Low-dose aspirin improves glucose uptake and attenuates inflammation in rats fed high-fat diet

Ahmed Abdelsadik[a], Mohamed M. Amin[b]

[a]Department of Zoology, Faculty of Science, [b]Department of Microbiology and Immunology, Faculty of Medicine, Aswan University, Aswan, Egypt

Correspondence to Ahmed Abdelsadik, PhD, Department of Zoology, Faculty of Science, Aswan University, Aswan, 81528, Egypt. Tel: +20 103 333 6675; fax: 0973480447; e-mail: aabdelsadik@aswu.edu.eg

Received 9 April 2018
Accepted 1 August 2018

Egyptian Pharmaceutical Journal 2018, 17:171–179

Background
Type 2 diabetes (T2D) is hallmarked by insulin resistance and pancreatic gradual islet β-cell dysfunction and hyperglycemia. Consumption of large quantities of high-fat diet (HFD) activates the caspase-pathway. This leads to apoptotic degradation of pancreatic β-cell and eventually progression to T2D or worsening of diabetes.
Aims
We aimed to assess the antidiabetic effects of aspirin when administered at low doses on insulin sensitivity and glucose uptake.
Materials and methods
A rat model fed HFD has been reported as an approved model of T2D. A total of 40 animals were assigned into four groups: controlled diet, HFD, controlled diet with aspirin, and HFD with aspirin. Hyperglycemia was confirmed by estimating the blood glucose levels. The diabetic rats were orally treated with low dose of aspirin 20 mg/kg body weight, for 70 days. At the end of the experiment, some biochemical parameters, the oxidative stress, and gene expression levels were quantified.
Results
Current data indicated that aspirin improved different physiological and immunological parameters. These include reduction of low-density lipoprotein and total cholesterol and improved glucose uptake via up-regulation of GLUT4 expression. Moreover, aspirin reduced fat peroxidation and obliterated hemeoxygenase-1-dependent fat down-regulation and reestablished the expression of LUT4 and hemeoxygenase-1 to the basal level.
Conclusions
In conclusion, this study confirmed the anti-inflammatory effect of aspirin and suggested that low-dose aspirin enhanced the metabolism of glucose and increased insulin sensitivity by suppression of inflammation and oxidative stress.

Keywords:
aspirin, glucose uptake, hemeoxygenase-1, high-fat diet, type 2 diabetes, inflammation, reactive oxygen species

Egypt Pharmaceut J 17:171–179

1687-4315

Introduction

Lifestyle interventions, genetic variations, and socio-economic considerations are major players in the progression of type 2 diabetes (T2D) [1–3]. Together, these factors induce free radical production and cellular and DNA damage, which terminally ends with gradual β-cell dysfunction, numbness for insulin, and ultimately T2D [4].

Interestingly, high glucose intake itself does not induce nuclear factor kappa-B (NF-kB) in rat or human β-cells *in vitro* or *in vivo* and consequently does not induce β-cell apoptosis [5]. Conversely, consumption of high amounts of fats increases blood triglycerides (TGs) and free fatty acids (FFAs), which can cause β-cell apoptosis via endoplasmic reticulum stress [6,7]. So, the consequence of high-fat diet (HFD) intake is potentiating insulin insufficiency to conquer insulin resistance and eventually T2D progression [8–10]. Moreover, chaperoned hormonal secretion variability such as reduction of the incretin glucagon-like peptide 1, as well as adiponectin and leptin secretion also contributes to hyperglycemia and insulin resistance [11].

Obese people predominantly have higher levels of plasma FFAs, which were identified as the main cause of peripheral insulin resistance and reactive oxygen species (ROS) overproduction, which consequently induce inflammasomes and cytokine production. Together, these factors induce lipotoxicity, reduction of insulin secretion, and β-cell dysfunction [12]. Previously mentioned observations suggest nutrients and inflammations as key components in pathogenesis of T2D.

Aspirin is an ideal candidate drug that can reduce the inflammatory response in case of obese patients and can prevent gradual decrease in insulin sensitivity, which is a worthy demand can reduce T2D progression without the risk of hypoglycemia [13].

Although administration of aspirin at high doses decreased risk for pancreatic cancer and abolished hypertriglyceridemia, long-term use causes gastrointestinal bleeding. However, the effects of low-dose aspirin are still a scientific and clinical debate [14].

Considering oxidative stress a direct byproduct of hyperglycemia, accumulation of ROS in turn causes cytotoxic effects, so it is considered a rudimentary source for cellular injury and microvascular and macrovascular diseases in diabetic patients [15]. As obese individuals have high levels of ROS, inflammasomes, and cytokine, we tackled the effects of using low-dose aspirin on ROS, inflammation, and glucose uptake in obese rats.

Materials and methods

Chemicals and reagents

Aspirin

Acetyl salicylic acid was purchased from Southern Egypt Drug Industries Company (6th October, Egypt) and 2', 7'-dichlorofluorescein ACS reagent from Sigma (St. Louis, MO, USA).

Animals

A total of 40 juvenile white rats (40–50 g) were obtained from Qena breeding center (Qena, Egypt) and were housed in individual tub cages at 25±1°C and 12 h light/12 h dark cycle. All animals had free access to food and water *ad libitum* for 1 week before being assigned to the experimental groups. All procedures were approved by the internal animal care and use committee of the University of Aswan, and complied with the guide for care and use of laboratory animals.

Diets

Standard diet consisted of 12.3% lipids, 63.3% carbohydrates, and 24.4% proteins, and HFD consisted of 41.5% lipids (19 g of butter oil and 1 g of soybean oil to provide essential fatty acids), 40.2% carbohydrates, and 18.3% proteins as described earlier elsewhere [16].

