Effect of high fat diet (HFD)-induced obesity on gene expression of adipose tissue macrophage markers in male rats with different ages: Role of AMPK/SIRT1 pathway.

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Abstract

Obesity and aging are associated with adipose tissue (AT) inflammation and a higher risk of chronic diseases. However, the underlying mechanism remains largely unknown. Here, we investigated the effect of HFD-induced obesity on gene expressions of ATM polarization markers and inflammatory cytokines in male rats with different ages and the possible role of AMPK/SIRT1 pathway in mediating this effect. Sixty male wistar rats were divided into young, adult and old-age groups. Rats of each group were either fed standard diet or HFD. The present results revealed that HFD feeding in all age groups resulted in increased body weight, lipids, visceral adiposity and induced hyperglycemia and insulin resistance compared to controls. Findings revealed significant higher AT IL-6 levels and lower IL-10 levels with significant upregulation of CD11c and CD206 mRNA expressions in all age groups. The histological findings showed increased inflammation and presence of crown like structures in adult and old HFD groups. Moreover, the HFD-induced obesity in groups resulted in significant reduction in p-AMPK levels and SIRT1 expression in AT as compared to controls. AMPK and SIRT1 was positively correlated with IL-10 and CD206 and negatively correlated with TG, HOMA-IR, IL-6 and CD11c in obese groups of different ages. In conclusion, HFD- induced obesity in all ages leads to ATMs polarization and increased inflammatory response that may contribute to metabolic dysfunction. In addition, AMPK/SIRT1 pathway is suggested to be a mediator of ATMs polarization. Thus, targeting this pathway may be promising for treating obesity and aging- related diseases.

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1. Introduction

Obesity and aging are significant health burdens on the adult population worldwide. Both conditions increase the risk of developing metabolic diseases such as insulin resistance, type II diabetes (T2D), and cardiovascular disease (CVD). Visceral adipose tissue (VAT) dysfunction is considered a key factor in the pathophysiology of obesity and aging[1,2].

Recently, it is known that changes in obesity and aging are caused by chronic low grade inflammation within VAT [3]. Adipose tissue macrophages (ATMs) are the most abundant type of leukocyte in AT and are key players in obesity-related inflammation and metabolic diseases [4]. ATMs are classified into two subpopulations based on their biological functions and expression of cell-surface markers. The first type is classically activated M1 macrophages, which cause inflammation by expressing proinflammatory genes. The other type is alternatively activated M2 macrophages, which have anti-inflammatory properties [4].

Adenosine monophosphate-activated protein kinase (AMPK) and silent information regulator 1 (SIRT1), have gained considerable attention asimportant nutrient sensors and inflammatory regulators [5]. AMPK boosts SIRT1 by elevating the NAD/NADH ratio and reduces inflammation [6]. AMPK and SIRT1 can regulate fatty acid oxidation and mitochondrial biogenesis by phosphorylating and deacetylating peroxisome proliferative activated receptor gamma coactivator 1 (PGC1)[7]. Previous research has found that activating AMPK/SIRT1 pathway protects against HFD-induced obesity and may be considered as a therapeutic target for the management of obesity and associated metabolic complications [5,8].

Therefore, the present study was designed to investigate the effect of HFD-induced obesity on the gene expression of ATMs surface markers and inflammatory cytokines in male rats with different ages and the possible role of AMPK/SIRT1 pathway in mediating this effect.

2. Methods

2.1. Animals and experimental design

All procedures and experimental protocols were carried outbased on the Guide for the Care and Useof laboratory animals (1985) [9] and the ARRIVE Guidelines 2.0 (Percie du Sert et al., 2020) [10].Throughout the experimental period, every effort was made to minimize the distress of the rats.

Sixty male Wistar rats were selected and divided according to age into 3 main groups: young (4 weeks), adult (17 weeks), and old-aged (72 weeks) [11]. All rats were obtained from the animal house andwere maintained in a controlled environment with room temperature of 25°C and 12 h light–12 h dark cycle,housed for one week prior to the experiment for acclimation and received diet and water *ad libitum*.

Each group was further subdivided into two subgroups; control subgroup receiving standard diet and an obese subgroup receiving high fat diet (HFD) for 12 weeks. The HFD contains 45% Kcal from fats (D12451; Research Diets, Inc., New Brunswick, NJ, USA) [12].

At the end of the experiment, final body weight and length were recorded to calculate both of body mass index (BMI),BMI= final body weight (g)/ (length^2) (cm^2) and lee index, Lee index= cube root of final body weight (g)/ length (cm) [13].

Rats were fasted for 12 hours at the conclusion of the experiment, and then they were sacrificed under anesthesia (intraperitoneal injection of ketamine 100 mg/kg and xylazine 10 mg/kg). To separate the sera for biochemical analysis, blood samples were takenand centrifuged at 1000 xg for 20 min at 4°C.

Visceral adipose tissues (VATs) (epididymal, mesenteric,perirenal, intraperitoneal) of all groups were gently extracted, cleaned with saline, and weighed with calculation of adiposity index. Adiposity index= (total body fat/final BW) × 100[14].

The extracted VATswere divided into three parts. The first part was used for RNA extraction to analyze gene expression, the second for histopathology,and the third for homogenizing in phosphate buffer (pH 7.4). For cytokines determination, the homogenates were centrifuged at 1000 ×g for 20 min at 4°C and the supernatants were collected and kept at −80°C.

2.2. Biochemical serum Analysis

Serum triglycerides (TG), total cholesterol (TC) and high density lipoprotein cholesterol (HDL-C) were determined using kits purchased from Biosystems S.A. Costa Brava 30, Barcelona, Spain. The Friedewald equation was used to calculate the low density lipoprotein cholesterol (LDL-C), LDLC = TC-(HDL-C+1/5 TGs) [15].Serum glucose level was assessed using Spinreact colorimetric reagent kit (Spinreact, Spain), and insulin by enzyme-linked immunosorbent assay (ELISA) kit (Abnova, Taiwan). The following formula was used to determine the homeostasis model assessment index for insulin resistance (HOMA-IR), HOMA-IR= [fasting glucose (mmol/l) × fasting insulin (µU/ml)] /22.5 [16].

