Original Article

Impaired Glucose Homeostasis Accompanies Cellular Changes in Endocrine Pancreas after Atorvastatin Administration

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Abstract

Atorvastatin (ATOR) has been reported to increase the risk for diabetes mellitus. Therefore, in the current study, we focused on studying the effect of ATOR on the structure of islets of Langerhans including their various cellular components as well as on glucose homeostasis. We detected a statistically significant increase ($P < 0.05$) in β-cell mass and percentage with a significant decrease in α-cell area and percentage in animals that received ATOR compared to control ones. In addition, a statistically significant increase ($P < 0.05$) in the β-cell proliferation was observed in the ATOR group with negligible change in expression of inflammatory cytokines of the islets. A significant downregulation in apoptosis alongside a significant upregulation in anti-apoptosis were detected in islets of animals treated with ATOR. Moreover, there was a significant impairment in various parameters of glucose homeostasis in the ATOR-treated group. Therefore, ATOR may induce insulin resistance-like state that was demarcated at cellular as well as at biochemical levels with little or no inflammatory response.

Keywords: Apoptosis, atorvastatin, diabetes, insulin resistance, islets neogenesis, β-cell proliferation, β-cells

Highlights

- Atorvastatin (ATOR) significantly increases most of the islet and β-cell parameters probably as a compensatory response to the induced insulin resistance-like state
- ATOR significantly decreases α-cell percentage and area in the islets
- ATOR significantly increases the proliferative activity of β-cells in the islets with suppression of apoptosis and upregulation of antiapoptosis
- The local inflammatory cytokines are insignificantly changed by ATOR
- Glucose homeostasis parameters are significantly impaired by ATOR.

Introduction

Statins are widely prescribed and recommended for an increasing number of patients that exceeds 56 million in the US.[1,2] Atorvastatin (ATOR) has become a mainstay drug in the prevention and treatment of atherosclerosis.[3] Its key pleiotropic effect is the anti-inflammatory one which may account for the cardiovascular (CV) risk reduction.[4] Despite the diabetogenicity of different statins, their benefits in reducing CV events evidently outweigh the risk of diabetes.[5,6] Therefore, they should be continued in acute coronary syndrome.[7] including acute myocardial infarction, even with the possible vulnerability to new-onset diabetes (NOD).[2] Nonetheless, statins as primary prevention without CV risk is still debatable with a need for further studies putting in mind NOD as a key adverse effect.[8]

Statins are reported to be associated with type 2 diabetes (T2D).[9] which is considered a global epidemic.[3]

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Their increased risk of diabetes is widely variable in different studies and may reach 27%.[7] with a potential underestimation.[5] Although statins are generally safe and well-tolerated, their potential diabetogenic effect differs among various statins.[10] The risk of diabetes is highest with lovastatin, ATOR, simvastatin, and fluvastatin, whereas pravastatin seems to be the least diabetogenic.[5,11,12] Moreover, ATOR is associated with increased glycosylated hemoglobin (HbA1c) levels in patients receiving intensive but not moderate therapy.[2] with the recommendation to include a warning of the increased risk of diabetes on its labels.[10,12] Yet, the exact mechanism for diabetogenicity of statins remains unclear.[6,13]

There is conflicting evidence regarding the effects of statins on insulin sensitivity and the worsening of insulin resistance (IR) and secretion.[14] with few studies investigated statin-associated NOD.[12] *In vitro* studies demonstrate that statins may impair insulin sensitivity and insulin secretion by multiple mechanisms.[5] Statins as other HMG-CoA reductase inhibitors may impair insulin signaling, decrease adipocyte differentiation and inhibit insulin secretion from β-cells.[9] Interestingly, the proposed mechanism of statin-associated muscle inflammation and statin-induced IR may be closely related.[1]

The objective of this study was to clarify the impact of ATOR on the structure of islets of Langerhans (IOL) focusing on their hormone-secreting cells and key local apoptotic and inflammatory markers along with the assessment of glucose homeostasis in animals without cardiac or metabolic risk.

Materials and Methods

Ethical approval

Approval of all procedures of this research was obtained from the Ethical Committee of Mansoura College of Medicine; the proposal Code is MS.18.05.161, and approved on 06/06/2018.

Experimental animals and design of the study

Adult male Sprague–Dawley rats (n = 36, 235 ± 30 g) were brought from Mansoura College of Pharmacy Animal House. During the whole period of the experiment, rats were housed in standard plastic cages (2 rats in each cage) for 12 weeks with 12–12 h of light and dark cycles and temperature of 24°C ± 2°C. They were equally divided into two groups (n = 18 in each group); control, and ATOR (normal rats that received ATOR) groups. Both groups were fed standard animal chow. Rats of the control group received daily doses of vehicle (0.9% NaCl) and to those of the ATOR group ATOR was administrated (50 mg/kg) by intragastric gavage for 12 weeks. ATOR was freshly prepared by dissolving the purchased tablets (ATOR 10 mg; Eipico, Egypt) to 5 mg/ml in 0.9% NaCl. All solutions were administered at 10 ml/kg. From each animal of the two groups, the pancreas was dissected out at the end of the experiment. In addition, to perform the current study, an approval (Code MS.18.05.161) was issued by Mansoura College of Medicine Ethical Committee.