Ethical approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Dietary manipulations and experimental groups

Male rats were matched by basic body weight and allocated for 70 days under different treatments. A low-dose aspirin (20 mg/kg) daily was intragastrically administrated as hereunder described.

Group I had control diet (CD), group II had HFD, group III had control diet+20 mg aspirin/kg/day (CDA), and group IV had HFD+20 mg aspirin/kg/day (HFDA). First, we fed the rats with HFD to induce obesity and insulin resistance as previously shown [13] (Table 1).

Serum sampling

Animals were fasted for 10–12 h, anaesthetized, and killed. Blood samples were collected, and sera were obtained by centrifugation for 15 min at the speed of 4000*g* at 4°C.

Biochemical assays

To assess the effect of low-dose aspirin on the levels of glucose in the serum, insulin plasma level was assessed, and homeostatic model assessment of insulin

Table 1 Experimental study design and doses of aspirin used in the experiment

Groups	Food	Drug	Dosage	Concentration
Control diet	CD		–	–
High-fat diet	HFD		–	–
Control diet+aspirin	CDA	Aspirin	20 mg/kg	5 mg/mL
High-fat diet+aspirin	HFDA	Aspirin	20 mg/kg	5 mg/mL

The experimental groups were divided into four groups, with eight rats each. First group is the control group that received control diet (CD), the second group fed control diet in combination with 20 mg/kg/day aspirin (CDA). The other groups received high-fat diet with or without aspirin HFD and HFDA, respectively. Drug applied according to the individuals' body weight for 12 weeks.

Table 2 Blood lipids levels after drugs administration for 12 weeks

Variables	CD	HFD	CDA	HFDA
Body weight (g)	265±12	318±23	255±15	324±19
TG (mmol/L)	0.54 ±0.02	0.81±0.04^{a}	0.5±0.02	0.76 ±0.03^{b,cc}
HDL (mmol/L)	0.56 ±0.08	0.56±0.05^{a}	0.63 ±0.06	0.63±0.07^{b}
LDL (mmol/L)	0.23 ±0.03	0.42 ±0.07^{aa}	0.2±0.03	0.3 ±0.03^{bb,cc}
TCH (mmol/L)	1.14 ±0.11	1.98 ±0.32^{aa}	1.06 ±0.27^{cc}	1.77 ±0.09^{bb}
FFAs (mmol/L)	0.49 ±0.05	0.88 ±0.20^{aa,cc}	0.46 ±0.07	0.58±0.10^{b}

Blood lipids profile were measured in model groups that include; Triglyceride TG, high density lipoprotein HDL, low density lipoprotein LDL, total cholesterol TCH and free fatty acids FFAs. The blood lipid levels were reduced in groups treated with Aspirin in various susceptibilities. $^{a}P< 0.05$, $^{aa}P< 0.01$ significant difference compared to control group; $^{b}P<0.05$, $^{bb}P<0.01$ significant difference compared to HFD group; $^{c}P< 0.05$, $^{cc}P<$ 0.01 significant difference compared to CDA.

resistance (HOMA-IR) was computed from glucose and insulin levels as previously reported [17]. TG, total cholesterol (TCH), low-density lipoprotein (LDL), high-density lipoprotein (HDL), and bilirubin were determined using commercial assay kits from the Egyptian company for Biotechnology (Cairo, Egypt) according to the manufacture's instruction. Total serum FFAs were determined as previously reported [18]. In brief, to determine the serum FFAs, lipids were extracted from serum into chloroform; the phospholipids were precipitated with acetone and $MgCl_2$ in water bath; and the FFA concentration in the supernatant was determined by formation of copper soaps. The copper was assayed colorimetrically at 440 nm (Table 2).

Insulin-tolerance tests

Rats from each subgroup were inhibited from feeding for 5–6 h at the beginning of the light period. Blood was sampled before and 0, 30, 60, 90, and 60 min after an intraperitoneal injection of regular insulin (0.5 pool/kg in 1 ml saline), and glucose concentration was determined with a handheld glucometer (Contour next; Bayer Vital Co., Basel, Switzerland). Area under the curve (AUC) and HOMA-IR values were calculated as formerly described [19].

Determination of gene expression in skeletal muscles

Gene expression of hemeoxygenase-1 (HO-1), GLUT4, and NF-kB was assessed in the soleus muscles using qRT-PCR studies. Reactions were done in triplicate using a Gene Amp PCR system 400 thermal cycler (Perkin-Elmer Corp., Norwalk, Connecticut, USA), using specific primers as previously described [20–22], as shown in Table 3. All data were normalized to fold changes, using $2^{-\Delta\Delta C}$, compared with control.

Detection of reactive oxygen species in skeletal muscles

Levels of ROS in soleus muscles were assessed as previously described. In brief, 2',7'-dichlorofluorescein dye was used as a probe, and the fluorescence was detected at 490/520 nm. The optical density (a relative fluorescence unit) was adjusted to samples protein concentrations and was defined as relative fluorescence unit/μg protein [23].

Statistical analysis

Data were analyzed by parametric statistics (analysis of variance and repeated measures analysis of variance and *t* tests, with Tukey's test used as appropriate) as described for each experiment, with set at *P* value less than 0.05, two-tailed.

Results

Effects of low-dose aspirin on body weight

The group that was fed HFD exhibited a severe gain in body weight when compared with those of the CD group. The group at 6 weeks after HFD feeding reached a statistical difference significance ($P \leq 0.01$) relative to control. However, aspirin gavages for 12 weeks for HFDA and CDA groups showed no difference when compared with nonaspirin gavaged rats for HFD and CD groups ($P < 0.7358$ and < 0.3376, respectively).