Interleukin-6 (IL-6) and interleukin-10 (IL-10)levels were evaluated in VAT homogenates using sandwich ELISA kits purchased from (Cusabio, USA and MyBioSource, Inc., California, USA), respectively. Competitive ELISA was used for assessment of phospho-AMP-activated protein kinase (pAMPK) levels in adipose tissue (MyBioSource, Inc., California, USA).

2.3. Gene expression and RT-PCR

Total RNA was extracted from VAT using the RNeasy Mini Kit (Qiagen, Hilden, Germany) following manufacturer's instructions.Reverse transcription into cDNA was carried out using miScript II RT kit. The Rotor-Gene SYBR Green PCR Kit (Qiagen®, Germany) and Rotor-Gene SYBR Green RT-PCR master mix were used to perform the PCR.The conditions were as follows: pre-denaturation at 95°C for 5 min, followed by denaturation (95°C, 5s) and combined annealing/extension (60°C, 10s). To determinethe mRNA expressions of cluster of differentiation 11c (CD11c), cluster of differentiation 206 (CD206) and SIRT-1, specific primers were used (Table 1).Data were analyzed using Ct method andthe expressions of these genes were quantified relative to -actinexpression as a reference gene [17].

2.4. Histopathological studies

Adipose tissue was collected, fixed for 72 h at room temperature in buffered 10% formalin, and then washed under running tap water. They underwent a graded series of ethanol- induced dehydration. Tissues were dehydrated, rinsed in xylene for five to ten minutes and then put into a

paraffin-embedding mixture. Using a rotary microtome, 5-mm-thick serial sections were obtained. The histology of adipose tissue was examined under a light microscope using hematoxylin and eosin (H&E) staining procedure.

2.5. Statistical analysis

The data were analyzed statistically using Statistical Package for Social Sciences (SPSS) program version 20. Data were expressed as mean ± SD. Analysis of variance (ANOVA) test was used to compare between the different studied groups and Post Hoc test (Tukey) was used for pairwise comparison. Twoway ANOVA test was used to analyze the effect of obesity, age, and the possible interaction between obesity and age variation on all the studied parameters.Additionally, Pearson correlation was carried out between both p-AMPK and SIRT1withthe other parameters under investigation. P values equal to or lower than 0.05 were considered significant for all statistical tests.

3. Results

3.1. Anthropometric and adiposity indices:

Weight recorded every week of all the studied groups was shown in figure (1).Table (2) shows means ± SD of anthropometric parameters (weight and BMI), lee index, adiposity index and visceral fat weight of the studied groups. Significant difference was found between groups in all parameters except lee index as shown by ANOVA test (F= 34.827, 6.061,24.397, 61.801, respectively and p<0.001). HFD induced a significant increase of all studied parameters as compared to their corresponding controls.Final body weight and visceral fat weight were significantly increased in old HFD group compared to young and adult HFD groups.

3.2. Biochemical parameters:

As shown in Table (3), results showed significant differences in TG, TC, LDL-C and HDL-C levels among all studied groups (F=73.811, 121.174, 73.909 and 40.190, respectively and P<0.001). The HFD caused a significant elevation in the serum levels of TG, TC and LDL-C in in all age groups compared to controls. However, a significant decrease in the serum levels of HDL-C was detected.

Findings revealed significant differences in glucose, insulin levels and HOMA-IR index between different studied groups (F=104.314, 377.718 and 246.707, respectively and P<0.001). The mean values of glucose, insulin and HOMA-IR were significantly increased by HFD in the all age groups compared to controls (Table 3).The highest levels of serum TG, TC, glucose and HOMA-IR index were detected in adult HFD group.

Table 1: The primer sequences of CD11c, CD206, SIRT-1 and -actin genes.

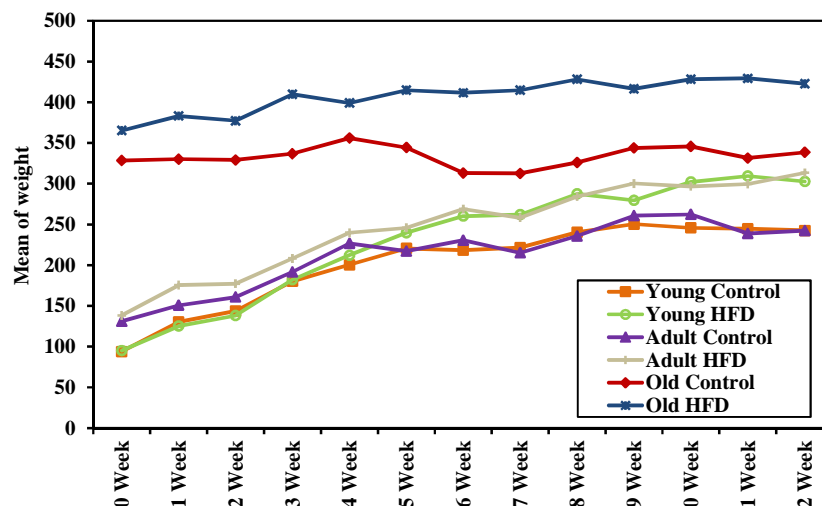| Gene	Forward Primer	Reverse Primer
CD11c	5-CATCCGTTATGCAATTGGGG-3	3-TCCATTTATCTGAGGCAGCA-5
CD206	5-TACAGTCTCAACACCGCTAC-3	3-TGAGCAGCTTACGTGGTTAT-5
SIRT-1	5-ACCCAAGACCATTCTTCAAGT-3	3-CTGCTCATGAATGCTGAGTT-5
-actin	5-ATCATTGCTCCTCCTGAGCG-3	3-GAAAGGGTGTAAAACGCAGCTC-5

Fig. 1: The mean body weights of young, adult and old age control and HFD groups during the experiment.

