3.5 [a,b,c,d]
BWG (%)	33.3 ± 5.2	112.6 ± 8.2 [a]	49.8 ± 5.2 [a, b]	50.8 ±4.7 [a, b]	40.2 ± 4.1 [a,b,c,d]
Food intake (g/day/rat)	11.99 ± 0.54	32.5 ± 2.5 [a]	18.71± .53 [a, b]	18.95±.38 [a, b]	15.01± 1.3 [a,b,c,d]
BMI (g/cm^2)	0.49 ± .03	0.88 ± .11 [a]	0.60 ± .04 [a, b]	0.64± .07 [a, b]	0.57± .03 [a,b,d]
Fat pad mass (g)& % from final BW	39.62 ±1.5 (15%)	178.56 ± 4.4 [a] (42%)	85.34± 2.8 [a, b] (29%)	89.2 ± 2.8 [a, b, c] (29%)	35.77 ± .45 [a,b,c,d] (13%)
LBM (g)& its % from final BW	217.92 ± 8.3 (82.5 %)	238.08 ± 5.9 [a] (56%)	206.0 ± 6.8 [a, b] (70%)	206.1± 4.7 [a, b] (70%)	233.9± 2.9 [a,c,d] (85%)

Data are expressed as mean ± standard deviation SD; $n = 7$, [a] $P < 0.05$ significant difference compared with the Group 1; [b] $P < 0.05$ significant difference compared with the Group 2; [c] $P < 0.05$ significant difference compared with Group 3; [d] $P < 0.05$ significant difference compared with the Group 4. HFD; High-fat diet, EX; Exercise, LP-CUR; Liposomal curcumin, BW; body weight, BWG; body weight gain percentage, BMI; body mass index, LBM; lean (fat-free) body mass

Table 3 Glucose homeostasis-related parameters in the experimental groups

Parameters	Experimental groups				
	Group 1 Control	Group 2 HFD	Group 3 HFD+EX	Group 4 HFD+LP-CUR	Group 5 HFD+LP-CUR+EX
Glucose (mg/dl)	90.57 ± 6.6	190.3 ± 7.6^a	$136.7 \pm 5.6^{a, b}$	$138.8 \pm 7.4^{a,b}$	$97.4 \pm 1.7^{a,b,c,d}$
Insulin (μU/mL)	$7.87 \pm .68$	$18.0 \pm .67^a$	$10.2 \pm .39^{a, b}$	$10.69 \pm .68^{a,b}$	$8.6 \pm .38^{a,b,c,d}$
HOMA-IR	$1.76 \pm .27$	$8.47 \pm .63^a$	$3.44 \pm .18^{a, b}$	$3.67 \pm .33^{a, b}$	$2.08 \pm .10^{b,c,d}$
QUICKI	$0.50 \pm .01$	$0.43 \pm .00^a$	$0.46 \pm .00^{a, b}$	$0.46 \pm .00^{a, b}$	$0.49 \pm .00^{a,b,c,d}$

Data are expressed as mean ± standard deviation SD; *n* = 7, [a]P < 0.05 significant difference compared with the Group 1; [b] P < 0.05 significant difference compared with the Group 2; [c]P < 0.05 significant difference compared with Group 3; [d]P < 0.05 significant difference compared with the Group 4. HFD; High-fat diet, EX; Exercise, LP-CUR; Liposomal curcumin,HOMA-IR, homeostasis model assessment of insulin resistance, QUICKI; Quantitative insulin-sensitivity check index

Table 4 Lipid profile in the experimental groups

Parameters	Experimental groups				
	Group 1 Control	Group 2 HFD	Group 3 HFD+EX	Group 4 HFD+LP-CUR	Group 5 HFD+LP-CUR+EX
TGs (mg/dl)	35.29 ± 3.4	119.57 ± 5.3^a	$57.28 \pm 4.3^{a, b}$	$67.86 \pm 4.8^{a,b,c}$	$42.29 \pm 2.7^{a,b,c,d}$
TC (mg/dl)	48.0 ± 3.2	125.2 ± 1.5^a	$79.4 \pm 4.2^{a,b}$	$95.8 \pm 3.1^{a,b,c}$	$68.4 \pm 3.9^{a,b,c,d}$
LDL (mg/dl)	15.3 ± 2.5	89.1 ± 4.2^a	$39.0 \pm 2.9^{a,b}$	$54.7 \pm 4.4^{a,b,c}$	$22.8 \pm 2.5^{a,b,c,d}$
VLDL (mg/dl)	$7.05 \pm .67$	23.9 ± 1.08^a	$11.5 \pm .87^{a,b}$	$13.6 \pm .96^{a,b,c}$	$8.45 \pm .53^{a,b,c,d}$
HDL (mg/dl)	29.7 ± 2.1	14.57 ± 1.72^a	$23.3 \pm 3.14^{a,b}$	$22.5 \pm 1.39^{a,b}$	$26.8 \pm 3.13^{a,b,c,d}$

Data are expressed as mean ± standard deviation SD; *n* = 7, [a] P < 0.05 significant difference compared with the Group 1; [b] P < 0.05 significant difference compared with the Group 2; [c] P < 0.05 significant difference compared with Group 3; [d] P < 0.05 significant difference compared with the Group 4. HFD; High-fat diet, EX; Exercise, LP-CUR; Liposomal curcumin, TGs; Triglycerides, TC, Total cholesterol, LDL, Low-density lipoprotein-cholesterol; VLDL; very low-density lipoprotein-cholesterol, and HDL; high-density lipoprotein-cholesterol

Table 5 Oxidative stress-related parameters in the experimental groups

Parameters	Experimental groups				
	Group 1 Control	Group 2 HFD	Group 3 HFD+EX	Group 4 HFD+LP-CUR	Group 5 HFD+LP-CUR+EX
MDA (nmol/l)	$5.01 \pm .56$	$10.7 \pm .56^a$	$7.9 \pm .41^{a, b}$	$8.4 \pm .45^{a, b}$	$5.53 \pm .40^{b,c,d}$
SOD (pg/ml)	64.8 ± 3.38	21.0 ± 2.16^a	$39.5 \pm 3.40^{a, b}$	$41.1 \pm 1.57^{a, b, c}$	$61.6 \pm 1.9^{a,b,c,d}$
Catalase (IU/L)	54.9 ± 2.96	17.0 ± 2.16^a	$30.3 \pm 2.69^{a, b}$	$32.5 \pm 2.63^{a, b}$	$50.2 \pm 3.74^{a,b,c,d}$

Data are expressed as mean ± standard deviation SD; *n* = 7, [a] P < 0.05 significant difference compared with the Group 1; [b] P < 0.05 significant difference compared with the Group 2; [c] P < 0.05 significant difference compared with Group 3; [d] P < 0.05 significant difference compared with the Group 4. HFD; High-fat diet, EX; Exercise, LP-CUR; Liposomal curcumin, MDA; malondialdehyde, and SOD; superoxide dismutase

Effect of HFD, EX, and LP-CUR on densitometric expression of ER stress-related proteins, CHOP and GRP78 (Fig.1)