Histological procedure

The dissected out pancreas from rats of each group was fixed in formaldehyde (10%) and its tiny parts were

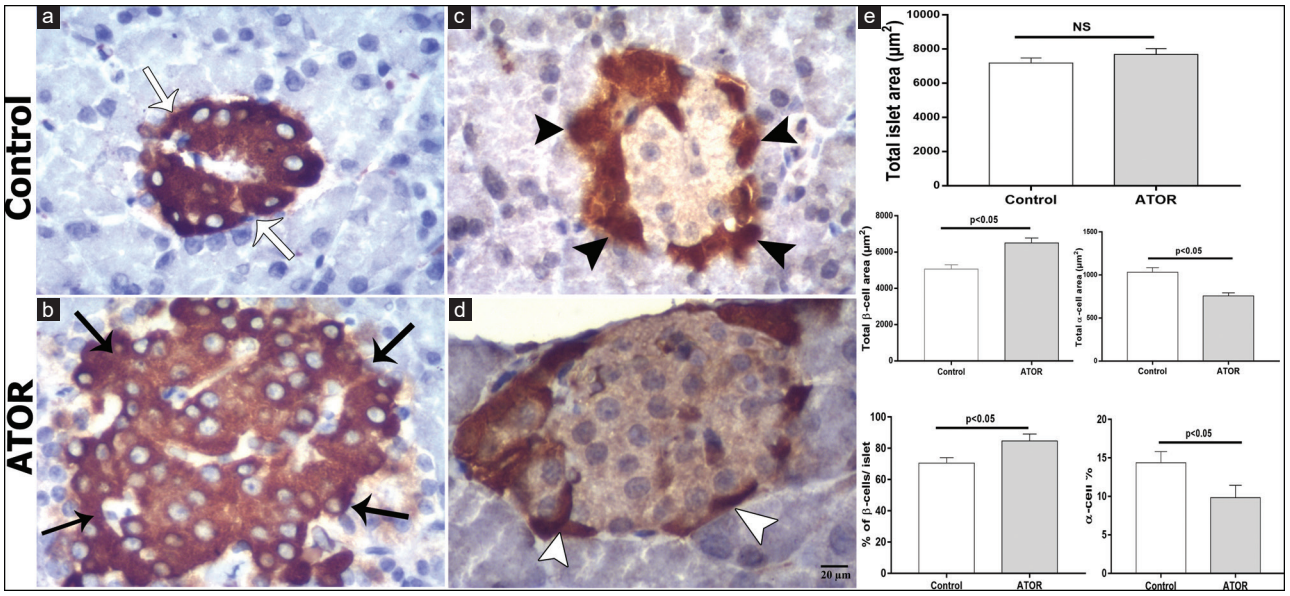

Figure 1: Immuno-histology of the islets in various groups. (IHC [a and b] anti-insulin; [c and d] anti-glucagon; bar is 20 μ). Data of the statistical morphometric analysis including total islet area (μm^2), β-cell area (μm^2), α-cell area (μm^2), β-cells percentage and α-cells percentage are represented on the right panel (e). Islet expansion was demarcated in the ATOR group with dominance of β-cell mass and relative shrinkage of α-cells (white arrow heads) compared to the control group (black arrow heads) (a-d). Although the total islet area was not significantly increased but we detected a statistically significant increase in the β-cell area, percentage of the β-cell per islet (black arrows vs. white arrows) with a decrease in the α-cell area and the percentage of α-cells in animals treated with ATOR compared to those that were not (e). Data are expressed as mean ± SEM (n = 18 rats in each group). $P < 0.05$ is significant. ATOR: Atorvastatin, SEM: Standard error of the mean, IHC: Immunohistochemistry

utilized to prepare paraffin blocks. From the latter, 3–4 μm sections were cut to be evaluated histologically as well as immunocytologically.

Immunocytology and morphometric assessment

Ki-67 is considered a reliable marker for proliferative activity in various settings including inflammatory and premalignant ones.[15,16] To evaluate the proliferation status together with cells secreting insulin (β) and those secreting glucagon (α), sections of the pancreas were stained specifically by the corresponding antibodies: Anti-Ki-67, anti-insulin, as well as antiglucagon antibodies respectively[17] [Supplementary Data, S1]. In addition, we performed morphometric assessments of various areas (i.e., that of the islet, β-cells as well as α-cells), β-cell percentage, α-cell percentage, mass of islets (mg), and mass of β-cells (mg) by Image J program (NIH's National Institute of Mental Health, Bethesda, Maryland) as formerly reported [Supplementary Data, S2].

β-cell proliferation evaluation

The proliferative activity was assessed previously by Ki67 antibodies in various metabolic[18,19] and non-metabolic diseases.[20] For β-cell proliferation assessment, $Ki67^{+}$ nuclei in the islets were counted and divided by the islet's total nuclei number. Moreover, we evaluated the neogenesis of β-cells by identifying islet cell frequency that immuno-stained by anti-insulin antibodies and buds from nearby pancreatic ducts as reported by literature.[21]

Local inflammatory cytokine assessment

The role played locally by cytokines has been demonstrated in pre-diabetes, diabetes[22] as well as various inflammatory[23] and pre-malignant conditions.[24,25] The pancreas was isolated from three animals in each study group; the supernatant from its islets was processed to measure the level of inflammatory cytokines which included: Interleukin (IL)-1 β, IL-6, IL-10, tumor necrosis factor-alpha (TNF-α), and transforming growth factor beta 1 (TGF-β1) through the ELISA procedure[17] [Supplementary Data, S3].

Glucose homeostasis evaluation

The levels of the fasting blood glucose (FBG), insulin, IR homeostasis model assessment index (HOMA-IR), and β-cell function homeostatic model assessment-%β were assessed at the end of the experiment.[26] In addition, the area under the curve (AUC) and oral glucose tolerance test (OGTT) were performed after administration of glucose (2.5 g/kg) by oral gavage.[27]

Real-time quantitative polymerase chain reaction assessment

Total RNA was extracted from islets of the pancreas by employing reagent for RNA isolation to evaluate key local inflammatory, apoptotic and anti-apoptotic genes. Afterward we performed reverse transcription by oligo(dt) priming according to instructions of the manufacturer. We added the used primer sequences in the supplementary data [Table S1].