Table 2: The Anthropometric parameters in young, adult and old age control and HFD induced obesity groups.

	Young		Adult		Old		F (p)
	Control (n = 10)	HFD (n = 10)	Control (n = 10)	HFD (n = 10)	Control (n = 10)	HFD (n = 10)	
Final body weight (g)	$242.6^c \pm 12.4$	$302.7^b \pm 37.5$	$242.5^c \pm 30.2$	$313.3^b \pm 28.2$	$338.5^b \pm 39.9$	$422.8^a \pm 54.45$	34.827* (<0.001*)
BMI (g/cm^2)	$0.55^c \pm 0.04$	$0.69^{ab} \pm 0.11$	$0.59^{bc} \pm 0.10$	$0.68^{ab} \pm 0.08$	$0.67^{ab} \pm 0.11$	$0.74^a \pm 0.09$	6.061* (<0.001*)
Lee index	$0.30^a \pm 0.01$	$0.32^a \pm 0.02$	$0.31^a \pm 0.02$	$0.32^a \pm 0.01$	$0.31^a \pm 0.02$	$0.31^a \pm 0.01$	2.289 (0.059)
Adiposity index	$2.14^b \pm 0.64$	$4.82^a \pm 0.92$	$3.32^b \pm 0.81$	$5.46^a \pm 1.33$	$2.54^b \pm 0.73$	$5.22^a \pm 0.92$	24.397* (<0.001*)
Visceral fat weight (g)	$5.20^c \pm 1.55$	$14.45^b \pm 2.57$	$7.93^c \pm 1.72$	$16.90^b \pm 3.41$	$8.43^c \pm 1.88$	$21.81^a \pm 3.44$	61.801* (<0.001*)

[a]Means in the same raw with small common letters are not significant (i.e. Means with Different lettersare significant)
[b]Data was expressed by using Mean ± SDSD: Standard Deviation
[c]F: F for ANOVA test, Pairwise comparison between each 2 groups was done using Post Hoc Test (Tukey)
[d]p: p value for comparing between the different studied groups
*: Statistically significant at p 0.05

Table 3: Biochemical parameters in the young, adult and old age control and HFD induced obesity groups.

	Young		Adult		Old		F (p)
	Control (n = 10)	HFD (n = 10)	Control (n = 10)	HFD (n = 10)	Control (n = 10)	HFD (n = 10)	
Cholesterol (mg/dl)	$88.80^d \pm 5.3$	$114.6^b \pm 6.0$	$96.30^c \pm 3.8$	$126.0^a \pm 5.8$	$78.80^e \pm 2.4$	$98.40^c \pm 5.4$	121.174* (<0.001*)
Triglycerides (mg/dl)	$76.70^{cd} \pm 3.3$	$116.6^b \pm 5.1$	$84.90^{cd} \pm 10.9$	$177.2^a \pm 26.2$	$74.20^d \pm 8.4$	$95.0^c \pm 17.9$	73.811* (<0.001*)
HDL-C (mg/dl)	$48.90^a \pm 1.9$	$33.0^b \pm 4.3$	$48.30^a \pm 1.6$	$34.30^b \pm 4.1$	$47.10^a \pm 4.6$	$30.0^b \pm 7.1$	40.190* (<0.001*)
LDL-C (mg/dl)	$24.50^{cd} \pm 6.1$	$58.30^a \pm 8.9$	$31.0^c \pm 4.5$	$56.30^{ab} \pm 7.4$	$16.80^d \pm 5.1$	$49.30^b \pm 5.9$	73.909* (<0.001*)
Glucose (mg/dl)	$88.65^{de} \pm 5.6$	$127.2^b \pm 7.4$	$101.1^c \pm 2.1$	$147.7^a \pm 13.6$	$81.20^e \pm 8.1$	$96.40^{cd} \pm 5.3$	104.314* (<0.001*)
Insulin (µIU/ml)	$3.34^e \pm 0.7$	$5.84^d \pm 0.7$	$7.89^c \pm 0.3$	$14.07^b \pm 0.5$	$5.02^d \pm 0.5$	$16.13^a \pm 1.7$	377.718* (<0.001*)
HOMA-IR	$0.73^d \pm 0.16$	$1.84^c \pm 0.27$	$1.98^c \pm 0.09$	$5.15^a \pm 0.62$	$0.99^d \pm 0.13$	$3.85^b \pm 0.46$	246.707* (<0.001*)

[a]Means in the same raw with small common letters are not significant (i.e. Means with Different lettersare significant)
[b]Data was expressed by using Mean ± SDSD: Standard Deviation
[c]F: F for ANOVA test, Pairwise comparison between each 2 groups was done using Post Hoc Test (Tukey)
[d]p: p value for comparing between the different studied groups
*: Statistically significant at p 0.05

3.3. Inflammatory cytokines and ATMs surface markers:

Results revealed significantly elevated levels of IL-6 with a significant reduction in IL-10 levels in VAT of young, adult and old age obese groups as compared to controls (Figure 2 A, B). Moreover, the HFD feeding caused a significant upregulation of CD11c and CD206 mRNA expressions in all groups in comparison to controls (Figure 3 A, B).

3.4. AMPK and SIRT1:

Findings revealed that HFD feeding in all age groups significantly reduced p-AMPK levels and SIRT1 mRNA expression in VAT compared to control groups (Figure 4).