Comparing the HFD group to the control group, there was noticeably higher levels of densitometric expression of CHOP, but a substantial decline in GRP78 ($P < 0.05$). The HFD + EX, HFD + LP-CUR, and HFD + LP-CU+ EX groups had significantly lower levels of CHOP while, significantly higher levels of GRP78 ($P < 0.05$). Also, better values were observed in the combined EX + LP-CUR regimen with HFD-fed group compared to either EX or LP-CUR alone regimen with HFD ($P < 0.05$).
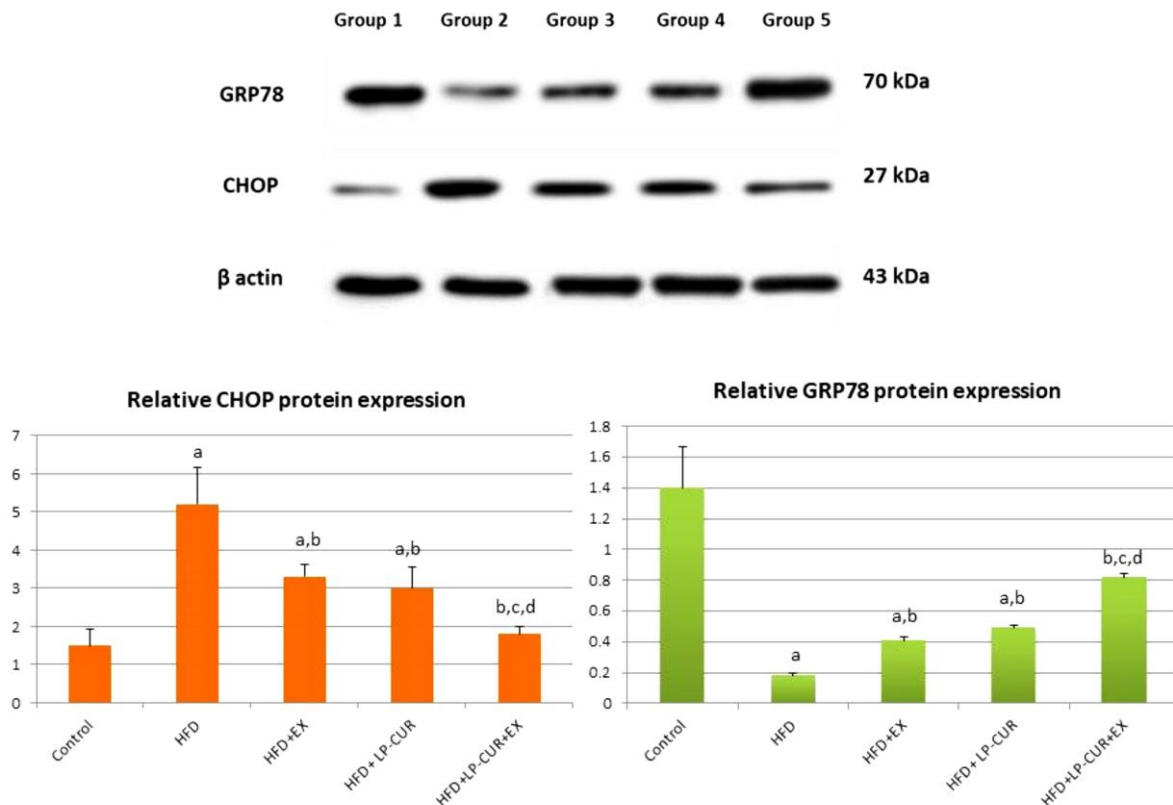
Figure 1 Western blotting with densitometric quantification of CHOP and GRP78 expression in soleus muscle
Representative blots of CHOP and GRP78 protein expression in soleus muscle with their densitometric quantifications. Data are expressed as mean ± standard deviation SD; $n = 7$, [a] P < 0.05 significant difference compared with the Group 1; [b] P < 0.05 significant difference compared with the Group 2; [c] P < 0.05 significant difference compared with Group 3; [d] P < 0.05 significant difference compared with the Group 4. HFD; High-fat diet, EX; Exercise, LP-CUR; Liposomal-curcumin, CHOP, CCAAT-enhancer-binding protein homologous protein; GRP78, Glucose-regulated protein 78

The effect of HFD, EX, and LP-CUR on FBN1/asprosin/OLFR734 pathway (Fig.2 and Table 6)

Interestingly, the HFD group exhibited an up-regulation in the asprosin pathway. There was a rise in the serum asprosin level (Table 6) in line with a significant increase in its synthesizing gene FBN1 (Fig. 2A) in the WAT ($P < 0.05$). Furthermore, the asprosin-targeted receptor OLFR734 (Fig. 2B) was substantially up-regulated in soleus muscle of HFD group with respect to the control one ($P < 0.05$).

EX training and LP-CUR considerably suppressed the asprosin pathway. Significant lower levels of serum asprosin, WAT FBN1, and soleus OLFR734 were documented in the HFD + EX, HFD + LP-CUR, and HFD + LP-CU+ EX groups in comparison to the HFD group ($P< 0.05$). Moreover, much lower values were recorded in the combined EX and LP-CUR regimen with respect to the single one ($P< 0.05$).

Effect of HFD, EX, and LP-CUR on SLN and SERCA proteins (Table 6)

To explore the prospect of EX and LP-CUR as a strategy for increasing thermogenesis and energy expenditure in skeletal muscle, we investigated the SLN/ SERCA levels. Our data revealed that soleus muscle SLN and SERCA protein levels were greatly suppressed in HFD group when compared to the control group ($P < 0.05$). With respect to the HFD group, EX training or LP-CUR ingestion with HFD groups prevented such decline and exhibited significant increases in their levels ($P < 0.05$).In Addition, more increase was observed in the combined regimen HFD + LP-CUR + EX group in relation to the only one regimen ($P < 0.05$).

Effect of HFD, EX, and LP-CUR on soleus muscle mitochondrial markers (Table 7 and Fig. 2)

Our findings suggested that HFD ingestion reduced indices of muscle mitochondrial biogenesis and activity, while EX and LP-CUR improved them significantly ($P < 0.05$).To explore the underlying signal transduction involved in such effect, the mRNA expression levels of the transcriptional regulator of mitochondrial content and biogenesis, PGC1α, and the mitochondrial uncoupler thermogenic marker, UCP1 were assessed in addition to the CS activity as a marker for mitochondrial functions. HFD-fed group exhibited significant down-regulation in mRNA expression levels of PGC1α and UCP1 (Fig. 2C & 2D), as well as reduced the CS activity (Table 7) in comparison to the control ($P < 0.05$). On the contrary, EX or LP-CUR, although of concomitant HFD ingestion, displayed marked up-regulation in their expressions and CS activity *versus* HFD group ($P < 0.05$). Their combination improved these parameters further more ($P < 0.05$).

Effect of HFD, EX, and LP-CUR on the soleus muscle SDH activity, serum FFA, and blood lactate (Table 7)

To explore the muscle substrate utilization preference, fuel handling, during HFD ingestion and EX or LP-CUR management, we investigated SDH activity and FFAs levels (indicators for the oxidative metabolic capacity of the skeletal muscle) in addition toblood lactate (a biochemical parameter related to anaerobic glycolysis and fatigue).

The HFD considerably decreased SDH activity in line with significant increase in the serum FFAs and blood lactate levels compared to the normal diet in the control group ($P < 0.05$), indicating lower aerobic and enhanced anaerobic capabilities. On the contrary, the EX training or LP-CUR administration along with the HFD ingestion significantly prevented such changes and enhanced SDH activity, reduced FFAs and blood lactate levels with respect to the HFD group ($P < 0.05$). These findings indicated a shift toward higher oxidative potential and less reliance on anaerobic glycolysis. The combination of both produced the more significant effect than single interference ($P < 0.05$).
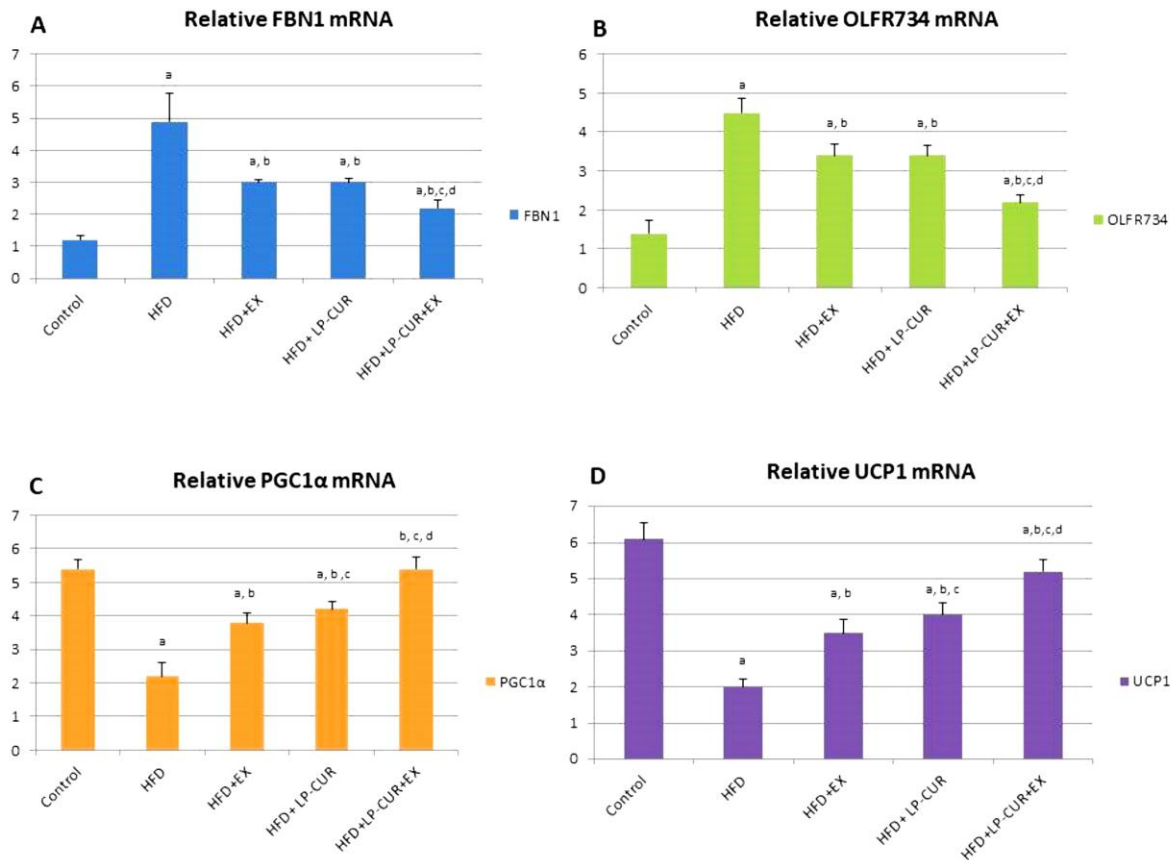
Figure 2 Relative mRNA expressions of FBN1, OLFR734, PGC1α, and UCP1 in the experimental groups