Effects of low-dose aspirin on lipid profile

Currently, displayed data showed that HFD elevated basal plasma FFAs levels by 1.8 fold, and this elevation was diminished 1.2 and 1.5 times in HFDA and CDA, respectively. During the experiment, the plasma FFA levels in fat feeding groups were 80% higher in comparison with those in the CD group ($P \leq 0.001$). Although there were no significantly changes in CD and CDA aspirin-treated groups, the FFAs levels were decreased 34% in HFDA relatively to HFD group ($P \leq 0.01$), as shown in Table 2.

The high-fat intake was accompanied with hyperlipidemia that included increase in serum TG, TCH, and LDL, whereas no change in the levels of HDL. However, aspirin slightly improved the levels of HDL and reduced the levels of LDL by 12% as well as the levels of TG, TCH, and LDL.

Generally, the HFD group showed a noteworthy rise in most blood lipid indexes in comparison with the control group ($P < 0.001$). On the contrary, the HFDA group showed slightly improvement in the lipid profile compared with CD and CDA, as shown in Table 2.

Table 3 Sequence of the primers used in the qRT-PCR experiments

Target gene	Forward	Reverse	References
β-actin	5′-TCATCACTATCGGCAATGAGCGGt-3′	5′-ACAGCACTGTGTTGGCATAGAGGt-3′	[20]
GLUT4	5′-AATGAGCGGTTTGAATGGGACCTG-3′	5′-AACCGCCCTGTCTCTGTCATCTA-3′	[20]
NF-kB	5′-CATGCGTTTCCGTTACAAGTGCGA-3′	5′-TGGGTGCGTCTTAGTGGTATCTGT-3′	[21]
HO-1	5′-CGTGCAGAGAATTCTGAGTTC-3′	5′-AGACGCTTTACGTAGTGCTG-3′	[22]

HO-1, hemeoxygenase-1; NF-kB, nuclear factor kappa-B.

Low-dose aspirin improves insulin sensitivity

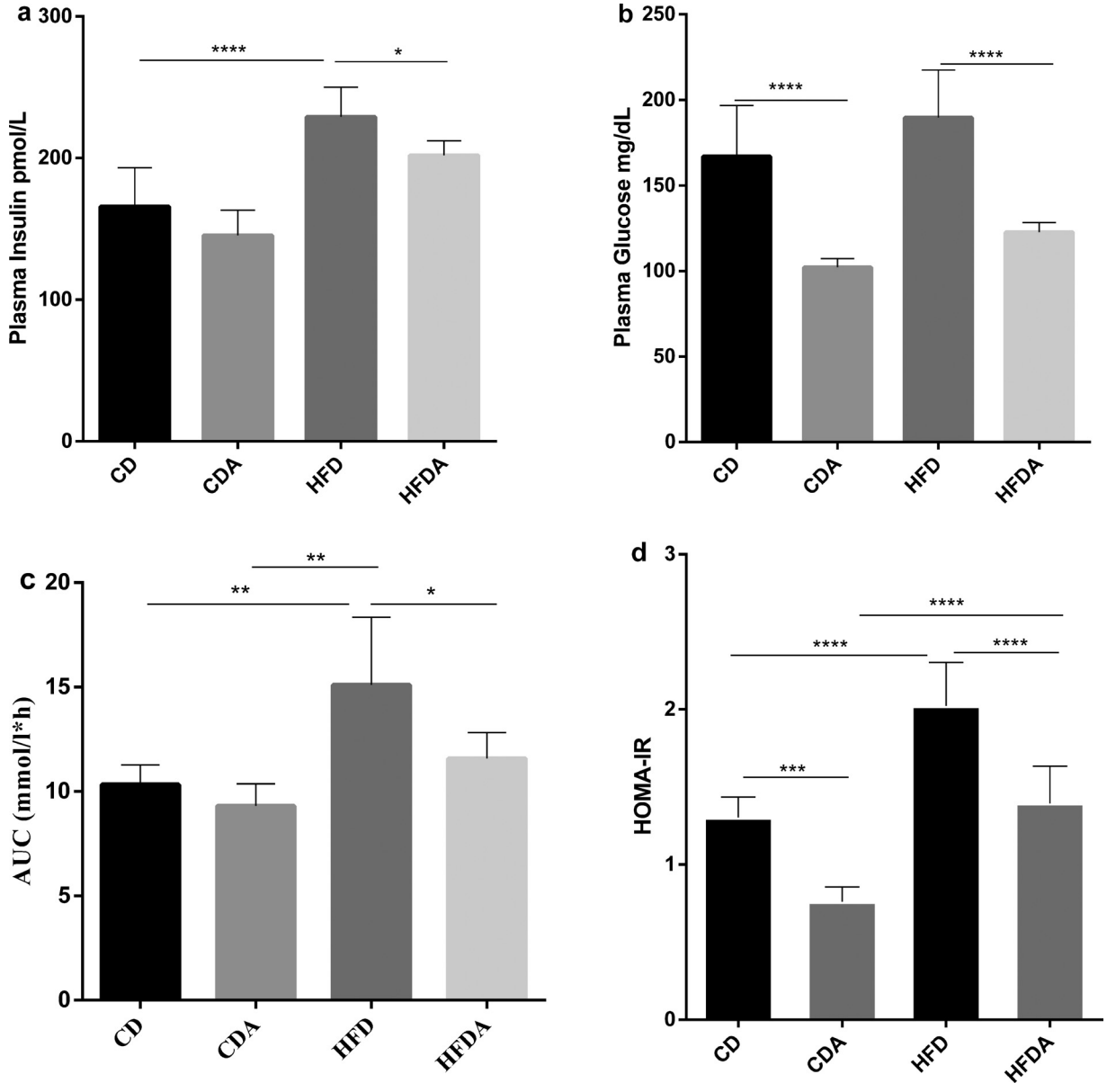
We found that low-dose aspirin in control groups had no observed effect on the level of glucose, because the difference between control and aspirin-treated groups were not significant as expected (Fig. 1). However, a significant effect can be observed between CD and HFD groups shown by Oral Glucose Tolerance Test curve (Fig. 2), and it seems the difference is owing to high-fat intake, not aspirin administration. Meanwhile, AUC is significantly higher in HFD when compared with HFDA ($P \leq 0.001$), and CD group was less significant ($P \leq 0.01$) compared with CDA group.