Using two-way ANOVA,results of most parameters including TG, TC, glucose, insulin, HOMA-IR, IL-6, IL-10, CD11c, CD206 and SIRT1 showed significant effect of obesity, significant effect of age and their combination (Table 4).
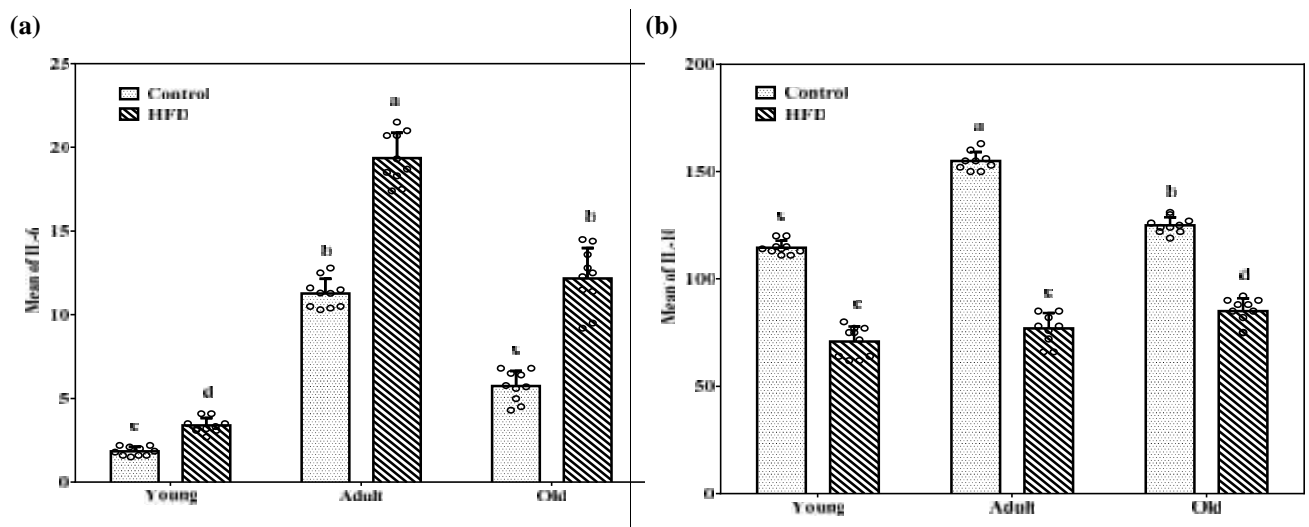
(a) (b)

Fig. 2: Levels of IL-6(pg/ml) (a) and IL-10 (pg/ml) (b) in VAT of controls and HFD induced obesity in male rats with different ages.

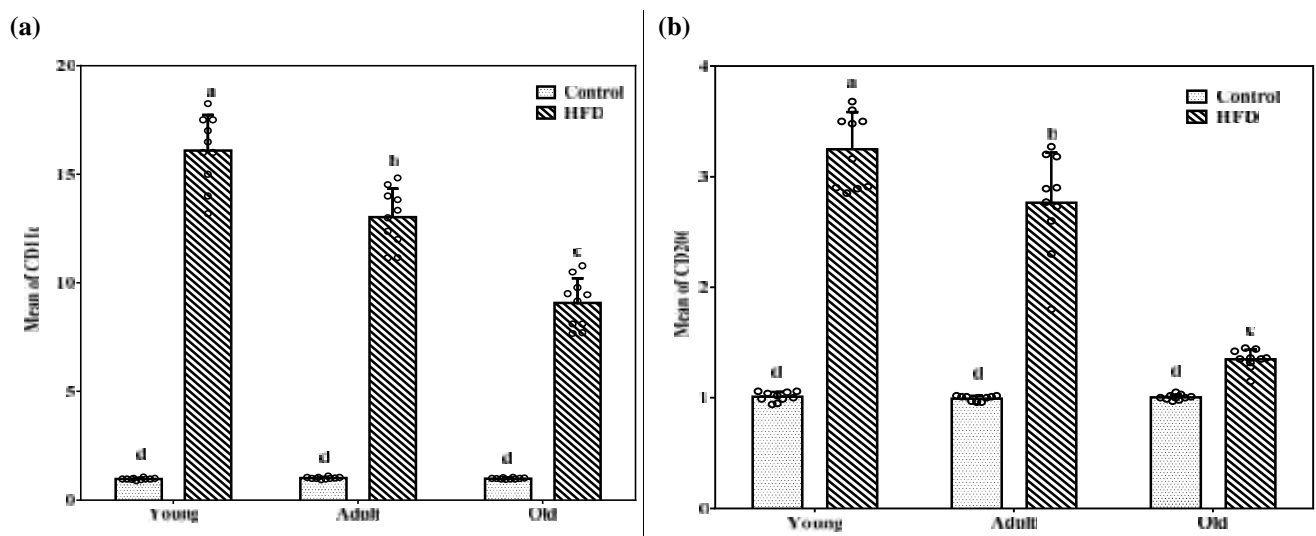(a) (b)