Data are expressed as mean ± standard deviation SD; $n = 7$, [a] $P < 0.05$ significant difference compared with the Group 1; [b] $P < 0.05$ significant difference compared with the Group 2; [c] $P < 0.05$ significant difference compared with Group 3; [d] $P < 0.05$ significant difference compared with the Group 4. HFD; High-fat diet, EX; Exercise, LP-CUR; Liposomal-curcumin, FBN1; Fibrillin, OLFR734, olfactory receptor 734; PGC1α, peroxisome proliferator activated receptor gamma coactivator-1 alpha; UCP1, uncoupling protein 1

Table 6 Serum asprosin and soleus muscle SLN and SERCA proteins levels in the experimental groups

Parameters	Experimental groups				
	Group 1 Control	Group 2 HFD	Group 3 HFD+EX	Group 4 HFD+LP-CUR	Group 5 HFD+LP-CUR+EX
Asprosin (ng/ml)	5.94 ± .22	18.8 ± .71[a]	12.0 ± .34[a,b]	12.6 ±.40[a, b, c]	7.99 ±.38[a,b,c,d]
SLN (ng/ml)	8.42 ± .62	3.31 ±.32[a]	5.82 ±.38[a, b]	5.24 ± .44[a, b, c]	7.27 ±.52[a,b,c,d]
SERCA (ng/ml)	7.22 ± .41	2.36 ±.21[a]	4.71 ± .26[a, b]	4.38 ± .25[a, b, c]	5.14 ± .21[a,b,c,d]

Data are expressed as mean ± standard deviation SD; $n = 7$, [a] $P < 0.05$ significant difference compared with the Group 1; [b] $P < 0.05$ significant difference compared with the Group 2; [c] $P < 0.05$ significant difference compared with Group 3; [d] $P < 0.05$ significant difference compared with the Group 4. HFD; High-fat diet, EX; Exercise, LP-CUR; Liposomal curcumin, SLN; Sarcolipin, SERCA; Sarco/endoplasmic reticulum Ca^{2+}-ATPase,

Table 7 Soleus muscle CS and SDH enzyme activities, serum FFA, and blood lactate levels in the experimental groups

Parameters	Experimental groups				
	Group 1 Control	Group 2 HFD	Group 3 HFD+EX	Group 4 HFD+LP-CUR	Group 5 HFD+LP-CUR+EX
CS (nmol/min/mg protein)	315.4 ± 11.5	199.4 ± 6.7^{a}	$241.7 \pm 7.4^{a,b}$	$242.5 \pm 7.5^{a, b}$	$309.3 \pm 3.3^{b,c,d}$
SDH (nmol/min/mg protein.	$4.55 \pm .62$	$1.71 \pm .16^{a}$	$3.67 \pm .63^{a, b}$	$3.45 \pm .48^{a, b}$	$4.46 \pm .31^{b,c,d}$
FFA (mEq/L)	$0.22 \pm .03$	$1.1 \pm .05^{a}$	$0.36 \pm .02^{a, b}$	$0.40 \pm .03^{a, b}$	$0.25 \pm .03^{b,c,d}$
Blood lactate (mmol/L)	$9.33 \pm .49$	$13.7 \pm .43^{a}$	$11.4 \pm .37^{a,b}$	$11.6 \pm .26^{a, b}$	$9.37 \pm .32^{b,c,d}$

Data are expressed as mean ± standard deviation SD; $n = 7$, [a] $P < 0.05$ significant difference compared with the Group 1; [b] $P < 0.05$ significant difference compared with the Group 2; [c] $P < 0.05$ significant difference compared with Group 3; [d] $P < 0.05$ significant difference compared with the Group 4. HFD; High-fat diet, EX; Exercise, LP-CUR; Liposomal curcumin, CS; citrate synthase, SDH; succinate dehydrogenase, FFA; Free fatty acids

Effect of HFD, EX, and LP-CUR on core body temperature and endurance performance (Fig. 3)

On measuring the rectal body temperature, the HFD showed substantially reduced body temperature in comparison to the control group ($P < 0.05$). Conversely, when we considered the effect of either EX and/or LP-CUR, there was a significant rise in the core temperature compared to the HFD group with the highest recorded temperature in the combined group ($P < 0.05$). These findings indicated the thermogenic effect of both EX training and LP-CUR administration (Fig. 3A).

Most importantly, time to exhaustion, as an index for physical endurance, was profoundly lessened in the HFD group versus the control group ($P < 0.05$). When we considered the effect of EX and/or LP-CUR with HFD ingestion, there was a significant increase in time to exhaustion among these groups, with LP-CUR+ EX group showed the longest time to exhaustion in comparison to other managed groups ($P < 0.05$). This referred to the improved physical activity and endurance (Fig. 3B).

The impact of HFD, EX, and LP-CUR on the histopathological features of soleus muscle (Fig. 4)

The histopathological finding of the control group revealed polygonal soleus muscle fibers arranged in fascicles with peripheral nuclei (Fig. 4A). On the contrary, HFD ingestion for 8 weeks resulted in marked disruption and disorganization of soleus fibers, wide inter cellular spaces, inflammatory cells between fibers and congestion in blood vessels in the HFD group (Fig. 4B1 &4B2). Moreover, the EX training or LP-CUR beside HFD ingestion caused little HFD-induced muscle changes; there were less widening in the inter-cellular spaces, few inflammatory cells infiltration between the hypotrophied and disorganized fibers, and normal blood vessels in HFD + EX and HFD + LP-CUR groups(Fig. 4C and 4D) respectively. Furthermore, the combined in the HFD+LP-CUR+EX group preserved the soleus muscle fibers architecture more or less near to the normal with

less inter-cellular spaces and fewer inflammatory cells infiltrate (Fig. 4E).

In addition, the morphometric analysis for the fiber-surface area (Fig. 4F), the number of nuclei per fiber (Fig. 4G), and number of inflammatory cells (Fig. 4H) were executed. There was a significant decline in the fiber-surface area and the number of nuclei per fiber but an increase in the number of inflammatory cells values in HFD group compared with the control group ($P < 0.05$). On the other side, intervention by EX, LP-CUR, or their combination resulted in notable higher values of fiber-surface area and the number of nuclei per fiber while, lower inflammatory cells *versus* the HFD group ($P < 0.05$). These data indicated a conservative effect of either EX training or LP-

CUR administration in line with HFD ingestion with better impact of their combination on fiber surface area and nuclei number.

Correlation between the assessed parameters (Fig. 5)

Serum asprosin and other parameters showed significant correlations ($P < 0.05$). Importantly, it had positive correlations with serum glucose (r = 0.984), insulin (r = 0.943), Food intake (r = 0.965), TGs (r = 0.964), MDA (r = 0.963), and CHOP (r = 0.910) levels. On the contrary and most interestingly, asprosin showed a negative correlation with QUICKI (r = -0.975), HDL (r = - 0.910), GRP78 (r = - 0.865), SHD (r = - 0.891), SLN (r = - 0.955), and SERCA (r = - 0.944).
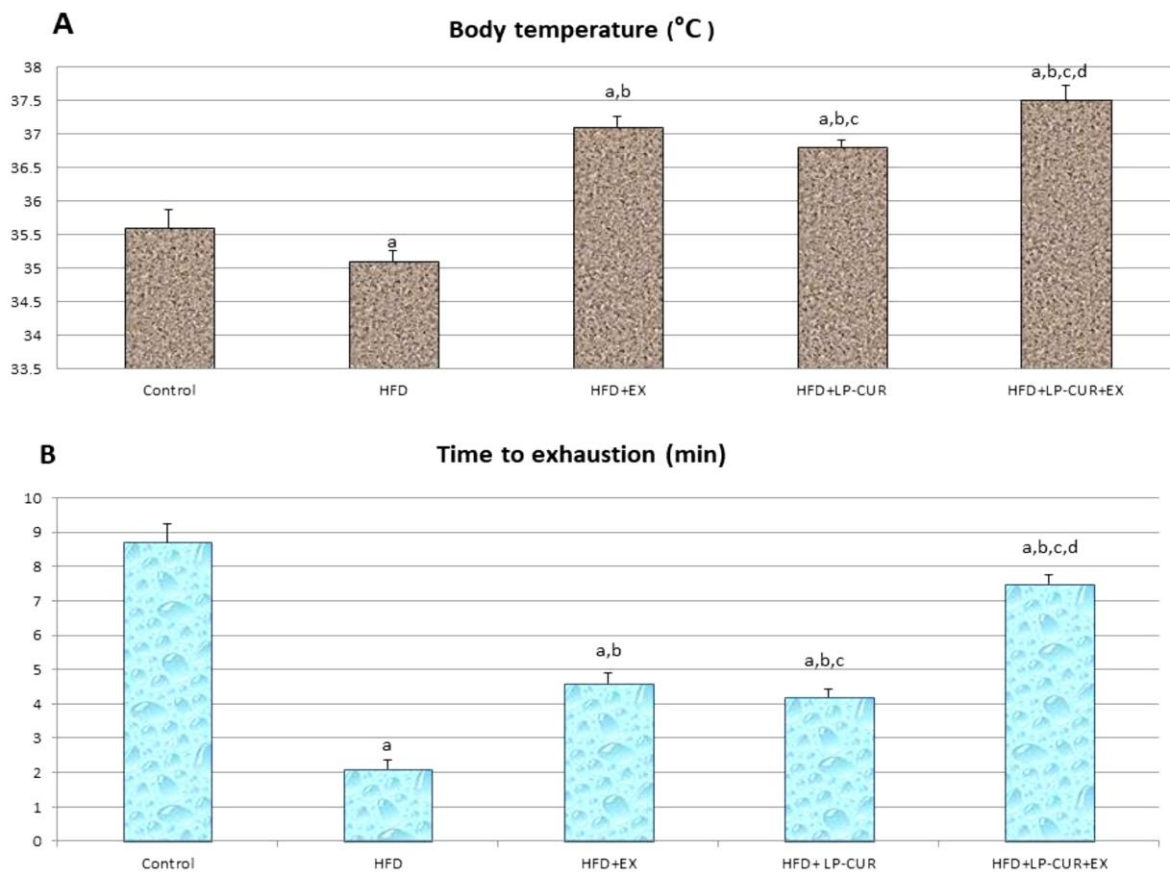