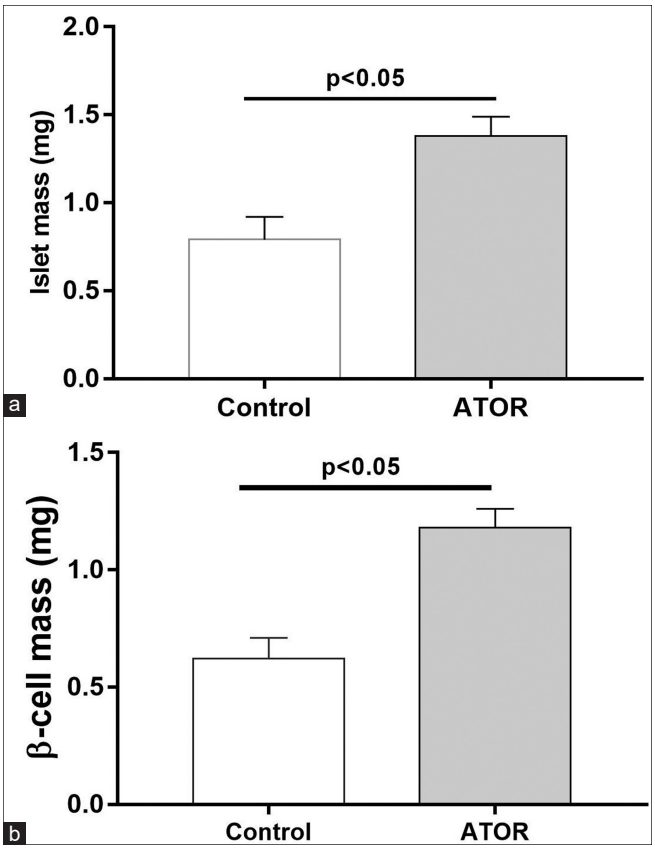

Figure 2: Representative graphs of islet mass (mg) and β-cell mass (mg) in various groups. They show a statistically significant increase in the mass of the islets (a) and β-cells (b) in animals treated with ATOR compared to control ones. Data are expressed as mean ± SEM (n = 18 rats in each group). $P < 0.05$ is considered significant. ATOR: Atorvastatin, SEM: Standard error of the mean

Then we carried out the protocol of polymerase chain reaction using the gene of β-actin as a housekeeping gene. In addition, we performed all reactions in a triplicate fashion [Supplementary Data, S4].

Statistical analysis

In the current study, we expressed data as mean ± standard error of the mean or standard deviation (SD). In addition, Student's t-test was employed to compare between data of different groups with considering $P < 0.05$ as statistically significant.

Results

Effect of atorvastatin on histology of the islets

We did not detect any obvious histological changes in the routine histology of the islets of the group treated with ATOR compared to those of the control apart from moderate increase in the size of islets but without any obvious inflammatory infiltration [Supplementary Figure 1].

Effect of atorvastatin on the cellular parameters of the islets

To semi-quantitatively assess the effect of ATOR on morphometric parameters of islets, β-cells, and α-cells, we

utilized the Image J program. Islet expansion was demarcated in the ATOR group with the dominance of β-cell mass and relative shrinkage of α-cells compared to the control group [Figure 1a-d]. This was statistically confirmed by the significant increase in the mass of the islets and β-cells in animals treated with ATOR compared to control ones [Figure 2 and Table S2]. Although the total islet area was not significantly increased, we detected a statistically significant increase in the β-cell area, percentage of the β-cell per islet with a decrease in the α-cell area, and the percentage of α-cells in animals treated with ATOR compared to those that were not [Figure 1e and Table S2].

Effect of atorvastatin on the proliferation of β-cells

To assess the effect of ATOR on β-cell proliferation, we used the Ki-67 marker as a proliferation index. We observed a statistically significant increase ($P < 0.05$) in the β-cell proliferation and neogenesis in the ATOR group compared with the control one [Figure 3].

Effect of atorvastatin on local inflammatory markers of the islets

Although there was a trend of rise in the level of local islet inflammatory cytokines in the group treated with ATOR, it did not reach a significant level ($P > 0.05$) compared to the control group [Figure 4a]. To confirm these data, we evaluated mRNA expression of the inflammatory markers (TNF-α, IL-6, and TLR-4) which revealed no significant differences between both groups [Figure 5].

Effect of atorvastatin on local apoptotic markers of the islets

To assess the effect of ATOR on local apoptosis of the islets, we evaluated levels of the key markers of apoptosis and anti-apoptosis, namely caspase-3 and Bcl-2. A statistically significant reduction in apoptosis represented by caspase-3 with a significant rise in the anti-apoptotic protein represented by Bcl-2 were detected in islets of animals treated with ATOR compared to those which did not [Figure 4b and c]. These data were further validated by assessing mRNA of caspase-3 and Bcl-2 which displayed significant downregulation of the former and a significant upregulation of the latter [Figure 6 and Table S3].

Effect of atorvastatin on glucose homeostasis parameters

There was a significant rise ($P < 0.05$) in the parameters of glucose homeostasis in ATOR-treated animals which included FBG level as well as that of insulin, HOMA-IR, and AUC accompanied by a significant decrease in percent function of β-cells compared to those of the control group. Moreover, OGTT impairment was observed in the ATOR-treated animals which exhibited a significant rise ($P < 0.05$) in blood glucose (BG) levels at different checked times [Figure 7 and Table S4].