Fig. 3:Gene expression of CD11c (a) and CD206 (b) in control and HFD induced obesity male rats with different ages.
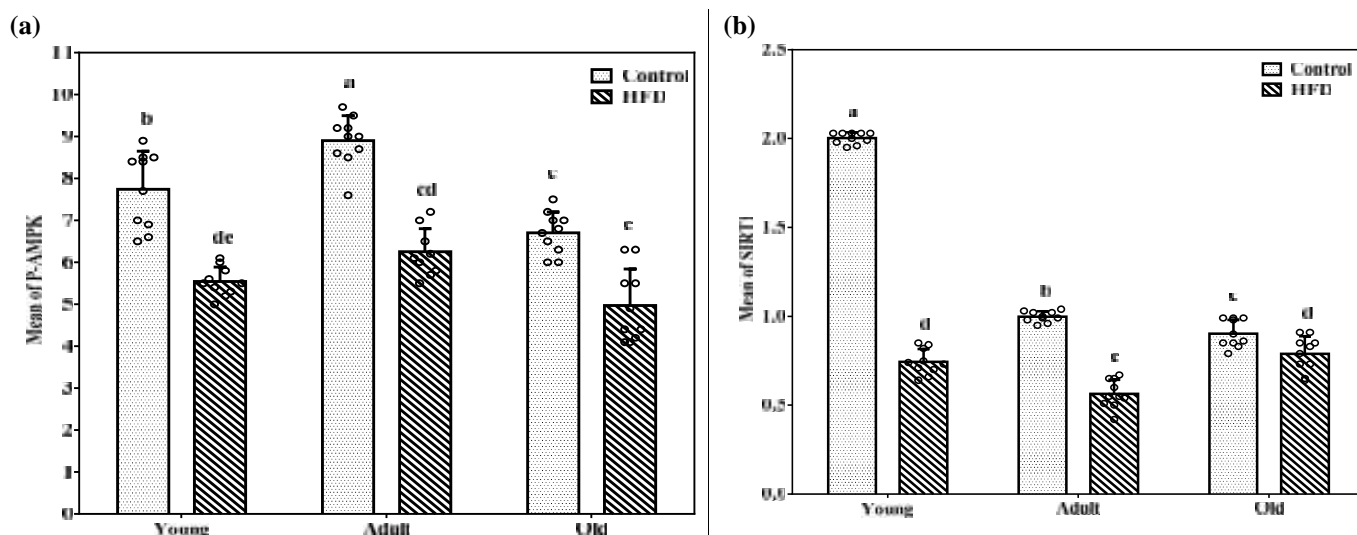
(a)

(b)

Fig.4: Levels of p-AMPK (a) and gene expression of SIRT1 (b) in VAT of control and HFD induced obesity in male rats with different ages.

Table (4): Two way ANOVA results for all studied parameters

	Obesity		Age		Obesity* Age	
	F	**P**	**F**	**P**	**F**	**P**
Glucose	272.360	<0.001[*]	103.022	<0.001[*]	21.583	<0.001[*]
Insulin	911.976	<0.001[*]	357.810	<0.001[*]	130.497	<0.001[*]
HOMA-IR	701.405	<0.001[*]	214.742	<0.001[*]	51.323	<0.001[*]
Triglycerides	189.553	<0.001[*]	56.458	<0.001[*]	33.292	<0.001[*]
Cholesterol	385.100	<0.001[*]	105.071	<0.001[*]	5.314	0.008[*]
HDL-C	194.912	<0.001[*]	2.373	0.103	0.647	0.528
LDL-C	334.670	<0.001[*]	14.929	<0.001[*]	2.508	0.091
IL-6	345.705	<0.001[*]	648.622	<0.001[*]	46.708	<0.001[*]
IL-10	1486.275	<0.001[*]	92.402	<0.001[*]	74.179	<0.001[*]
CD11c	2192.100	<0.001[*]	65.293	<0.001[*]	65.978	<0.001[*]
CD206	578.765	<0.001[*]	89.369	<0.001[*]	89.325	<0.001[*]
SIRT1	1123.580	<0.001[*]	436.004	<0.001[*]	360.284	<0.001[*]
P-AMPK	165.155	<0.001[*]	34.711	<0.001[*]	2.422	0.098

3.5. Correlation studies:

The correlations of p-AMPK and SIRT1 with other studied parameters in young, adult and old HFD groups are represented in Table (5). P-AMPK was positively correlated with IL-10 and CD206 in young, adult and old obese groups (IL-10, r= 0.754, p= 0.012& r=0.878, p=0.001 & r= 0.795, p=0.006, respectively) and (CD206, r=0.803, p=0.005 & r=0.823, p=0.003 & r=0.774, p=0.009, respectively). P-AMPK was negatively correlated with TG, HOMA-IR, IL-6 and CD11c in young, adult and old HFD groups.

SIRT1 was positively correlated with IL-10 and CD206 and negatively correlated with TG, HOMA-IR, IL-6 and CD11c in young, adult and

old obese groups.In addition, SIRT1 was negatively correlated with visceral fat weight in old obese group.

3.6. Histopathological results:

H&E staining of VAT showed a normal morphological structure with normal size adipocytes in control groups of different ages (Figure 5a, 6a,7a), respectively.

However, histological examination of young HFD group, VAT showed adipocyte hypertrophy with few inflammatory cells (Figure 5b).While, adult and old HFD groups revealed irregular shaped adipocytes with more inflammation and crown-like structures (Figure 6b,c and Figure 7b,c), respectively.

Table 5: Correlation of P-AMPK and SIRT1 with different parameters in young, adult and old age HFD induced obesity male rats.

		P-AMPK			SIRT1		
		Young (n = 10)	Adult (n = 10)	Old (n = 10)	Young (n = 10)	Adult (n = 10)	Old (n = 10)
Triglycerides	R	-0.796	-0.873	-0.735	-0.712	-0.708	-0.661
	P	0.006*	0.001*	0.015*	0.021*	0.022*	0.037*
HOMA-IR	R	-0.970	-0.675	-0.828	-0.882	-0.817	-0.950
	P	<0.001*	0.032*	0.003*	0.001*	0.004*	<0.001*
IL-6	R	-0.820	-0.937	-0.734	-0.846	-0.825	-0.795
	P	0.004*	<0.001*	0.016*	0.002*	0.003*	0.006*
IL-10	R	0.754	0.878	0.795	0.652	0.784	0.962
	P	0.012*	0.001*	0.006*	0.041*	0.007*	<0.001*
CD11c	R	-0.761	-0.971	-0.875	-0.829	-0.839	-0.902
	P	0.011*	<0.001*	0.001*	0.003*	0.002*	<0.001*
CD206	R	0.803	0.823	0.774	0.682	0.835	0.842
	P	0.005*	0.003*	0.009*	0.030*	0.003*	0.002*
Visceral fat weight	R	-0.249	0.120	0.092	-0.388	0.345	-0.747
	P	0.488	0.740	0.801	0.268	0.329	0.013*
SIRT1	R	0.915	0.897	0.897			
	P	<0.001*	<0.001*	<0.001*			