Figure 3 Body temperature and time to exhaustion in the experimental groups
Data are expressed as mean ± standard deviation SD; *n* = 7, [a] P < 0.05 significant difference compared with the Group 1; [b] P < 0.05 significant difference compared with the Group 2; [c] P < 0.05 significant difference compared with Group 3; [d] P < 0.05 significant difference compared with the Group 4. HFD; High-fat diet, EX; Exercise, LP-CUR; Liposomal-curcumin
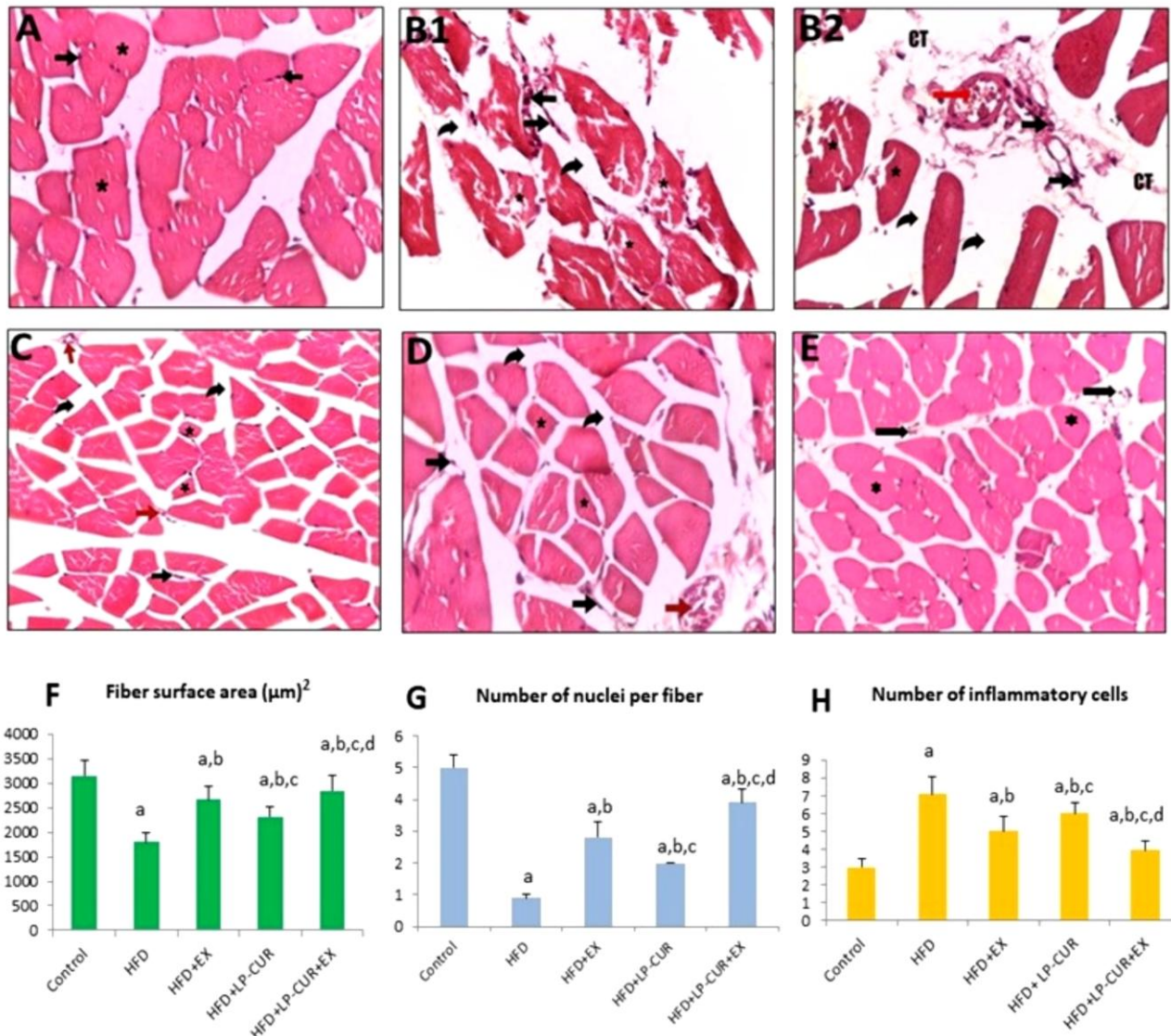
Figure 4 Photomicrographs of cross sections in rat's soleus muscle fibers stained with Hematoxylin and Eosin *(H&E X400).*

Fig. **4A** of control group that shows polygonal fibers (stars) arranged in fascicles with peripheral nuclei (black arrows); Fig. **4B1 and B2** represents HFD group that shows disruption and disorganization of fibers (stars), wide inter cellular spaces (curved black arrows), inflammatory cells between fibers (black arrows), and congestion in blood vessels (red arrow). Fig. **4C** of HFD + EX group showing hypotrophied (stars) and disorganized fibers, wide inter cellular spaces (curved black arrows), few inflammatory cells infiltration between fibers (black arrow), and normal blood vessels (red arrows). Fig.**4D** of HFD + LP-CUR group showing disorganized and hypotrophied fibers (stars), wide inter cellular spaces (curved black arrows), inflammatory cells infiltration between fibers (black arrows) and congested blood vessels (red arrow). Fig. **4E** HFD+LP-CUR+EX group shows fibers that are near to the normal (stars), with a little widening in inter-cellular spaces and few inflammatory cells infiltrate (black arrows). Fig. **4F**, Fig. **4G,** and Fig. **4H** shows the morphometric analysis for the fiber surface area, the number of nuclei per fiber, and number of inflammatory cells, respectively in the experimental groups. Data are expressed as mean ± standard deviation SD; $n = 7$, [a] P < 0.05 significant difference compared with the Group 1; [b] P < 0.05 significant difference compared with the Group 2; [c] P < 0.05 significant difference compared with Group 3; [d] P < 0.05 significant difference compared with the Group 4. HFD; High-fat diet, EX; Exercise, LP-CUR; Liposomal-curcumin
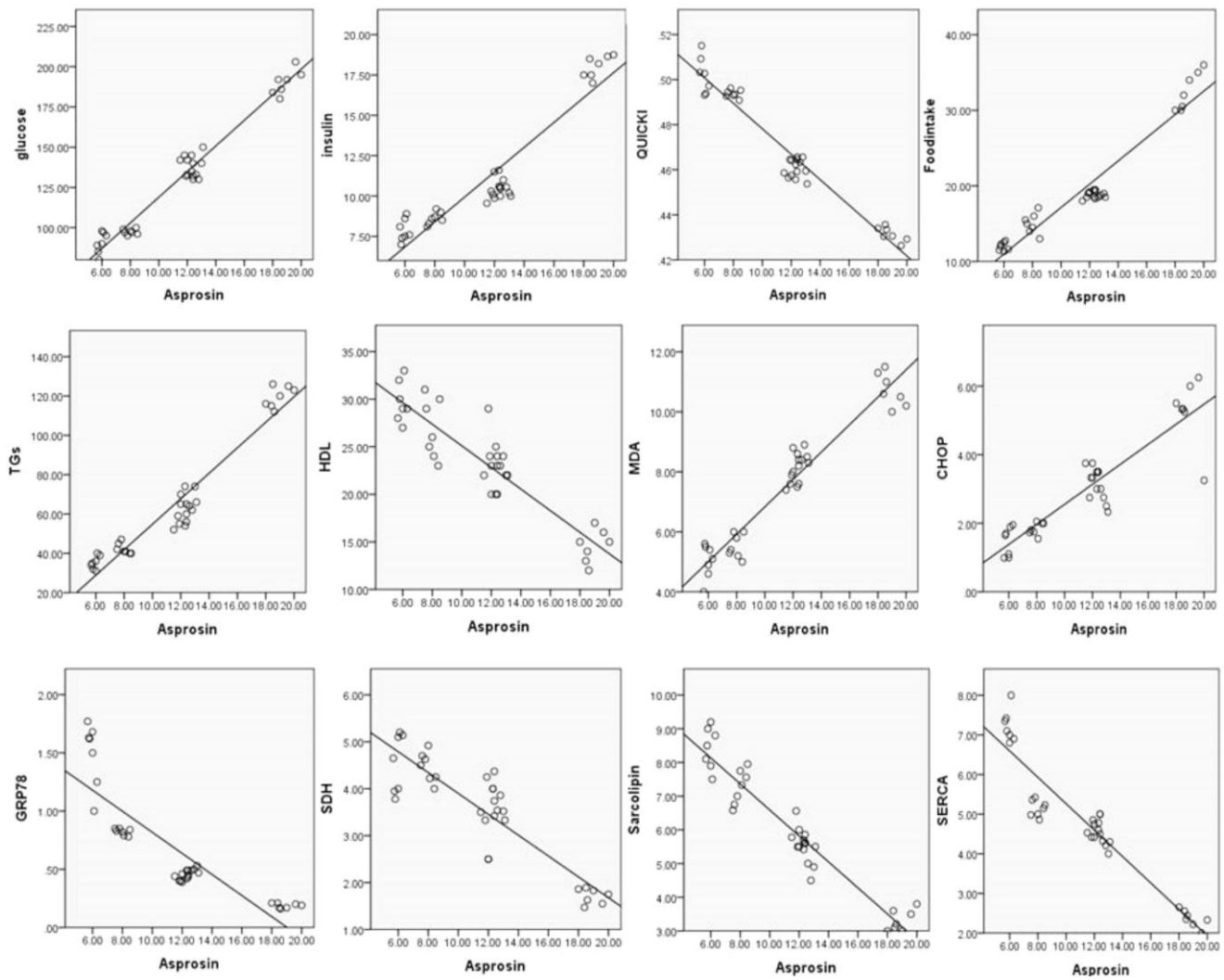
Figure 5 Correlation analyses between asprosin and glucose, insulin, QUICKI, Food intake, TGs, HDL, MDA, CHOP, GRP78, SDH, SLN, and SERCA parameters