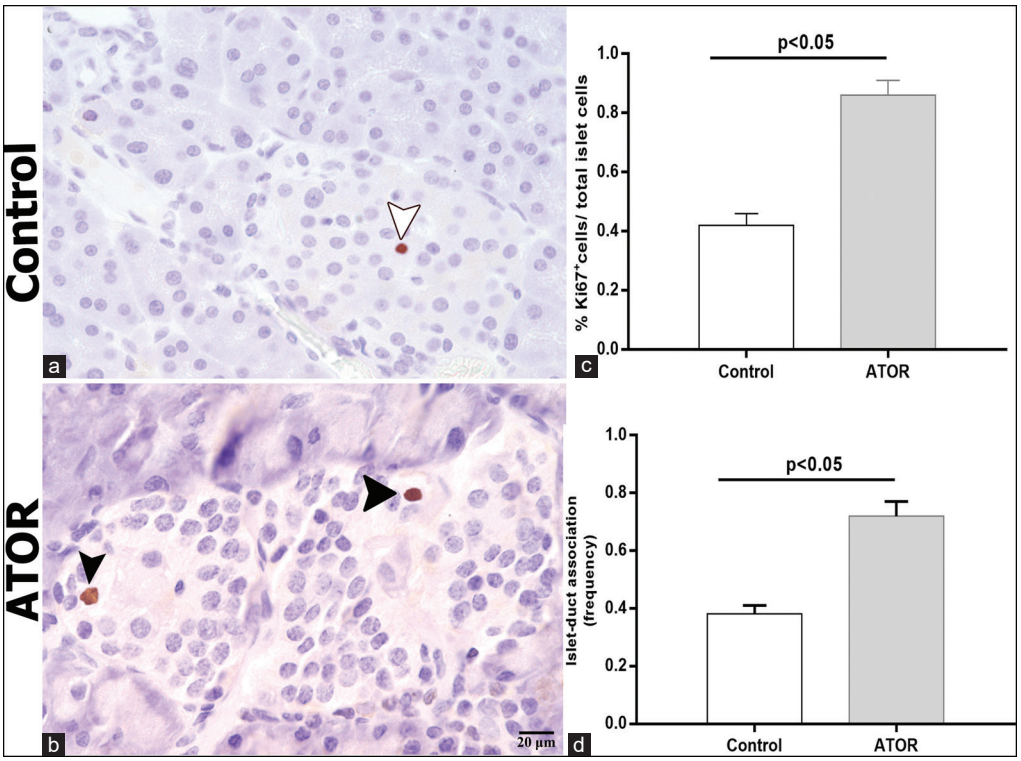

Figure 3: Representative graphs of β-cell proliferation index in various groups (a and b) with statistical evaluation of ki-67$^+$ cells per total islet cells and islet duct association (frequency) on left panel (c and d). A statistically significant increase ($P < 0.05$) in the β-cell proliferation and neogenesis was observed in the ATOR group (black arrow heads) compared with the control one (white arrow heads) (Anti-Ki-67 IHC; Bar is 20 μ). Data are expressed as mean ± SEM ($n = 18$ rats in each group). $P < 0.05$ is considered significant. ATOR: Atorvastatin, SEM: Standard error of the mean, IHC:

Discussion

We did not detect obvious histological changes in the routine histology of the islets of the group treated with ATOR from those of the control apart from moderate increase in the size of the islets but without any remarkable inflammatory infiltration. On contrary in the conventional prediabetes mellitus model induced by high-fat diet, low-grade inflammation is a key feature both local in the islets as well as in the adipose tissue.[21]

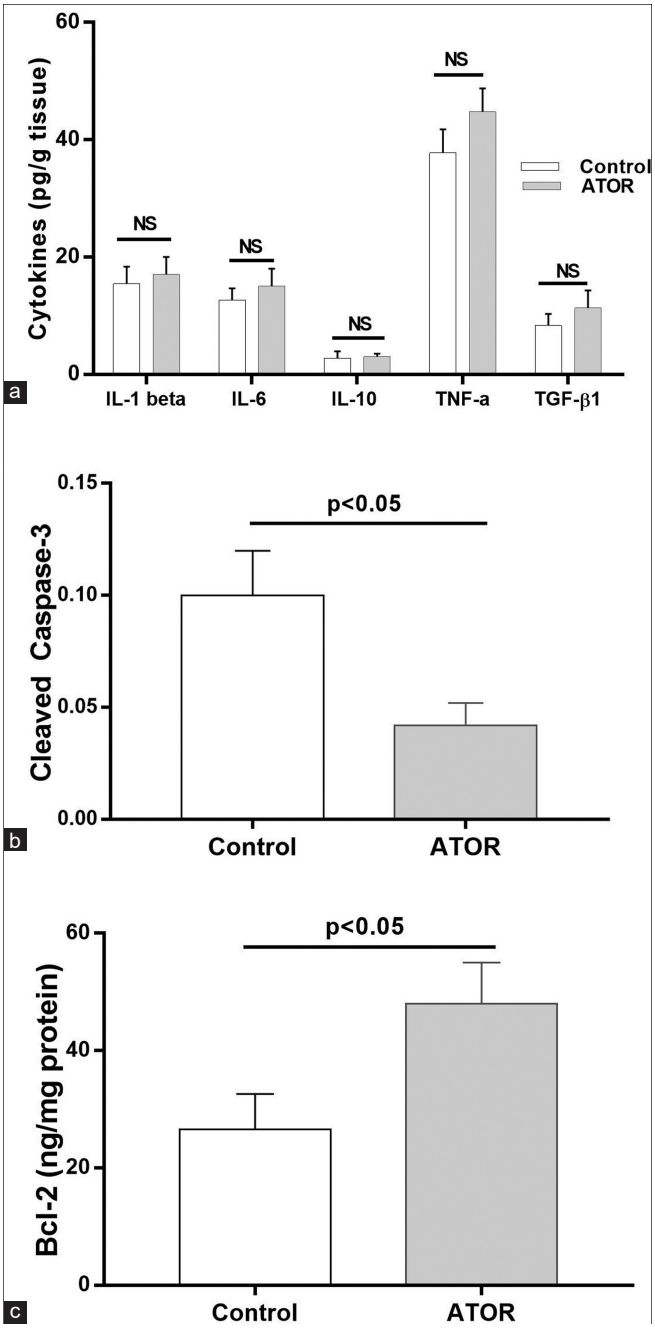

Figure 4: Graphs representing levels of IL-1 β, IL-6, IL-10, TNF-α and TGF-β1 (pg/g tissue). They show no significant differences between in islets of various groups (a). A statistically significant reduction in apoptosis represented by caspase-3 (b) with a significant rise in the anti-apoptotic protein represented by Bcl-2 are detected in islets of animals treated with ATOR compared to those which did not (c). The assessment of cleaved caspase-3 as an apoptotic marker is in fold alterations in the OD. Whereas that Bcl-2 as the anti-apoptotic one (c) was in ng/mg protein. Expression of data is as mean ± SD ($n = 3$ rats). $P < 0.05$ is considered significant. IL: Interleukin, TNF-α: Tumor necrosis factor-alpha, TGF-β: Transforming growth factor beta, ATOR: Atorvastatin, OD: Optical density