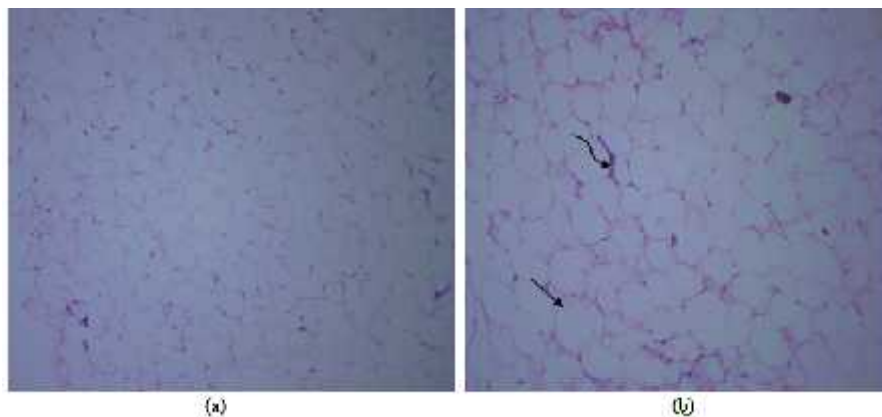r: Pearson coefficient, *: Statistically significant at p ≤ 0.05

Fig. 5: Photomicrograph of haematoxylin and eosin-stained sections of white adipose tissue from young control and obese rats: **(a)** the sections of young control rat showing normal size adipocytes (H & E, x100). **(b)**the sections of young rats provided with high fat diet showing widespread adipocyte hypertrophy ( ) with few inflammatory cells (wavy arrow) (H & E, x100).
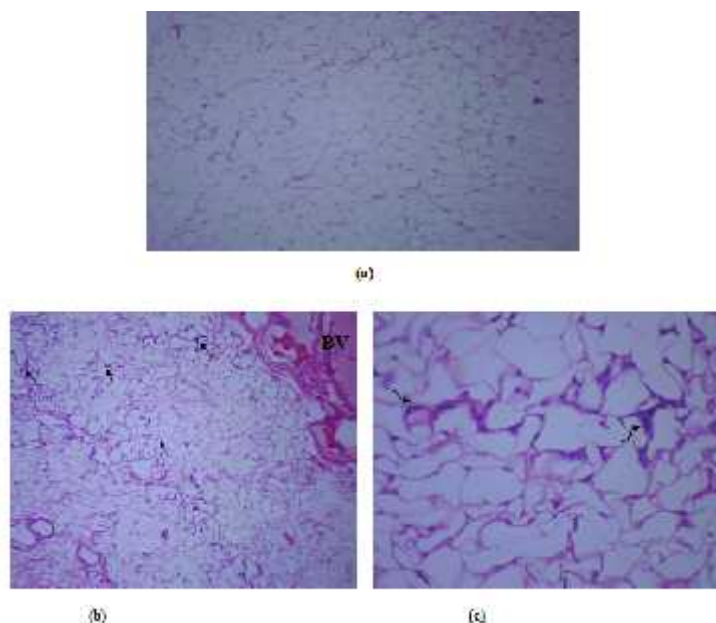

Fig. 6: Photomicrograph of haematoxylin and eosin-stained sections of white adipose tissue from adult control and obese rats: **(a)** the sections of adult control rat showing normal size adipocytes (H & E, x100). **(b)**the sections of adult rat provided with high fat diet showing irregular shaped adipocytes with moderate inflammation (wavy arrow) (H & E, x100). **(c)** In adult HFD sections, many crown-like structures (dash arrow) and large congested blood vessel (BV) are observed (H & E, x400).
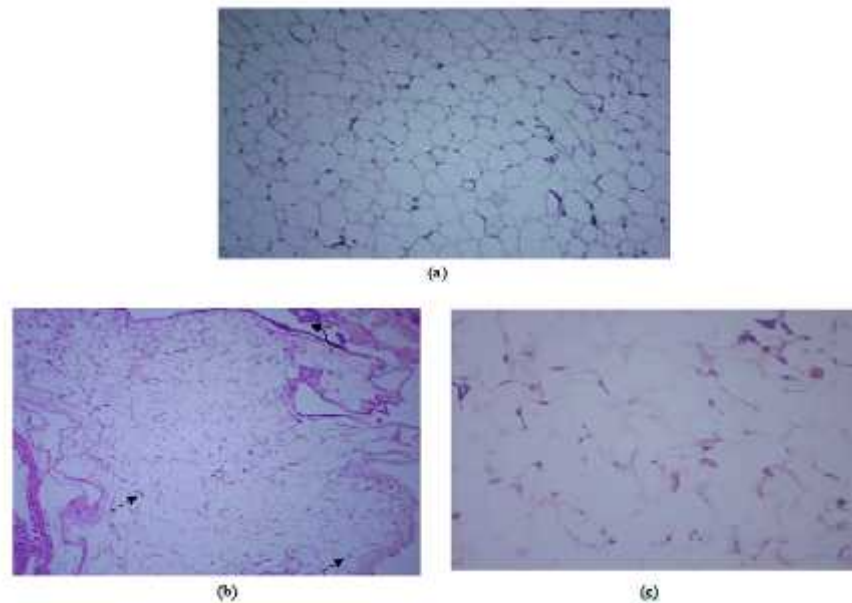
Fig. 7: Photomicrograph of haematoxylin and eosin-stained sections of white adipose tissue from old control and obese rats: (a) the sections of old control rat showing normal size adipocytes (H & E, x100). (b) the sections of old rat provided with high fat diet showing irregular shaped adipocytes with mild inflammation (wavy arrow) (H & E, x100). (c) In old HFD sections, few crown-like structures (dash arrow) are observed (H & E, x400).