It was analyzed using Pearson's correlation coefficient (r) 2-tailed test. A p-value of less than 0.05 was considered statistically significant. QUICKI; Quantitative insulin-sensitivity check index, TGs; triglycerides, HDL; high-density lipoproteins, MDA; malondialdehyde, CHOP, CCAAT-enhancer-binding protein homologous protein; GRP78, Glucose-regulated protein 78, SDH; Succinate dehydrogenase, SLN; Sarcolipin, SERCA; Sarco/endoplasmic reticulum Ca2+-ATPase

Discussion

Increasing energy expenditure and reducing fat synthesis is the key in tackling obesity and the related co-morbidities. The data presented in this study revealed the anti-adiposity and insulin-sensitizing impact of either EX or LP-CUR *via* up-regulation of SLN/SERCA-mediated skeletal muscle-based thermogenesis. In addition, they exerted a negative impact on the fibrillin 1/asprosin/OLFR734 pathway, the major contributor of HFD-induced obesity and insulin resistance. They, in addition, preserved redox homeostasis and ER functions.

In the current work, HFD ingestion for 8 weeks resulted in an obvious rise in BWG%, BMI, fat pad mass, but a decline in the LBM compared to control group. HFD-fed rats exhibited metabolic dysfunctions; dyslipidemia, insulin resistance, oxidative imbalance, and WAT expansion in addition to soleus muscle fibers disruption and

disorganization. These findings agreed with others [15, 31].

Novel adipokines secreted from WAT are being discovered every day, and their association with diabetes pathogenesis or prognosis is increasing. In line with these interests, our data revealed that the adipokine asprosin, at both mRNA and protein levels, was substantially overexpressed in the HFD group with respect to the control. This is because hyperlipidemia stimulates asprosin mRNA expression [32].

In addition, there were positive correlations between asprosin levels and serum glucose, insulin, and HOMA-IR levels while negatively correlated with QUICKI, insulin sensitivity index. Our results were as in plenty of other studies that documented higher asprosin levels in obese mice and humans with insulin resistance; in addition, they recommended the possibility of asprosin levels being taken as a biomarker for the early diagnosis of diabetes and monitoring its progression [33–35]. In support of these findings, when specific asprosin antibodies were constructed, the insulin resistance was ameliorated and blood glucose and insulin levels were normalized in animal models [36]. However, we should consider another research that did not confirm a positive correlation between asprosin and HOMA-IR, but this finding could be explained by the abnormal metabolic behavior related to the pathological pregnancies as gestational diabetes and preeclampsia [37].

The FBN1/asprosin/OLFR734 pathway mechanistically stimulates hepatic gluconeogenesis by using cyclic adenosine monophosphate (cAMP) as a second messenger [36]. Interestingly, OLFR734 deficiency also dramatically attenuates HFD-induced glucose production [6]. Based on the aforementioned, these data were expressing the value of the asprosin/OLFR734 pathway in glucose homeostasis and resulted in the characterization of asprosin as a glucogenic hormone.

To get further insight into the molecular mechanisms underlying the asprosin daibetogenic effect, we investigated the possible involvement of the SERCA pump being a critical factor in the maintenance of sarco/endoplasmic reticulum calcium balance and homeostasis as well as important for the insulin signaling and utilization in skeletal muscle.

Herein, HFD-induced asprosin surge resulted in a decline in soleus SERCA protein expression and augmented ER stress-related markers as indicated by GRP78 depression and CHOP overexpression in comparison to the control group. Furthermore, serum asprosin was positively correlated with CHOP while negatively correlated with GRP78. In support of this, Jung and coauthors [32] proved that asprosin diminishes SERCA mRNA expression in the soleus muscle of mice fed HFD and in C2C12 cells and eventually augments ER stress. These results demonstrate that asprosin promotes skeletal muscle insulin resistance via a SERCA-dependent ER stress-dependent pathway.

Moreover, dyslipidemia and oxidative stress are also major triggering factors for ER stress. In our model, HFD-associated oxidative radicals, due to decreased antioxidant enzymes, SOD and catalase, activity and increased MDA levels in addition to dyslipidemia in the form of an increase in serum TGs, TC, VLDL, and LDL, but HDL decline in the HFD-fed group *versus* the control group, were documented. So, they trigger ER stress besides the

SERCA suppression. These eventually disrupt insulin signaling and impair skeletal muscle insulin sensitivity. Since skeletal muscle stands the foremost organ for glucose uptake by insulin, therefore SERCA up-regulation could recover the deterioration of skeletal muscle insulin sensitivity, which is vital for managing diabetes.

Food intake, BWG%, and BMI of rats in the HFD group were noticeable higher than in the control group. In addition, food intake was positively correlated with serum asprosin. In support of our findings, Mishra et al. have found that asprosin neutralization with a specific antibody was reported to cause a simultaneous decline in food intake and body weight in a mouse model of metabolic syndrome [38]. Thus, asprosin could be identified as an orexigenic hormone.

The mitochondrial biogenesis and functions as indicated by PGC1α, UCP1, and CS activity were significantly diminished in the HFD group with respect to the control. Those findings led to reduced thermogenesis that eventually resulted in the reduction in the rectal body temperature in the HFD group *versus* the control. The reduced energy expenditure allows WAT expansion and the increase in fat pad mass at the expense of LBM. In support of our data, Miao and coauthors revealed that the asprosin overexpression group exhibited a prominent decline in the thermogenic genes UCP1 and PGC1α in addition to rising adipogenic gene expressions that aggravate lipid deposition, allowing WAT enlargement. They also reported that asprosin overexpression made mice more difficult to maintain their body temperature due to decreases in energy expenditure and thermogenesis [39]. Thus, asprosin negatively regulated thermogenesis and promoted adipogenesis in a

vicious circle mechanism, being an adipokine released from WAT. Considering these data, a strategy based on augmented thermogenesis could effectively abrupt this vicious circle and be crucial to the treatment of adiposity and its related comorbidities.

Since exercise can recover the outcomes of metabolic disorders, it was reasonably expected to affect the asprosin release and its metabolic sequels. Interestingly, in support of this data, the asprosin-encoding gene FBN1 was reported to be plentifully expressed in mesoangioblasts derived from myocytes, osteoplastic cells, and mesenchymal stem cells [7]. This indicates the potential and promising role of the musculoskeletal system in the regulation of asprosin. Herein, compared to the HFD group, the exercise-trained HFD-fed rats exhibited lower asprosin pathway at both the mRNA and protein levels, in line with lower glucose, insulin, HOMA-IR, food intake, BWG%, fat pad mass, MDA, and CHOP protein expression. On the other hand, there were evidently higher QUICKI indexes, a good lipid profile, a rise in LBM, increased antioxidant enzymes, and GRP78 protein expression. In support of our findings, the majority of available literature detected the lowering impact of exercise training on asprosin, lipid profile, insulin, and glucose levels [14, 40].