Expansion of IOL was demarcated in the ATOR-treated animals with the dominance of β-cell mass and relative shrinkage of α-cells compared to control animals. In addition, we detected a statistically significant increase in the β-cell area, percentage of β-cells with a decrease in α-cell area, and percentage of α-cells in the ATOR group. These findings of the cellular parameters in islets of the ATOR-treated animals resemble those detected in the IR state of prediabetes. We have previously revealed expansion of islets together with enhanced β-cell parameters and suppressed α-cell ones in prediabetes.[21,28] ATOR may induce an IR-like state accompanied by a compensatory cellular response which may delay the occurrence of diabetes.

Statins have been reported to increase HbA1c and FBG.[10,12] and risk for T2D.[7,9] This may be more manifested in females and the elderly.[29] and might be attributed to low-density lipoprotein cholesterol (LDL) gene variants.[5] Interestingly, mutations of the LDL-receptor gene in patients with familial hypercholesterolemia protect them against statin-induced diabetes.[13]

In the current study, we observed a statistically significant increase in the β-cell proliferation and neogenesis in the ATOR group compared with the control one. Moreover, a significant reduction in caspase-3 accompanied by a significant rise in Bcl-2 were detected in islets of animals treated with ATOR both at mRNA and protein levels. ATOR displayed a similar effect on the myocardium of diabetic cardiomyopathy by suppressing markers of apoptosis and upregulating those of anti-apoptosis.[30]

Although the exact mechanism for diabetogenicity of statins is not elucidated,[31] it includes impaired insulin secretion and increased IR.[32] β-cell function may be impaired by statin-inhibition of cholesterol synthesis leading to an increase in its cellular uptake.[6] In addition, statin-induced diabetes is dose-dependent.[3,8] High-intensity ATOR is associated with increased risk of diabetes than moderate-intensity ATOR.[32] Statins may adversely affect glucose homeostasis according to their HMG-CoA reductase inhibition capacity.[32] It is suggested that lipophilic statins are more diabetogenic than hydrophilic ones with particular predilection in those with a previous risk for diabetes.[9,32]

Unlike the proposed anti-inflammatory effect of statins, they are assumed to have pro-inflammatory effects in impaired metabolic conditions by induction of certain inflammasomes.[6] The pleiotropic effects of statins include decreased protein prenylation, which can alter immune function and increase IL-1 β in a caspase-dependent manner.[4] In the current study, although there was a trend of rise in the local islet inflammatory cytokines in the

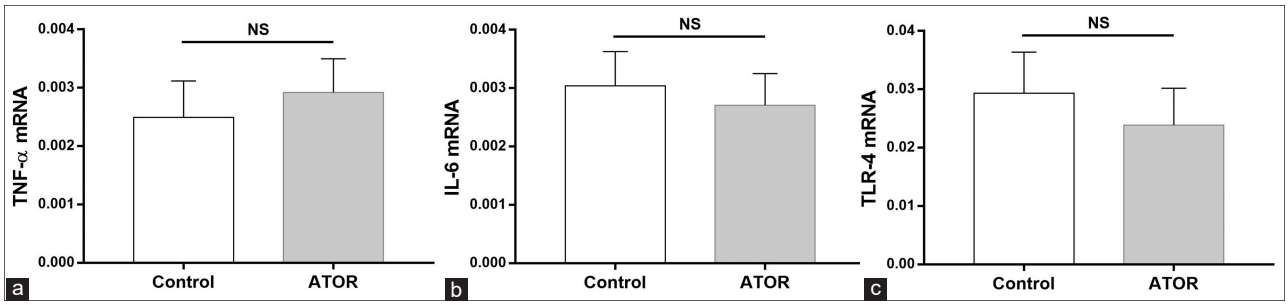

Figure 5: Representative graphs of the expression of mRNA of inflammatory cytokines (TNF-α, IL-6 and TLR-4) in islets of different groups (a-c). There is no significant (NS) differences between both groups. Expression of data is as mean ± standard deviation (SD; n=3 animal in each group); with β-actin gene as the housekeeping gene. *P* value less than 0.05 is considered significant