Serum insulin level was higher in the HFD group when compared with the HFDA group ($P \leq 0.03$) and much lower in CDA when compared with CD group ($P < 0.001$) (Fig. 1). Moreover, HOMA-IR showed that HFD group showed 55 and 45% higher insulin resistance than CD and HFDA groups, and CDA has shown 42, 62, and 46% lower resistance than CD, HFD, and HFDA, respectively.

Aspirin ameliorates GLUT4 production in skeletal muscles

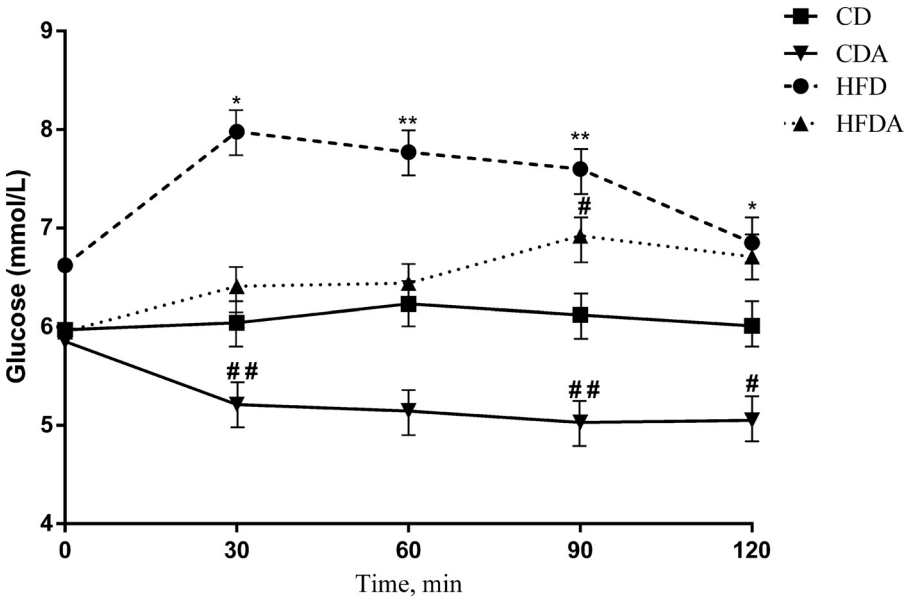
Transcriptional data indicated that aspirin enhances the GLUT4 expression in HFDA and CDA groups

Fig. 1



Plasma insulin (a), glucose (b), area under curve (c) and homeostatic model assessment of insulin resistance (d) were measured or calculated as previously described. Data are presented as mean±standard error of the mean. *$P<0.01$; **$P<0.01$; and ****$P<0.0001$. CD, control diet; CDA, control diet+20 mg aspirin/kg/day; HFD, high-fat diet; HFDA, HFD+20 mg aspirin/kg/day.

Fig. 2



Oral Glucose Tolerance Test (OGTT). Insulin curve of OGTT in rats fed control diet only (CD) or with aspirin (CDA), high-fat diet only (HFD) or combined with aspirin (HFDA).CD group showed no response to insulin when compared to HFD group, while CDA group showed greater response to insulin injection $^{\#}P<0.05$, $^{\#\#}P< 0.01$. Group fed high fat showed moderate response and HFD group showed the lowest response to insulin $^{*}P< 0.05$, $^{**}P< 0.01$. Data are presented as mean±standard error of the mean.

about 2.66 and 1.16 folds in comparison with their compartments, respectively (Fig. 3a).

Aspirin ameliorates high-fat diet-induced reactive oxygen species and suppresses activation of nuclear factor kappa-B

Data revealed that HFD group showed 1.46 fold increasing in NF-kB of the muscle in comparison with HFDA ($P\leq0.0392$). It is worthwhile, noting that the level of NF-kB of the HFDA with aspirin has been restored to the basal levels as in CD group ($P\leq0.9789$), suggesting a great sensitivity of NF-kB toward aspirin treatment (Fig. 3b).

As shown in Fig. 3c, HFD induced ~45% elevation of serum malondialdehyde (MDA) level, whereas aspirin gavages completely reversed this elevation to approximately the basal levels of MDA in CD group ($P\leq0.9142$). Furthermore, the ROS level was increased in the skeletal muscle of HFD-fed rat ~1.45-fold when compared with that in the CD rat. Aspirin administration decreased muscular ROS content to the level that was comparable with that in the CD rat (Fig. 3d). Furthermore, aspirin treatment abolished muscular ROS content in comparison with HFD.