Unfortunately, a limitation to engaging in a regular physical activity may exist either due to obesity-related movement sluggishness or lifestyle sedentary. So, searching for an alternative or complementary tool is also required. Curcumin has a potential role in the treatment and prevention of various chronic diseases [41]. However, little is known about its effect on the energy metabolism

in muscle. Liposomal preparation of curcumin increases its efficacy. In the present study, LP-CUR-treated HFD-fed rats, when compared to the HFD group, reported an improvement in anthropometric parameters, glucose, insulin, HOMA-IR, and lipid profiles, with a greater increase in HDL levels, antioxidant levels, and mitochondrial and ER-stress-related markers. Our data were in agreement with previous studies that referred to anti-hyperglycemic, anti-hyperlipidemia, and redox homeostatic effects in type 2 diabetic rats and patients upon curcumin usage [42, 43]. Another supporting result reported that CUR attenuates ER stress and suppresses the cAMP/protein kinase A pathways and thus inhibits asprosin-mediated hepatic gluconeogenesis [44]. Moreover, Gokdemir et al. [45] have verified that CUR is a hypoglycemic compound and has similar effects on glucose metabolism as metformin or gliclazide anti-diabetic drugs.

Most importantly, the discovery of SLN as an uncoupling protein of the SERCA pump provides a promising target to increase muscle-based thermogenesis. SLN performs a double regulation of muscle metabolism. This includes stimulation of useless SERCA pump activity, creating an energy demand state, and exerting a pivotal role in muscle thermogenesis. In addition, it is fundamental for mitochondrial biogenesis and fatty acid oxidation.SLN-knockout muscles showed less mitochondrial count and cristae and a rise in the glycolytic enzymes. That is because SLN modulates high local cytosolic Ca^{2+} concentration, allowing flux of Ca^{2+} into the mitochondria, and causes recruitment and activation of PGC1α, increasing mitochondrial biosynthesis and ATP production. Thus, SLN/SERCA interaction creates

a state of chronic increase in energy demands by futile ATP hydrolysis [46]. Thus, SLN mimics the exercise effect to increase energy expenditure, and a higher SLN provides resistance against diet-induced obesity.

In line with these SLN-mediated physiological mechanisms, it was found that SLN-knockout mice have become obese on HFD due to lost muscle-based thermogenesis. On the contrary, SLN-overexpressing mice significantly gained less weight than wild-type mice and were resistant to HFD-induced obesity [47]. Moreover, these SLN-overexpressing mice showed improved mitochondrial health and enhanced exercise performance compared to control mice [48, 49].

More interestingly, the data of the current study revealed overexpression in the SLN and SERCA proteins on either EX or LP-CUR treatment compared to the HFD group, which means they can counteract the asprosin-mediated HFD effects also through SLN/SERCA up-regulation. This was in agreement with an increase in the SLN gene upon voluntary wheel running, indicating that exercise training can recruit SLN-mediated signaling [4]. In addition, LP-CUR augmented mitochondrial biogenesis and function as well as SLN/SERCA proteins. This denotes increased thermogenesis and reduced fat mass with an increase in LBM that eventually reduced asprosin levels. A recently published study has reported CUR beneficial impacts on the diaphragmatic muscle dystrophy, mechanistically acting for SERCA up-regulation [50]. In addition, Choi and his colleges have revealed that CUR motivated thermogenesis by activating the SERCA/SLN axis and mitochondrial uncoupling in WAT [51]. Furthermore, it was demonstrated that curcumin

supplementation of 100 mg/kg body weight for 3 days significantly increased the activity of mitochondrial enzymes in the skeletal muscle of rats with chronic obstructive pulmonary disease [52].

For the sequel of up-regulated soleus muscle SLN/SERCA, PGC1α, and UCP1 mRNA, and increased CS activity on muscle substrate utilization preference, fuel handling, during HFD ingestion, and EX or LP-CUR management, we investigated blood lactate and FFAs levels in addition to the SDH activity. Our results concerning blood lactate levels, as proof for increased anaerobic glycolysis, were augmented in the HFD group *versus* the control while it was decreased on intervention by EX and/or LP-CUR. In addition, EX and/or LP-CUR promoted an increase in the soleus muscle PGC1α, UCP1, and citrate synthase activity as biomarkers for increased mitochondrial biogenesis and functions, in addition to increased SDH activity as an indicator of the oxidative metabolic capacity, concomitantly with the reduction of serum FFAs and increased body thermogenesis concluded from rectal thermometry. These data revealed improved oxidative metabolic capacity of skeletal muscles that eventually resulted in enhanced exercise performance in the LP-CUR and/or exercise-treated groups compared to the HFD group, as concluded from the swimming-until exhaustion test. One of the findings worth exploring is the synergistic effect of both EX training and LP-CUR, known to increase the energy expenditure in muscle, having a better impact on asprosin-induced changes in a combined therapy. Most importantly, no reported adverse effects due to augmented SLN exist in the muscle at both the structural and biochemical levels. It was consistent with that reported by Sopariwala et al. [49]. Rats in the EX and/or LP-CUR groups experienced a higher endurance capacity and improved muscle performance with a longer time to exhaustion.

An illustration of the underlying molecular mechanism was shown in Fig. 6

Conclusion

While much progress has been achieved in decoding the pathophysiological activities of asprosin in the setting of obesity and diabetes, we identified, for the first time, SLN/SERCA upgrading as a possible pathway to antagonize its effects. It was by targeting non-shivering thermogenesis in one of the largest organs in the body, skeletal muscle. These findings put forward SLN/SERCA as a new avenue to mimic or augment the effects of exercise. Therefore, asprosin lowering and SLN/SERCA upgrading can be used as effective strategic goals for the treatment of obesity and its related comorbidities
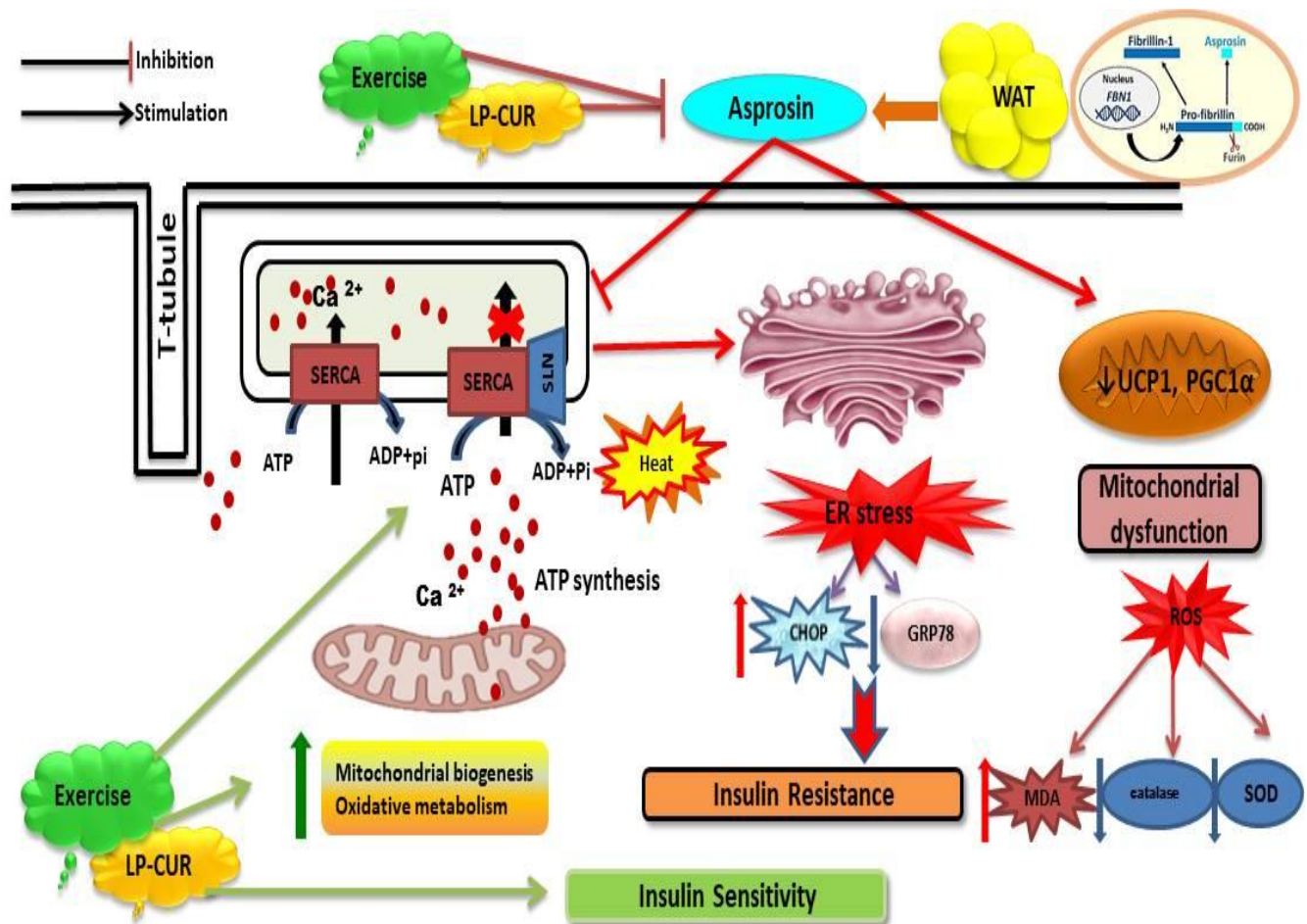
1

Figure 6 A schematic diagram for the molecular mechanism

WAT expansion, caused by HFD intake, resulted in excess asprosin production mediated by C-terminal cleavage of the pro-fibrillin protein. Asprosin reduced SERCA and initiated SERCA-dependent ER stress. It was indicated by a CHOP rise and GRP78 decline that eventually resulted in muscle insulin resistance. Asprosin also impairs mitochondrial functions and biogenesis due to the associated lowering of PGC1α and UCP1 and increased ROS production, depleting the antioxidant enzymes and increasing MDA.