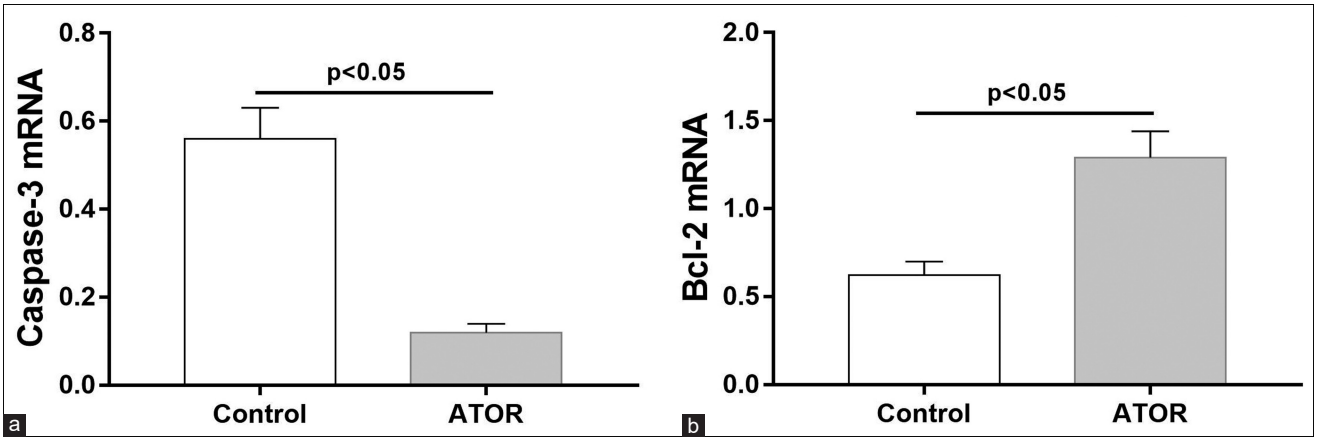

Figure 6: Representative graphs of the mRNA expression of caspase-3 for apoptosis (a) and Bcl-2 for anti-apoptosis (b) in various study groups. A statistically significant reduction in apoptosis represented by caspase-3 mRNA expression (a) with a significant rise in the anti-apoptotic mRNA represented by Bcl-2 (b) are detected in islets of animals treated with ATOR compared to those which did not. Expression of data is as mean ± SD (three animals in each study group); with β-actin gene as the housekeeping gene. *P* < 0.05 is considered significant. ATOR: Atorvastatin, SD: Standard deviation

group treated with ATOR, it did not reach a significant level compared to the control group. This was confirmed by evaluating the mRNA expression of the inflammatory markers (IL-6, TLR-4, and TNF-α) which revealed the insignificant change in both groups.

Statins may impair glucose homeostasis and elevate BG levels through muscle inflammation.[33] which might be a proposed mechanism for statin-associated muscle symptoms as well as for the potential IR.[1] Interestingly, it is hypothesized that metformin, by reducing IR may decrease the frequency of these muscle symptoms with a decrease in diabetes and CV risk.[1] In addition, it has been recently displayed the positive impact of metformin on the remodeling of the cardiac muscles in diabetes.[34]

In the current experiment, we detected a significant rise in the parameters of glucose homeostasis of the ATOR-treated animals accompanied by a significant decrease in the function of β-cells. Moreover, OGTT impairment was observed in the ATOR-treated animals which exhibited a significant rise in BG levels at different checked times. Almost all previous studies were *in vitro* and demonstrated that statins may impair insulin sensitivity and secretion by multiple mechanisms.[14] They may affect insulin signaling pathways which can affect insulin sensitivity, β-cell function, and adipokine secretion.[8] ATOR may inhibit insulin synthesis by inhibiting Ras complex (Ras, Raf-1, and p-CREB) in β-cells. In addition, ATOR, fluvastatin,[35] and simvastatin[36] may inhibit insulin secretion by decreasing adult treatment panel content in pancreatic islets and this may be dependent on the degree of lipophilicity.

Conclusion

In summary, ATOR had induced IR-like state which was remarkable at the cellular level of the endocrine pancreas as well as on the biochemical level in terms of glucose homeostasis. The latter was impaired together with corresponding changes that occurred in insulin, glucagon secreting cells, and other cellular parameters of the islets. Although these changes may be compensatory in nature, further research is required to unveil their mechanistic basis and importantly their natural history. In addition, wide-scale randomized control trials are recommended to weigh the