Low-dose aspirin induces hemeoxygenase-1 activity

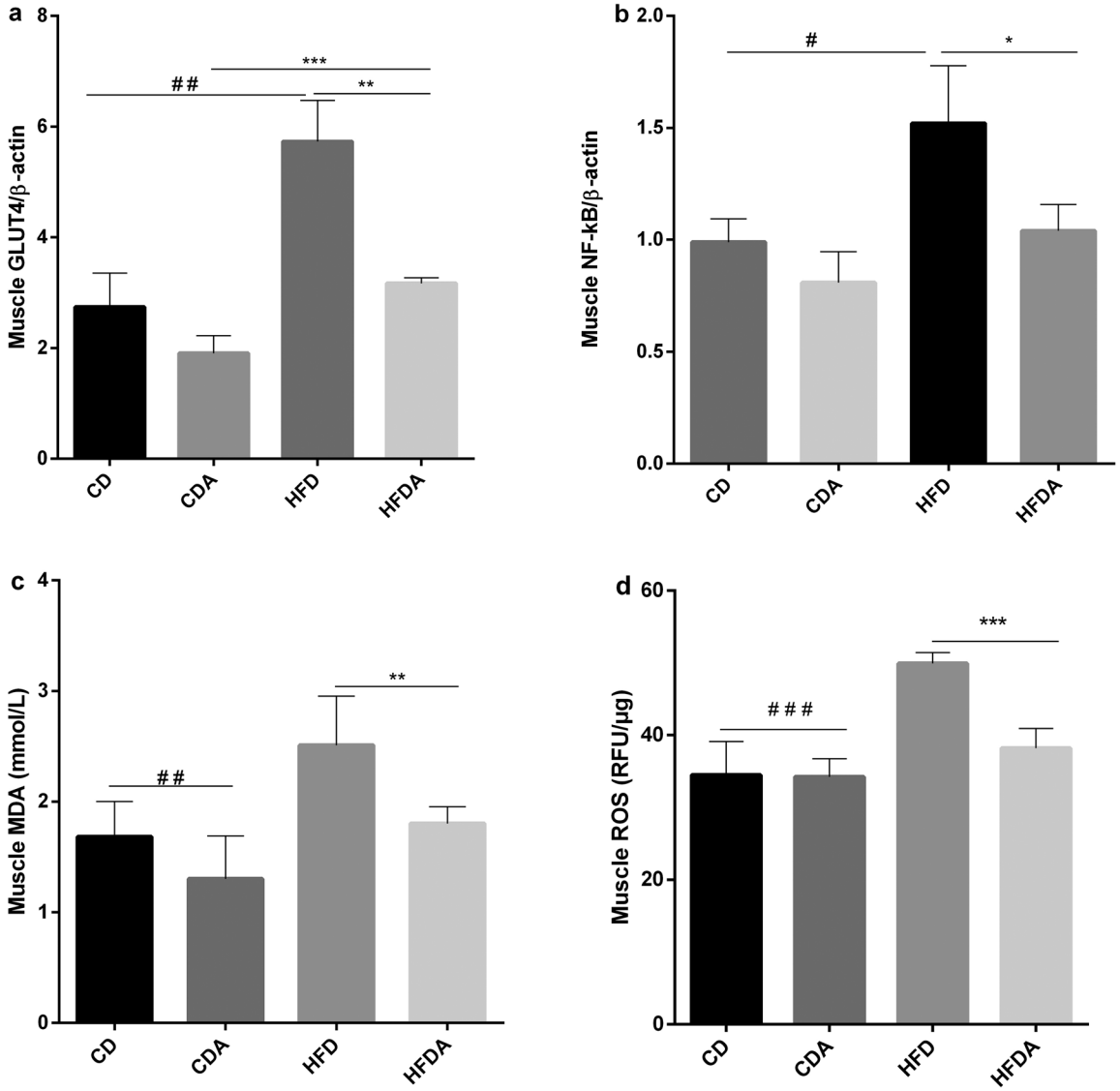
In the control group that received aspirin for 12 weeks, HO-1 mRNA levels were slightly changed in comparison with CD group ($P<0.05$). However, in the HFD group, the levels increased ~1.4 times compared with HFDA group ($P\leq0.001$) as shown in Fig. 4a. Furthermore, we analyzed bilirubin as an indicator for HO-1 activity in skeletal muscles [24]. However, HFD showed 30% lower bilirubin in comparison with CD ($P<0.0001$), and administration of aspirin increases bilirubin levels by 29% compared with HFD group ($P\leq0.0001$) displayed in Fig. 4b. Together these results evidenced that HFD downregulated HO-1 activity, and low-dose aspirin can restore HO-1 to its basal levels. Therefore, aspirin has a potential protective effect against tissue insult by activation of HO transcription and by exerting antioxidative effects.

Discussion

Aspirin is one of the most commonly consumed drug among NSAIDs and is characterized by high antioxidant selectivity [25]. Obesity is a common health problem that is determined by several factors, including modus vivendi like physical inactivity and HFD intake [26]. It was reported that levels of TGs were reduced in obese man and rodents upon treatment with aspirin at high doses [27,28]. Conversely, it can cause gastrointestinal bleeding over long-term use [29]. However, so far, the mechanistic effects of low-dose aspirin on glucose uptake are still poorly understood.

In the present study, we addressed the changes in lipid profile and total FFAs in plasma of groups treated with HFD with or without aspirin. Our data confirm that