4. Discussion

Obesity and aging are associated with adipose tissue (AT) dysfunction and low-grade inflammation, which play a major role in the development of metabolic diseases [2]. A better understanding of the mechanisms underlying AT inflammation in aging with diet-induced obesity is of great importance.

In the present study, it is clearly evident that young, adult and old-age rats fed a HFD showed significant increases in body weight, visceral fat, serum lipids, insulin resistance and inflammation as compared to their corresponding controls. The old obese group showed a higher increase in body weight and visceral fat weight, indicating abdominal AT expansion and higher fat deposition that occur with advanced age. This was explained previously by the redistribution of lipids from the subcutaneous to visceral fat compartment [2].

It has been reported that inflammation is caused by the inability of AT to buffer dietary lipids, leading to lipotoxicity mediated by excessive lipid deposition in the liver and skeletal muscle [18]. Oxidative stress and activation of serine threonine kinases such as c-jun N-terminal kinase (JNK), IkB kinase (IKK), and protein kinase C (PKC) are stimulated by lipotoxicity, resulting in disruption of insulin receptor signaling cascades and increased insulin resistance [19].

AT inflammation is characterized by increased infiltration and altered adipose tissue macrophages (ATMs)polarization, which results in systemic inflammation and peripheral insulin resistance in both obesity and aging[20]. ATMs have diverse biological roles and exhibit varying plasticity in lean versus obese subjects. Classically activated M1 macrophages (CD11c+) are the most common ATMs in obese people and dietary-induced obese animals and produce pro-inflammatory cytokines such as tumor necrosis factor (TNF), interleukin-1 (IL-1), and interleukin-6(IL-6). In lean mice, the alternatively activated M2

macrophages (CD11c-CD206+) are the predominant population of ATM and secrete anti-inflammatory cytokines such as interleukin-4 (IL-4), interleukin-10 (IL-10), and IL-1 receptor antagonist [21].

The present study showed that HFD-induced obesity in all groups demonstrated a significant higher expression of the M1 surface marker CD11c with significant higher levels of the pro-inflammatory cytokine IL-6 and lower levels of the anti-inflammatory cytokine IL-10 as compared to controls. These findings confirm the inflammatory state in obese groups as well as macrophage infiltration and phenotypic switch among resident macrophage towards the inflammatory phenotype. Surprisingly, the current findings showed a significant upregulation of the M2 surface marker CD206 expression in all age groups fed a HFD, which contradicts the majority of previous research[3,22]. These divergent results could be attributed to the selective induction or suppression of M2 genes induced by HFD feeding [23].

According to a prior study, increased CD11c expression promotes macrophage accumulation and/or activation in the arterial wall and contributes to the accumulation and activation of T cells, which accelerates AT inflammation and atherogenesis[24]. Additionally, mice lacking CD11c exhibited improvements in HFD-induced insulin resistance, glucose intolerance, and AT inflammation [24].

The molecular switches that control ATMs polarization are unclear. Wu et al., [25]proposed that eosinophils in mouse white AT could induce polarization of M2 macrophages via IL-4/IL-13-dependent pathway. Natural killer T cell activation

in obese AT could also induce polarization of alternatively activated M2 macrophage through IL-4/STAT6 signaling axis [26]. Adipocytes have also been suggested as a potential source of the Th2 cytokines that lead to the polarization of M2 macrophages in adipose tissues [27].

As a part of the inflammatory process, adipocyte hypertrophy and hypoxia can result in formation of a distinct histological structure known as a "crown-like structure," in which macrophages surround dead adipocytes to digest cell debris and residual lipids. This structure is used to quantify levels of inflammation in the adipose tissue [28]. At the histological level, the present findings showed more inflammation in the adult and old HFD groups. This is manifested by the presence of crown-like structures around dead adipocytes.

It has been suggested that numerous energy-sensing pathways and inflammation regulators play significant roles in obesity, ageing and theirassociated pathologies. These pathways are potential targets for anti-aging and anti-obesity medications.

AMPK and SIRT1 are critical nutrient sensors and inflammatory regulators. In obesity, they may serve as pathogenic factors that cause adipocyte formation and AT inflammation [5]. Through phosphorylation and deacetylation of peroxisome proliferative activated receptor gamma coactivator 1(PGC1), both AMPK and SIRT1 can enhance fatty acid oxidation and mitochondrial biogenesis [7].

Given the close association between AMPK and SIRT1 activation and their effects on inflammation related to obesity, the present study suggested that AMPK/SIRT1 pathway could have a role in obesity associating aging. Results showed

decreased expression of both AMPK and SIRT1 in the VAT of HFD induced obesity in different ages as compared to their corresponding control groups. Positive relationship between AMPK and SIRT1 in all HFD groups confirming the regulatory effect of AMPK on SIRT1.

In the VAT, decreased AMPK activity was closely associated with adiposity and inflammation in obese patients [29]or genetically and diet-induced obese rodents [30,31]. Previous studies have suggested that AMPK activation has metabolic functions in adipocytes that are associated with protection from obesity and related inflammation[5].

SIRT1 in adipocytes plays a more important role than SIRT1 in other tissues because it controls the expression and secretion of adipokines like adiponectin, MCP-1, and interleukin 4 [3].Recent research has shown close relationships between SIRT1 and inflammation, and inflammatory diseases have been linked to changes in SIRT1 expression and activity [6].The interplay between SIRT1 and AMPK is crucial in the inflammatory response. AMPK is a key inhibitor of NF-kB, and AMPK can inhibit NF- B indirectly through its downstream mediators such as SIRT1, forkhead box O (FoxO), and peroxisome proliferator-activated receptor gamma coactivator-1 (PGC-1)[32].