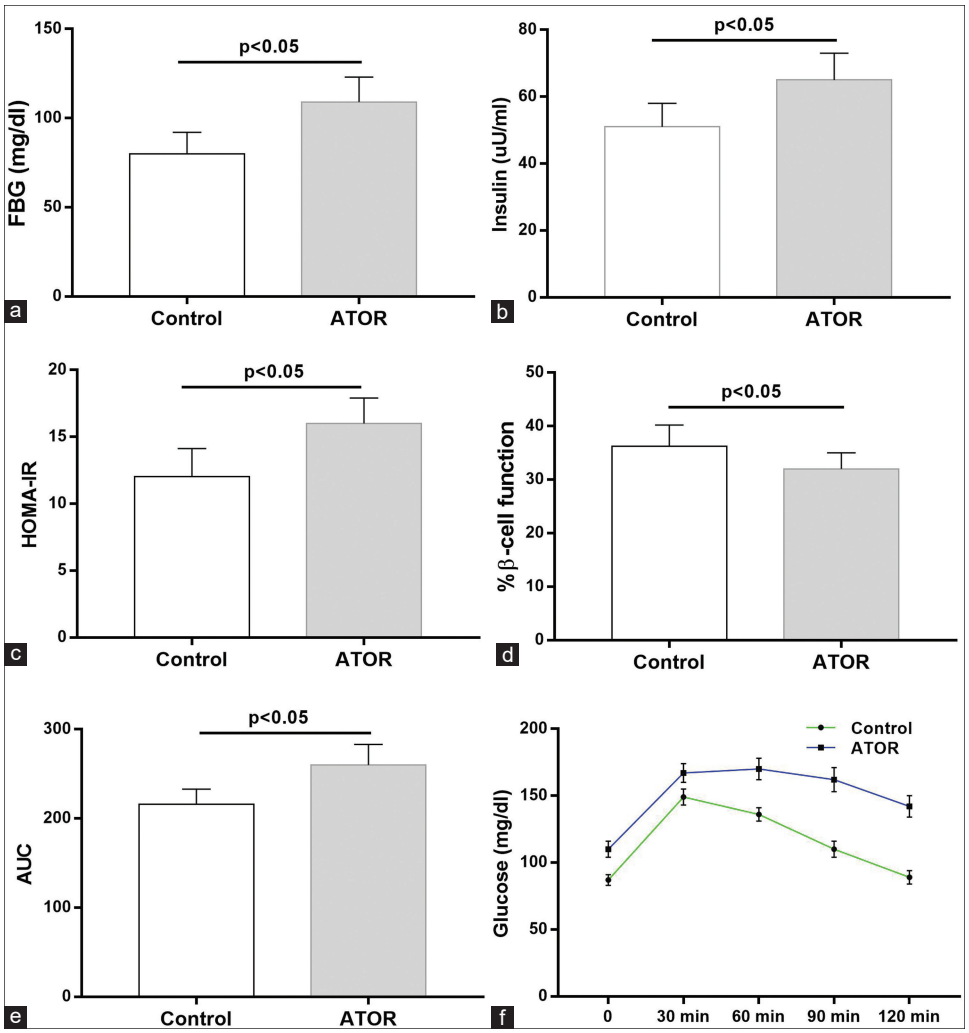

Figure 7: Representative graphs of glucose homeostasis data: (a) FBG (mg/dl), (b) insulin level (μU/ml), (c) HOMA-IR, (d) % β-cell function, (e) AUC, and (f) OGTT in different study groups. Expression of data is as mean ± SD (n = 18 rats in each group). $P < 0.05$ is considered significant. There is a significant rise ($P < 0.05$) in the parameters of glucose homeostasis in ATOR-treated animals which included FBG level as well as that of insulin, HOMA-IR and AUC (a-c and e) accompanied by a significant decrease in % function of β-cells (d) compared to those of the control group. Moreover, OGTT impairment is observed in the ATOR-treated animals which exhibited a significant rise ($P < 0.05$) in BG levels at different checked times (f). BG: Blood glucose, FBG: Fasting BG, HOMA-IR: Homeostasis model assessment index-insulin resistance, AUC: Area under the curve, OGTT: Oral glucose tolerance test, ATOR: Atorvastatin

risk-benefit of long-term statin administration as primary prevention for CV events in population without notable cardiac risk.

Recommendations

Based on the potential evidence of cellular changes in the islets besides the deranged glucose homeostasis induced by ATOR; we recommend wider experimental as well as prospective clinical studies to establish or deny this potential risk.

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Nil.

Conflict of interest

There is no conflict of interest.

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Supplementary Data

Supplementary Data S1: Immunocytological study

Paraffin sections from specimens of the pancreas were immuno-cytochemically stained by guinea pig anti-insulin (polyclonal, 1/100, A0564, Dako, Glostrup, Denamark), rabbit anti-glucagon (polyclonal, 1/50, ab8055, Abcam, Cambridge, MA) and mouse anti-Ki67 (monoclonal, 1/20, Clone MIB-5, Dako, Glostrup, Denmark) antibodies according to instructions provided by the manufacturer.

Supplementary Data S2: Morphometric evaluation

For the morphometric evaluation of various islet parameters, we followed what mentioned in previous literature (Oliveira *et al*. 2015; Abdel-Hamid and El-Firgany 2016). We selected three blocks from each pancreas which were serially cut (4 µm thickness, 20 section spaced). Then we randomly selected two sections per block for staining by anti-insulin and other two for anti-glucagon immunostaining (Carvalho *et al*. 2006; Oliveira *et al*. 2015). Afterwards, we performed morphometric assessment of the sections according to the previously described (Chintinne *et al*. 2012; Mezza *et al*. 2014; Oliveira *et al*. 2015). We selected all the detected islets of Langerhans (defined as a cluster of ≥5 β-cells) from six sections per animal which were photographed by a digital camera coupled to a microscope (CX 31, Olympus, Tokyo, Japan).

Morphometric assessment included various areas (of islet, β-cells and α-cells) with assistance of Image J software package (version 1.43, NIH). Similarly percentage of β-cell % was calculated by dividing the area of all insulin$^+$ cells by the islet area and then multiplying by 100, and that of α-cell area was evaluated by dividing the area of all glucagon$^+$ cells by the islet area and then multiplying by 100 as formerly reported by Oliveira *et al*. (2015). Furthermore Islet mass (mg) was evaluated by multiplying the weight of pancreas by the percentage of the total islet area per section, meanwhile β-cell mass (mg) was assessed by multiplying the islet mass by the percentage of β-cells/islet as previously mentioned (Abdel-Hamid and El-Firgany 2016).

Supplementary Data S3: Islets of Langerhans isolation

Isolation of the islets of Langerhans (IOL) from pancreata (3 animals in each group) was performed as described by Carter *et al*. (2009) and Pae *et al*. (2013). Briefly, the pancreas was perfused directly by a collagenase, as tissue dissociating enzyme, through a cannula inserted in the common bile duct. The removed pancreas was placed into a tube (for digestion at 37°C for 8 min) and washed by G-solution (1% BSA containing Hank's balanced salt solution, HBSS, ThermoFisher Scientific Inc.). Afterwards, the tissue was filtered through a Netwell Polyester Mesh (Corning Life Sciences). Then, centrifugation was performed and the pellet was re-suspended with Histopaque 1100 solution (Sigma–Aldrich) for gradient separation.

The supernatant was transferred into a new tube and washed with G-solution. The pellet was resuspended in RPMI 1640 media (ThermoFisher Scientific Inc.), supplemented with 10 % FBS and 1% penicillin–streptomycin mixture and cultured at 37°C and 5% CO_2 incubator for 4 h. After centrifuged for 5 min at 3000 rpm, supernatant was taken from IOL of each group for their cytokine assessment.

Local levels of interleukin (IL)-1β, IL-6, IL-10, tumor necrosis factor-α and transforming growth factor-β1 were assessed by ELISA kits guided according to instructions of the manufacturer (eBioscience; Vienna, Austria).

The pancreatic IOL caspase-3 was assayed by a caspase-3 colorimetric assay kit according to guidelines of the manufacturer's. The supernatant of IOL (50 µl) was added in a 96-well flat-bottom microplate. Caspase-3 colorimetric substrate (DEVD-pNA) is cleaved by caspase and the chromophore pNA released. The latter was estimated spectrophotometrically by a microplate reader (at a wave length of 405 nm). The results were expressed as fold increase in caspase-3 activity according to the corresponding alterations in the optical density (Ola *et al*., 2015).

Supplementary Data S4: Real-time quantitative polymerase chain reaction supplementary data

From the isolated islets of the pancreas total-RNA was extracted using an RNA isolation reagent, TRIzol (TRIzol® Reagent; Invitrogen Life Technologies). Then we performed reverse transcription by oligo(dt) priming according to instructions of the manufacturer (ThermoScript™ RT-PCR System; Invitrogen Life Technologies).