Fig. 3



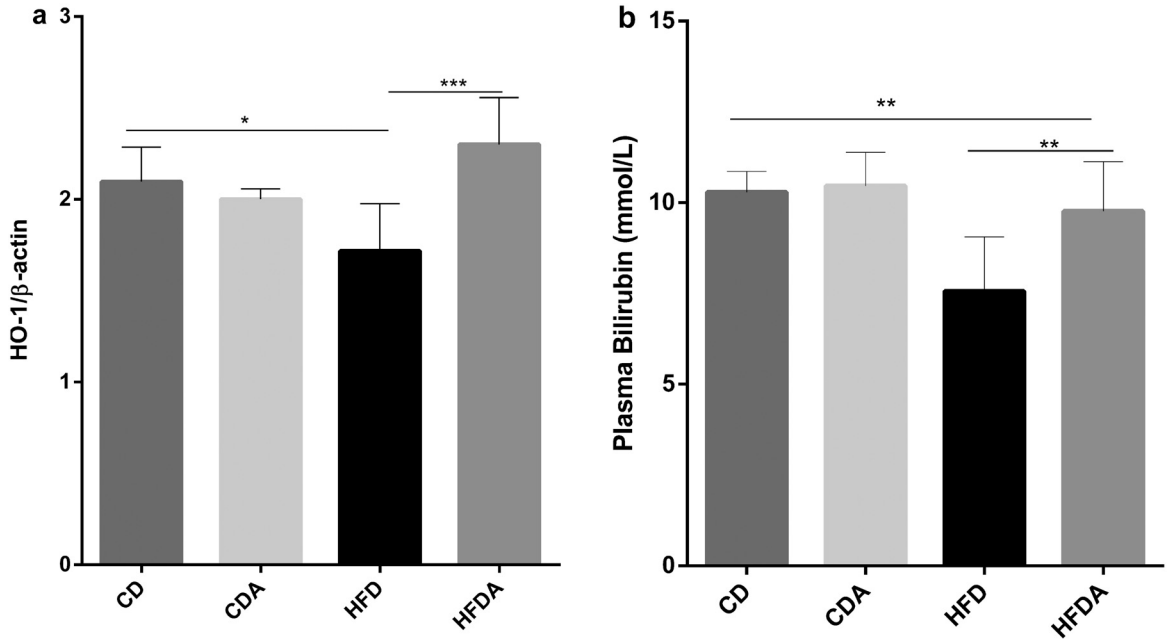
(a) Gene expression, MDA, and ROS in skeletal muscles. Low-dose aspirin restored GLUT4 to its approximate basal levels. (b) Gastrocnemius muscle NF-kB relative expression to beta-actin had the highest level in HFD group, which was reduced by aspirin treatment. (c) Gastrocnemius muscle MDA was evaluated, showing higher levels in the HFD. (d) Gastrocnemius muscle ROS was evaluated showing higher levels in the HFD. The results are presented as the mean±SE (n=8). To compare subgroup of the same treatment $^{*}P<0.05$, $^{**}P<0.01$, and $^{***}P<0.001$ and to compare with control $^{\#}P<0.05$, $^{\#\#}P<0.01$, and $^{\#\#\#}P<0.001$. CD, control diet; CDA, control diet+20 mg aspirin/kg/day; HFD, high-fat diet; HFDA, HFD+20 mg aspirin/kg/day; NF-kB, nuclear factor kappa-B; ROS, reactive oxygen species.

HFD worsens lipid profile by increasing LDL, TCH, and TG, in addition decreasing HDL levels in the plasma. On the contrary, aspirin treatment improves hypertriglyceridemia by reversing variables and reduced them approximately to the basal levels. These improvements in lipid profile are in consensus with former observations that indicated recovering from normal levels of plasma lipids subsequent to aspirin treatment in patients with T2D and in mice fed HFD [30].

A great reduction in FFAs has been recorded in the skeletal muscles upon treatment with low dosages of aspirin. This reduction in FFAs, which is a major link between obesity, insulin resistance, and T2D progression, is an indicator for the ameliorative outcome of aspirin in reduction the risk for insulin resistance and progression of T2D.

To test the hypothesis whether aspirin improves insulin sensitivity, levels of plasma insulin and glucose were measured and HOMA-IR was computed mathematically. It was observable that aspirin has a lowering effect on the level of glucose and this is owing to reducibility of aspirin on insulin levels and insulin resistance as shown by the decreased AUC and HOMA-IR. Together, these data indicated that aspirin gavages improved insulin sensitivity, reduced levels of serum insulin, and might have enhanced glucose uptake by the skeletal muscles [27,31,32].

Fig. 4



(a) Changes in HO-1 mRNA and levels of plasma bilirubin. HO-1 mRNA was decreased in HFD compared with CD, and aspirin ameliorates HO-1 activity. (b) Plasma bilirubin was decreased in HFD compared with CD, and aspirin ameliorates bilirubin activity. Data represent the mean±SE. *$P<0.05$, **$P<0.01$ and ***$P<0.001$. CD, control diet; CDA, control diet+20 mg aspirin/kg/day; HFD, high-fat diet; HFDA, HFD+20 mg aspirin/kg/day; HO-1, hemeoxygenase-1.

It would be interesting to elucidate the mechanism by which aspirin reduces the glucose clearance; therefore, we test GLUT4 transcription in skeletal muscles, the protein that allows glucose entry into muscle and adipose cells in presence of insulin in a cGMP-dependent mechanism [33]. It is noteworthy to mention that HFD reduces GLUT4 transcription and prevents its exocytosis in the skeletal muscles, which results in decreased GLUT4 levels at the plasma membrane and consequently prevents glucose shuttling into the cell.

The reduction of GLUT4 secretion in our study is matched by an increase in free radicals in rats fed HFD, suggesting that HFD reduces GLUT4 mRNA expression and increases free radicals activity and eventually inhibits glucose uptake in the peripheral tissues. In contrast to our data, Li *et al*. [34] suggest that ROS induces glucose uptake via AMPK pathway. We thought that this reduction in glucose uptake may be a defense mechanism against ROS-induced cell death, as release of energy by insulin is accompanied with production of macromolar quantities of H_2O_2, which triggered more free radicals and enviably leading to cell death. Our results, in accordance with previous reports, indicated hyperglycemia-dependent ROS production [35].

Hyperglycemia induces various detrimental effects through triggering oxidative stress and inflammation, which are involved in the pathogenesis of multiple metabolic syndromes [36]. Our data indicated hyperglycemia was associated with hyperinsulinemia and higher levels of NF-kB along with higher ROS activation. These effects were abolished by administering aspirin at low doses, and in case of NF-kB, levels were restored to the basal levels as in CD group, suggesting great sensitivity of NF-kB towards aspirin treatment.