On the other side, EX and/or LP-CUR in concomitant with HFD antagonized the asprosin effects. They caused overexpression of SERCA and SLN proteins. The latter causes futile cycling of the SERCA pump with ATP hydrolysis without Ca^{2+} entry into the ER. The increased sarcoplasmic Ca^{2+} fluxes into the mitochondria and causes recruitment and activation of PGC1α, increasing mitochondrial biogenesis, ATP production, enzymes for oxidative metabolism, and energy expenditure. These effects eventually improved skeletal muscle insulin sensitivity and reduced WAT expansion with subsequent asprosin decline.

HFD, high-fat diet; EX, exercise, LP-CUR, Liposomal curcumin, SERCA, Sarco/endoplasmic reticulum Ca2-ATPase; SLN, Sarcolipin; CHOP, CCAAT-enhancer-binding protein homologous protein; GRP78, Glucose-regulated protein 78;ER, Endoplasmic reticulum; FBN1, Fibrillin;OLFR734, olfactory receptor 734; PGC1α, peroxisome proliferator activated receptor gamma coactivator-1 alpha; UCP1, uncoupling protein 1; WAT, white adipose tissue; MDA, malondialdehyde, SOD, superoxide dismutase; ATP, adenosine triphosphate; ROS, reactive oxygen species.

References

1. **Muhammad, M. H., Elwai, S., &Abd El Rahman, S. M.** Anti-adiposity impact of phosphodiesterase-5 inhibitor, Sildenafil is possibly through browning of white adipose tissue and FGF21 in obese rats. *Bulletin of Egyptian Society for Physiological Sciences*, *39*(2), 143-157, 2019

2. **Kenney, W. L., Wilmore, J. H., &Costill, D. L.** *Physiology of sport and exercise*. Human kinetics. Eighth edition, Part II, Muscle Physiology, Ch. 5, 2021

3. **Chambers, P. J., Juracic, E. S., Fajardo, V. A., &Tupling, A.** R. Role of SERCA and sarcolipin in adaptive muscle remodeling. *American Journal of Physiology-Cell Physiology*, *322*(3), C382-C394, 2022

4. **Bal, N. C., &Periasamy, M**. Uncoupling of sarcoendoplasmic reticulum calcium ATPase pump activity by sarcolipin as the basis for muscle non-shivering thermogenesis. *Philosophical Transactions of the Royal Society B*, *375*(1793), 20190135, 2020

5. **Farrag, M., AitEldjoudi, D., González-Rodríguez, M., Cordero-Barreal, A., Ruiz-Fernández, C., Capuozzo, M., ...&Gualillo, O.** Asprosin in health and disease, a new glucose sensor with central and peripheral metabolic effects. *Frontiers in Endocrinology*, *13*, 1101091, 2023

6. **Li, E., Shan, H., Chen, L., Long, A., Zhang, Y., Liu, Y., ...& Wang, Y.** OLFR734 mediates glucose metabolism as a receptor of asprosin. *Cell metabolism*, *30*(2), 319-328, 2019

7. **Liu, L., Liu, Y., Huang, M., Zhang, M., Zhu, C., Chen, X., Bennett, S., Xu, J., &Zou,** J. The Effects of Asprosin on Exercise-Intervention in Metabolic Diseases. *Frontiers in physiology*, *13*, 907358, 2022

8. **Muthu ML & Reinhardt DP**. Fibrillin-1 and Fibrillin-1-Derived asprosin in adipose tissue function and metabolic disorders. *J Cell Commun Signal. 142:159–73, 2020*

9. **Coll, T., Eyre, E., Rodriguez-Calvo, R., Palomer, X., Sanchez, R. M., Merlos, M., …** Vazquez-Carrera, M. Oleate reverses palmitate-induced insulin resistance and inflammation in skeletal muscle cells. Journal of Biological Chemistry, 283(17), 11107–11116, 2008.

10. **Keshk, W. A., Elseady, W. S., Sarhan, N. I., &Zineldeen, D. H.** Curcumin attenuates cytoplasmic/endoplasmic reticulum stress, apoptosis and cholinergic dysfunction in diabetic rat hippocampus. *Metabolic brain disease*, *35*, 637-647, 2020

11. **Balaramnavar, V. P.** A comprehensive review of turmeric benefits. *South Asian Journal of Marketing & Management Research*, *11*(11), 169-174, 2021

12. **Khater, S.I.; Dowidar, M.F.; Abdel-Aziz, A.E.; Khamis, T.; Dahran, N.; Alqahtani, L.S.; Metwally, M.M.M.; Al-HadyAbd-Elrahamn, A.-S.; Alsieni, M.; Alosaimi, M.E.; et al.** β-Cell Autophagy Pathway and Endoplasmic Reticulum Stress Regulating-Role of Liposomal Curcumin in Experimental Diabetes: A Molecular and Morphometric Study. *Antioxidants11*, 2400, 2022.

13. **Gheit, R. E. A. E., Younis, R. L., El-Saka, M. H., Emam, M. N., Soliman, N. A., El-Sayed, R. M., Hafez, Y. M., AbuoHashish, N. A., Radwan, D. A., Khaled, H. E., Kamel, S., Zaitone, S. A., &Badawi, G. A.** Irisin improves adiposity and exercise tolerance in a rat model of postmenopausal obesity through enhancing adipo-myocyte

thermogenesis. *Journal of physiology and biochemistry*, 78(4), 897–913, 2022.

14. **Ko, J. R., Seo, D. Y., Kim, T. N., Park, S. H., Kwak, H. B., Ko, K. S., ... & Han, J.** Aerobic exercise training decreases hepatic asprosin in diabetic rats. *Journal of clinical medicine*, 8(5), 666, 2019

15. **Guo, X. X., Wang, Y., Wang, K., Ji, B. P., & Zhou, F**. Stability of a type 2 diabetes rat model induced by high-fat diet feeding with low-dose streptozotocin injection. *Journal of Zhejiang University. Science. B*, 19(7), 559–569, 2018

16. **Muhammad, M. H., &Allam, M. M.** Resveratrol and/or exercise training counteracts aging-associated decline of physical endurance in aged mice, targeting mitochondrial biogenesis and function. *The Journal of Physiological Sciences*, 68(5), 681-688, 2018.

17. **Ludgero-CorreiaA, Jr., Aguila MB, Mandarim-de-Lacerda CA, and Faria TS**. Effects of high-fat diet on plasma lipids, adiposity, and inflammatory markers in ovariectomized C57BL/6 mice. *Nutrition (Burbank, Los Angeles County, Calif)* 28: 316-323, 2012

18. **Rising R, and Lifshitz F.** Energy expenditures & physical activity in rats with chronic suboptimal nutrition. *Nutrition & metabolism* 3: 11., 2006

19. **Wu MV, Bikopoulos G, Hung S, and Ceddia RB.** Thermogenic capacity is antagonistically regulated in classical brown and white subcutaneous fat depots by high-fat diet and endurance training in rats:

impact on whole-body energy expenditure. *The Journal of Biological Chemistry* 289: 34129-34140, 2014

20. **Lott, J. A., & Turner, K.** Evaluation of Trinder's glucose oxidase method for measuring glucose in serum and urine. *Clinical chemistry*, 21(12), 1754-1760, 1975

21. **Hiroyuki, S., Masaaki, S., Yoshio, T., Ryuji, K., Masakatsu, H., &Nobuhisa, O.** An enzyme immunoassay system for measurement of serum insulin. *Molecular immunology*, 17(3), 377-381, 1980

22. **Matthews, D. R., Hosker, J. P., Rudenski, A. S., Naylor, B. A., Treacher, D. F., & Turner, R. C.** Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man.*Diabetologia*, 28(7), 412-419, 1985.