Activating SIRT1in mice throughgenetic manipulation or small pharmacological molecules has been shown to prevent HFD- or natural aging-associated metabolic dysfunctions [33,34]. The overexpression of a dominant-negative SIRT1 in the ATworsened hyperglycemia, dyslipidemia, ectopic lipid deposition, and insulin resistance, possibly as a result of aberrant chromatin remodelling and epigenetic modifications[35].Studies have reported that Sirt1 knockout in AT alters gene expression, contributing to obesity-related inflammation [36]. Moreover,it was also reported that SIRT1 activator can improve the health status and prolong life span of mice. In hypothalamus,aging candecrease SIRT activity, enhance leptin resistance and increase obesity[37]. Lu et al.[21]demonstrated that AMPK is a pro-longevity kinase and its activity in tissues declines with age, implying that AMPK activation is sufficient to regulate longevity and extend calorie restriction-induced lifespan in many organisms.

Despite the fact that many chemokines and factors have been proposed to trigger infiltration and activation of adipose-resident macrophages, the mechanisms behind increased production of these macrophage attractants remain unknown[38].

The present study suggested the role of AMPK/SIRT1 pathway as a triggering factor for macrophage infiltration. The reduced expression of both SIRT1and AMPK observed in AT of HFD groups could be considered as the driving force for macrophage infiltration and polarization and consequently increased inflammation and insulin resistance. This is clearly evident from the obtained negative association between both AMPK and SIRT1 with CD11c and IL-6 and HOMA-IR and also their positive association with CD206 and IL10.

It has been shown that AMPK has the ability tosuppress pro-inflammatory responses in macrophages and induce macrophage polarization toward an anti-inflammatory phenotype.In macrophages, anti-inflammatory cytokines including IL-10 and TGF can phosphorylate and

activate AMPK [19]. AMPK can boost SIRT1 by elevating NAD/NADH ratio and decreases ATMs infiltration and inflammation [39].

Similarly, Hui et al.[3] have reported that SIRT1 knockout in adipocytes but not macrophages results in aggravated infiltration of adipose-resident macrophages at an early stage of obesity. They clarified that such a change is caused byupregulated MCP1 expression and decreasedadiponectin production.

Previous in vitro experiments indicated that myeloid Sirt1 deletion induced M1 polarization and that SIRT1 expression was reduced in M1 macrophages and increased in M2 macrophages. After HFD feeding, mice showed increased number of F4/80, CD11b, and CD11c triple-positive ATMs. These findings suggested that Sirt1 deficiency induced a switch in the phenotype of ATMs to a more pro-inflammatory M1 subtype [40]. In human subjects, it was found that SIRT1 level is inversely associated with BMI and ATMs infiltration and that overexpressing SIRT1 significantly reduces obesity-induced ATMs infiltration [41].

The obtained findings indicate that reduced expression of AMPK/SIRT1 pathway in VAT is closely related to accumulation of VAT in HFD aged group. The observed negative association between SIRT1 and VAT weight in old HFD group could indicatethat aging associating obesity induces increased VAT inflammation with accumulation of pro-inflammatory immune cells that exacerbates the inflammatory status.

It has been reported that aging leads to a decline in immune competence, or "immunosenescence,"which contributes to or might be caused by chronic, low-grade inflammation,known as"inflammageing"[42].

Cellular senescencecould potentially link obesity and age-related VAT inflammation. Senescence is thought to be a distinct characteristic of aging, and active senescent cells can release various cytokines and chemokines in an NFKB dependent manner, which further trigger local inflammation[43].

During obesity, senescent cells have been shown to accumulate within the VAT, and their clearance is linked to improved metabolic parameters and reduced macrophage accumulation [44]. Expansion of visceral adipose depotsin morbid obesity is accompanied byan increased number of senescent-like macrophages, whichmay causeinflammation and a defective insulin signaling pathway in adipocytes, supporting a framework where senescent macrophages fuel obesity-induced systemic inflammationand maylead to the development of insulin resistance [45].

The severity of obesity- related metabolic disturbances in aging is debatable, with reduced [46,47,48]and aggravated severity reported [49]. In the present study, higher changes in the metabolic profile and IL-6 were more pronounced in the adult HFD group as compared to other HFD groups. However, the downregulation of CD11c expression, increased IL-10 levels and SIRT1 mRNA expression in old HFD rats indicate that old age is less vulnerable to metabolic dysfunction as compared to adults. Meanwhile, other inflammatory markers showed variable changes in each age group. A recent study by Moreno-Fernandez et al.[50] explained that increased splenic frequency of regulatory T (Treg) cells and circulating IL-10 levels were linked to less severe metabolic derangements in old mice. Thus,

increased metabolic dysfunction and inflammation in obese rats of different ages appear to be more linked to obesity than aging. Further studies are required to investigate the expression of other inflammatory genes and ATMs surface markers to specify changes in each age.

5. Conclusions

Findings of the present study indicate that aging associating diet-induced obesity is characterized by alterations of the immune landscape and display an activation of chronic inflammatory status, which may lead to metabolic dysfunctions. AMPK/SIRT1 pathway is suggested to be a crucial mediator of macrophage infiltration and polarization inducing inflammatory responses. Targeting this pathway through pharmacological activation or dietary control may be an effective anti-inflammatory therapeutic approach for treating obesity and aging–related diseases.

Abbreviations

Adenosine monophosphate-activated protein kinase (AMPK), Silent information regulator 1 (SIRT1), Adipose tissue macrophages (ATMs), cluster of differentiation 11c (CD11c), cluster of differentiation 206 (CD206).

Declarations:

Ethics approval and consent to participate:

The protocols used in this study were approved by the Research Ethical Committee of the Medical Research Institute of Alexandria University (Approval reference number: 0122272231).

Consent for publication:

Not applicable.

Availability of data and materials:

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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