Restoring redox homeostasis induced by high-fat intake requires activation of the antioxidative system. HO system plays major roles in limiting tissue injure initiated by redox disequilibrium [37]. It is a cellular stress response molecule responsible for heme breakdown and the generation of bilirubin or carbon monoxide [38]. Present data indicated that HO-1 gene expression and plasma bilirubin levels in HFD groups paralleled with reduce bilirubin levels compared with HFDA group. Together these results evidenced that HFD downregulates HO-1 activity, and low-dose aspirin can restore HO to its basal levels. Therefore, aspirin has potential protective effects against ROS-induced tissue insults by activation of HO to exert the antioxidative effects [39–42].

In conclusion, raised lipid profile and oxidative stress triggered by HFD feeding has been attenuated by treatment with aspirin. In this study, aspirin appears

to act as a nonenzymatic hypoglycemic agent. This hypoglycemic action seems to drive through different mechanisms by reducing free radical toxicity via modulation of bilirubin and enhances glucose uptake in skeletal muscles by upregulation of GLUT4. Together these findings would place aspirin as the supportive therapy for the treatment of hyperglycemia, as well as oxidative stress-related diseases.

Acknowledgements

Dr Abdelsadik was involved in designing the experiments, collecting and analyzing the data, and writing the manuscript. Dr Amin participated in data collection, data analysis, and writing of the manuscript.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1 Hsu C, Lee C-H, Wahlqvist ML, Huang H-L, Chang H-Y, Chen L, *et al.* Poverty increases type 2 diabetes incidence and inequality of care despite universal health coverage. Diabetes Care 2012; 35:2286–2292.

2 Karter A. Race and ethnicity. Vital constructs Diabetes Res 2003; 26:2189–2193.

3 Gamboa-Meléndez M, Huerta-Chagoya A, Moreno-Macías H, Vázquez-Cárdenas P, Ordóñez-Sánchez ML, Rodríguez-Guillén R, *et al.* Contribution of common genetic variation to the risk of type 2 diabetes in the Mexican Mestizo population Diabetes 2012; 61:3314–3321.

4 Asmat U, Abad K, Ismail K. Diabetes mellitus and oxidative stress – a concise review. Saudi Pharm J 2016; 24:547–553.

5 Benzler J, Ganjam GK, Pretz D, Oelkrug R, Koch CE, Legler K, *et al.* Central inhibition of IKKβ/NF-κB signaling attenuates high-fat diet-induced obesity and glucose intolerance. Diabetes 2015; 64:2015–2027.

6 Cnop M, Welsh N, Jonas J-C, Lenzen S, Eizirik DL. Mechanisms of pancreatic β-cell death in type 1 and type 2 diabetes: many differences, few similarities. Diabetes 2005; 54:S97–S107.

7 Sharma R, Alonso L. Lipotoxicity in the pancreatic beta cell: not just survival and function, but proliferation as well?. Curr Diab Rep 2014; 14:492–492.

8 Sakuraba H, Mizukami H, Yagihashi N, Wada R, Hanyu C, Yagihashi S. Reduced β-cell mass and expression of oxidative stress-related DNA damage in the islet of Japanese type II diabetic patients. Diabetologia 2002; 45:85–96.

9 Butler A, Janson J, Bonner-Weir S, Ritzel R, Rizza RA, Butler PC. Beta-cell deficit and increased beta-cell apoptosis in humans with type 2 diabetes. Diabetes 2003; 52:102–110.

10 Pataky M, Wang H, Yu CS, Arias EB, Ploutz-Snyder RJ, Zheng X, *et al.* High-fat diet-induced insulin resistance in single skeletal muscle fibers is fiber type selective. Sci Rep 2017; 7:13642.

11 Wang G-X, Zhao X-Y, Lin JD. The brown fat secretome: metabolic functions beyond thermogenesis. Trends Endocrinol Metab 2015; 26:231–237.

12 Boden G. Obesity and free fatty acids (FFA). Endocrinol Metabol Clin North Am 2008; 37:635–6.

13 Lin H, Yen HW, Hsieh SL, An L, Shen KP. Low-dose aspirin ameliorated hyperlipidemia, adhesion molecule, and chemokine production induced by high-fat diet in Sprague-Dawley rats. Drug Dev Res 2014; 75:97–106.

14 Saito Y, Okada S, Ogawa H, Soejima H, Sakuma M, Nakayama M, *et al.* Low-dose aspirin for primary prevention of cardiovascular events in patients with type 2 diabetes: 10-year follow-up of a randomized controlled trial. Circulation 2016; 135:659–670.

15 Fiorentino T, Prioletta A, Zuo P, Folli F. Hyperglycemia-induced oxidative stress and its role in diabetes mellitus related cardiovascular diseases. Curr Pharm Des 2013; 19:5695–5703.

16 Frauloub J, Ogg-Diamantino R, Fernandes-Santos C, Barbosa Aguila M, Mandarim-de-Larceda C. A mouse model of metabolic syndrome, insulin resistance, fatty liver and non-alcoholic fatty pancreas disease (NAFDP) in C57BL/6 mice fed a high fat diet. J Clin Biochem Nutr 2010; 46:212–223.

17 Salgado AL, Carvalho Ld, Oliveira AC, Santos VN, Vieira JG, Parise ER. Insulin resistance index (HOMA-IR) in the differentiation of patients with non-alcoholic fatty liver disease and healthy individuals. Arq Gastroenterol 2010; 47:165–169.

18 Anstall H, Trujillo J. Determination of free fatty acids in plasma by a colorimetric procedure: an appraisal of the method and comparison with other technics. Clin Chem 1965; 11:741–747.