Table S1: Primer sequences list

name	3	5
Caspase-3	F, CTGGACTGCGGTATTGAG	R, GGAACATCGGATTTGATT
Bcl-2	F, CTACCCAAGTTAGCATT CC	R, CAAAGTCCCTATTTATCCCT
TNF-α	F, TACTCCTCAGAGCCCCCAAT	R, TCAGCGTCTCGTGTGTTTCT
IL-6	F, CTCTCCGCAAGAGACTTCCAG	R, TGTGGGTGGTATCCTCTGTGA
TLR4	F, AATGCCAGGATGATGCCTCT	R, AGGAAGTACCTCTATGCAGGG
β-actin	F, AGCCA TGTACGTAGCCATCC	R, GCTGTGGTGGTG AAGCTGTA

TNF-α: Tumor necrosis factor-alpha, IL: Interleukin

Table S2: Comparison between data of various cellular parameters of the islets in both groups

parameter measured	Control	ATOR
Total islet area (μm²)	7190.48±292	7686±345 (NS)
Total β-cell area (μm²)	5065.69±240	6502.36±280*
Total alpha-cell area (μm²)	1033.27±52	756.69±36*
β-cells/islet (%)	70.45±3.49	84.6±4.52*
Alpha cell/islet (%)	14.37±1.461	9.845±1.599*
Islet mass (mg)	0.79±0.13	1.38±0.11*
β-cell mass (mg)	0.62±0.09	1.18±0.08*

*Significant versus control. NS: No significant, ATOR: Atorvastatin

Table S3: Comparison between apoptotic (caspase-3) and anti-apoptotic (Bcl-2) markers in both groups at both protein and mRNA level

parameter measured	Control	ATOR
Cleaved caspase-3	0.1±0.02	0.042±0.01*
Bcl-2 (ng/mg protein)	26.65±6	48±7*
Caspase-3 mRNA	0.56±0.07	0.12±0.02*
Bcl-2 mRNA	0.62±0.08	1.29±0.15*

*Significant versus control. ATOR: Atorvastatin

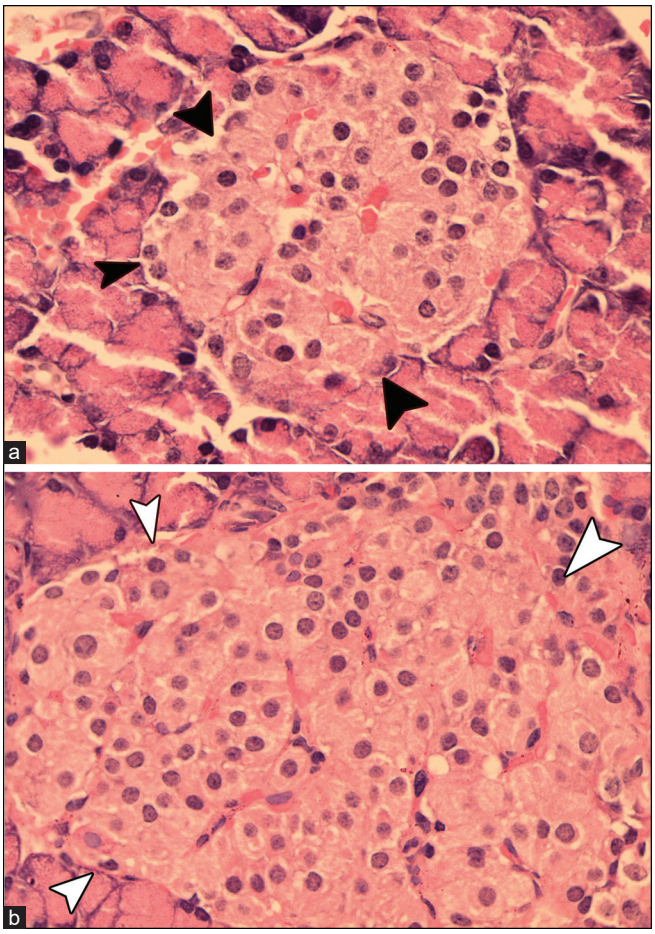
Table S4: Comparison between various glucose homeostasis parameters in both groups

parameter measured	Control	ATOR
FBG (mg/dl)	80±12	109±14*
Insulin (μU/ml)	51±7	65±8*
HOMA-IR	12.02±2.1	16±1.9*
Percentage β-cell function	36.24±4	32±3*
AUC	215.8±17	260±23*

*Significant versus control. ATOR: Atorvastatin, FBG: Fasting blood glucose, HOMA: Homeostatic model assessment, IR: Insulin resistance, AUC: Area under the curve

The primer sequence is mentioned below [Table S1]. Afterwards polymerase chain reaction (PCR) reaction was performed. The PCR reaction protocol was conducted at 94°C (5 min); 35 cycles (for caspase-3 and Bcl-2) or 30 cycles (for β-actin) of 30 s at 94°C, 30 s at 55°C, and 30 s at 72°C; with final elongation for 10 min at 72°C (Sun *et al*. 2012).

The primer sequences for the local inflammatory markers (TLR4, tumor necrosis factor-α and interleukin-6) are listed below. Amplification of 1 μL of each reverse transcription reaction product was performed in 20-μL PCR reaction. The PCR protocol was performed at 95°C for 30s and 40 cycles consisting of 5 s at 95°C, 34 s at 60°C (Wang *et al*. 2017). Moreover, we performed all reactions in triplicate manner and calculated the relative amounts of target gene transcripts by employing the comparative cycle time technique.



Supplementary Figure 1: Routine histology of the pancreas by H and E staining. Expansion of the islet of Langerhans is evident in the ATOR pancreas ([b] white arrowheads) compared to the control ([a] black arrowheads) one together with complete absence of inflammatory cell infiltrate

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