23. **Cacho, J., Sevillano, J., de Castro, J., Herrera, E., & Ramos, M. D. P.** Validation of simple indexes to assess insulin sensitivity during pregnancy in Wistar and Sprague-Dawley rats. *American journal of physiology-endocrinology and metabolism*, 295(5), E1269-E1276, 2008

24. **Muhammad, M. H., Hussien, N. I., &Elwia, S. K.** Vitamin D Replacement Mitigates Menopause-Associated Dyslipidaemia and Atherogenic Indices in Ovariectomized Rats; A Biochemical Study. *Experimental and Clinical Endocrinology & Diabetes*, 128(03), 144-151, 2020

25. **Friedewald WT, Levy RI, Fredrickson DS.** Estimation of the concentration of low-

density lipoprotein cholesterol in plasma, without the use of the preparative ultracentrifuge. *Clin Chem.*; 18: 499–502, 1972

26. **Foster LB, and Dunn RT.** Stable reagents for determination of serum triglycerides by colorimetric condensation method. ClinChemActa 19: 338–340, 1973

27. **Aebi H. Catalase in vitro.** In: Lester P (ed) Methods enzymol. Academic Press, San Diego, pp 121–126, 1984

28. Marklund S. Distribution of Cu-Zn superoxide dismutase and Mn-superoxide dismutase in human tissues and extracellular fluids. ActaPhysiolScandSuppl 492:19–23, 1980

29. **Gérard-Monnier D, Erdelmeier I, Régnard K, Moze-Henry N, Yadan JC, Chaudière J.** Reactions of 1-methyl-2-phenylindole with malondialdehyde and 4-hydroxyalkenals. Analytical applications to a colorimetric assay of lipid peroxidation. Chem Res Toxicol11:1176–1183, 1998

30. **Elnagar, A., El-Dawy, K., El-Belbasi, H. I., Rehan, I. F., Embark, H., Al-Amgad, Z., Shanab, O., Mickdam, E., Batiha, G. E., Alamery, S., Fouad, S. S., Cavalu, S., & Youssef, M.** Ameliorative Effect of Oxytocin on *FBN1* and *PEPCK* Gene Expression, and Behavioral Patterns in Rats' Obesity-Induced Diabetes. *Frontiers in public health*, *10*, 777129, 2022

31. **Lasker, S., Rahman, M. M., Parvez, F., Zamila, M., Miah, P., Nahar, K., ...&Alam, M. A.** High-fat diet-induced metabolic syndrome and oxidative stress in

obese rats are ameliorated by yogurt supplementation. *Scientific reports*, *9*(1), 20026, 2019.

32. **Jung, T. W., Kim, H. C., Kim, H. U., Park, T., Park, J., Kim, U., ... &Jeong, J. H.** Asprosin attenuates insulin signaling pathway through PKCδ-activated ER stress and inflammation in skeletal muscle. *Journal of cellular physiology*, *234*(11), 20888-20899, 2019

33. **Roh KH, Abdellrahim AA, Abo Elwafa RAH, &Moawad MG.** Study of the Relationship between Serum Asprosin, Endothelial Dysfunction and Insulin Resistance. Clin Diabetol.11:73–79, 2022

34. **Goodarzi, G., Setayesh, L., Fadaei, R., Khamseh, M. E., Aliakbari, F., Hosseini, J., &Moradi, N.** Circulating levels of asprosin and its association with insulin resistance and renal function in patients with type 2 diabetes mellitus and diabetic nephropathy. *Molecular biology reports*, *48*, 5443-5450, 2021.

35. **Zhang X, Jiang H, Ma X, & Wu H.** Increased serum level and impaired response to glucose fluctuation of asprosin is associated with type 2 diabetes mellitus. J Diabetes Investig. 11:349-55, 2020.

36. **Romere, C., Duerrschmid, C., Bournat, J., Constable, P., Jain, M., Xia, F., ...& Chopra, A. R.** Asprosin, a fasting-induced glucogenic protein hormone. *Cell*, *165*(3), 566-579, 2016

37. **Baykus, Y., Yavuzkir, S., Ustebay, S., Ugur, K., Deniz, R., &Aydin, S.** Asprosin in umbilical cord of newborns and maternal

blood of gestational diabetes, preeclampsia, severe preeclampsia, intrauterine growth retardation and macrosemic fetus. Peptides, 120, 170132, 2019

38. **Mishra, I., Duerrschmid, C., Ku, Z., He, Y., Xie, W., Silva, E. S., Hoffman, J., Xin, W., Zhang, N., Xu, Y., An, Z., & Chopra, A. R.** Asprosin-neutralizing antibodies as a treatment for metabolic syndrome. *eLife*, *10*, e63784, 2021

39. **Miao, Y., Qin, H., Zhong, Y., Huang, K., &Rao, C.** Novel adipokine asprosin modulates browning and adipogenesis in white adipose tissue. *Journal of Endocrinology*, *249*(2), 83-9, 2021

40. **Nakhaei, H., Mogharnasi, M., &Fanaei, H.** Effect of swimming training on levels of asprosin, lipid profile, glucose and insulin resistance in rats with metabolic syndrome. *Obesity medicine*, *15*, 100111, 2019

41. **Kunnumakkara, A. B., Hegde, M., Parama, D., Girisa, S., Kumar, A., Daimary, U. D., ...&Aggarwal, B. B.** Role of turmeric and curcumin in prevention and treatment of chronic diseases: Lessons learned from clinical trials. ACS Pharmacology & Translational Science, 6(4), 447-518, 2023

42. **Seo, K. I., Choi, M. S., Jung, U. J., Kim, H. J., Yeo, J., Jeon, S. M., & Lee, M. K.** Effect of curcumin supplementation on blood glucose, plasma insulin, and glucose homeostasis related enzyme activities in diabetic db/db mice. *Molecular nutrition & food research*, *52*(9), 995-1004, 2008

43. **Gutierres, V. O., Assis, R. P., Arcaro, C. A., Oliveira, J. O., Lima, T. F. O., Beretta, A. L. R. Z., ...** &Brunetti, I. L. Curcumin improves the effect of a reduced insulin dose on glycemic control and oxidative stress in streptozotocin-diabetic rats. *Phytotherapy Research*, *33*(4), 976-988, 2019

44. **Wang, L., Zhang, B., Huang, F., Liu, B., &Xie, Y.** Curcumin inhibits lipolysis via suppression of ER stress in adipose tissue and prevents hepatic insulin resistance [S]. *Journal of lipid research*, *57*(7), 1243-1255, 2016.

45. **Gokdemir, G. S., Gokdemir, M. T., Tasdemir, E., Yokus, B., &Baylan, M.** Importance of curcumin effect and asprosin level on glucose metabolism in diabetic rats. *Medicine Science*, *12*(1), 2023

46. **Maurya, S. K., Herrera, J. L., Sahoo, S. K., Reis, F. C., Vega, R. B., Kelly, D. P., &Periasamy, M.** Sarcolipin signaling promotes mitochondrial biogenesis and oxidative metabolism in skeletal muscle. *Cell reports*, *24*(11), 2919-2931, 2018

47. **MacPherson, R. E., Gamu, D., Frendo-Cumbo, S., Castellani, L., Kwon, F., Tupling, A. R., & Wright, D. C.** Sarcolipin knockout mice fed a high-fat diet exhibit altered indices of adipose tissue inflammation and remodeling. *Obesity (Silver Spring, Md.)*, *24*(7), 1499–1505, 2016

48. **Maurya SK, Bal NC, Sopariwala DH, Pant M, Rowland LA, Shaikh SA, Periasamy M.** Sarcolipin is a key

determinant of the basal metabolic rate, and its overexpression enhances energy expenditure and resistance against diet-induced obesity. J. Biol. Chem. 290, 10 840–10 849, 2015

49. **Sopariwala, D.H., Pant, M., Shaikh, S.A., Goonasekera, S.A., Molkentin, J.D., Weisleder, N., Ma, J., Pan, Z., and Periasamy, M.** Sarcolipin overexpression improves muscle energetics and reduces fatigue. J. Appl. Physiol. 118, 1050–1058, 2015.

50. **Gorza, L., Germinario, E., Vitadello, M., Guerra, I., De Majo, F., Gasparella, F., Caliceti, P., Vitiello, L., &Danieli-Betto, D.** Curcumin Administration Improves Force of *mdx* Dystrophic Diaphragm by Acting on Fiber-Type Composition, Myosin Nitrotyrosination and SERCA1 Protein Levels. *Antioxidants (Basel, Switzerland)*, *12*(6), 1181, 2023

51. **Choi, M., Mukherjee, S., & Yun, J. W.** Curcumin stimulates UCP1-independent thermogenesis in 3T3-L1 white adipocytes but suppresses in C2C12 muscle cells. *Biotechnology and Bioprocess Engineering*, *27*(6), 961-974, 2022

52. Saud Gany, S. L., Chin, K. Y., Tan, J. K., Aminuddin, A., &Makpol, S. Curcumin as a Therapeutic Agent for Sarcopenia. *Nutrients*, *15*(11), 2526, 2023