19 Xu J, Zhang W, Lu Z, Zhang F, Ding W. Airborne $PM_{2.5}$-induced hepatic insulin resistance by Nrf2/JNK-mediated signaling pathway. Int J Environ Res Public Health 2017; 14:piiE787.

20 Ndisang JF, Lane N, Jadhav A. The heme oxygenase system abates hyperglycemia in zucker diabetic fatty rats by potentiating insulin-sensitizing pathways. Endocrinology 2009; 150:2098–2108.

21 Ndisang JF, Lane N, Jadhav A. Upregulation of the heme oxygenase system ameliorates postprandial and fasting hyperglycemia in type 2 diabetes. Am J Physiol Endocrinol Metab 2009; 296:E1029–E1041.

22 Long J, Wang S, Zhang Y, Liu X, Zhang H, Wang S. The therapeutic effect of vascular endothelial growth factor gene- or heme oxygenase-1 gene-modified endothelial progenitor cells on neovascularization of rat hindlimb ischemia model. J Vasc Surg 2013; 58:756–765.e2.

23 He H, Wang GY, Gao Y, Ling WH, Yu ZW. Curcumin attenuates Nrf2 signaling defect, oxidative stress in muscle and glucose intolerance in high fat diet-fed mice. World J Diabetes 2012; 3:94–104.

24 McCoubrey W. Detection of heme oxygenase 1 and 2 proteins and bilirubin formation. Curr Protoc Toxicol 1999; 00:9.3.1–9.3.9.

25 Jian Z, Tang L, Yi X, Liu B, Zhang Q. Aspirin induces Nrf2-mediated transcriptional activation of haem oxygenase-1 in protection of human melanocytes from H2O2-induced oxidative stress. J Cell Mol Med 2016; 20:1307–1318.

26 Sartorius B, Veerman LJ, Manyema M, Chola L, Hofman K. Determinants of obesity and associated population attributability, South Africa: empirical evidence from a National Panel Survey, 2008-2012. PLoS ONE 2015; 10: e0130218.

27 Hundal R, Petersen KF, Mayerson AB, Randhawa PS, Inzucchi S, Shoelson SE, *et al.* Mechanism by which high-dose aspirin improves glucose metabolism in type 2 diabetes. J Clin Invest 2002; 109:1321–1326.

28 Yuan M, Konstantopoulos N, Lee J, Hansen L, Li ZW, Karin M, *et al.* Reversal of obesity- and diet-induced insulin resistance with salicylates or targeted disruption of Ikkbeta. Science 2001; 293:1673–1677.

29 29.Huang E, Strate L, Ho W, Lee SS, Chan AT. Long term use of aspirin and the risk of gastrointestinal bleeding. Am J Med 2011; 124:426–433.

30 Van Diepen J, Vroegrijk IO, Berbée JF, Shoelson SE, Romijn JA, Havekes LM, *et al.* Aspirin reduces hypertriglyceridemia by lowering VLDL-triglyceride production in mice fed a high-fat diet. Am J Physiol Endocrinol Metab 2011; 301:1099–1107.

31 Fernández-Real JM, Ló pez-Bermejo A, Ropero AB, Piquer S, Nadal A, Bassols J, *et al.* Salicylates increase insulin secretion in healthy obese subjects. J Clin Endocrinol Metabol 2008; 93:2523–2530.

32 Amiri L, John A, Shafarin J, Adeghate E, Jayaprakash P, Yasin J, *et al.* Enhanced glucose tolerance and pancreatic beta cell function by low dose aspirin in hyperglycemic insulin-resistant type 2 diabetic Goto-Kakizaki (GK) rats. Cell Physiol Biochem 2015; 36:1939–1950.

33 Leto D, Saltiel A. Regulation of glucose transport by insulin: traffic control of GLUT4. Nat Rev Mol Cell Biol 2012; 13:383–396.

34 Li M, Zhao J, Hu Y, Lu H, Guo J. Oxygen free radicals regulate energy metabolism via AMPK pathway following cerebral ischemia. Neurol Res 2010; 32:779–784.

35 Yao D, Brownlee M. Hyperglycemia-induced reactive oxygen species increase expression of the receptor for advanced glycation end products (RAGE) and RAGE ligands. Diabetes 2010; 59:249–255.

36 McCracken E, Monaghan M, Sreenivasan S. Pathophysiology of the metabolic syndrome. Clin Dermatol 2017; 36:14–20.

37 Yachie A, Niida Y, Wada T, Igarashi N, Kaneda H, Toma T, *et al.* Oxidative stress causes enhanced endothelial cell injury in human heme oxygenase-1 deficiency. J Clin Invest 1999; 103:129–135.

38 Ansley D, Wang B. Oxidative stress and myocardial injury in the diabetic heart. J Pathol 2013; 229:232–241.

39 Bansal S, Biswas G, Avadhani NG. Mitochondria-targeted heme oxygenase-1 induces oxidative stress and mitochondrial dysfunction in macrophages, kidney fibroblasts and in chronic alcohol hepatotoxicity. Redox Biol 2014; 2:273–283.

40 Lin CY, Hsiao WC, Huang CJ, Kao CF, Hsu GS. Heme oxygenase-1 induction by the ROS–JNK pathway plays a role in aluminum-induced anemia. J Inorg Biochem 2013; 128: 221–228.

41 Vijayan V, Wagener FADTG, Immenschuh S. The macrophage heme-heme oxygenase-1 system and its role in inflammation. Biochem Pharmacol 2018; 153:159–167.

42 Hill-Kapturczak N, Thamilselvan V, Liu F, Nick HS, Agarwal A. Mechanism of heme oxygenase-1 gene induction by curcumin in human renal proximal tubule cells. Am J Physiol Renal Physiol 2001; 281:F851–F859.

(DUPHAT 2019: www.duphat.ae)