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# Statins reduce testicular and ocular VEGF: A potential compromise to microcirculation

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ABSTRACT

Microcirculation has great importance in eye and testicular tissue and is necessary to have adequate and appropriate amount of angiogenesis. It is known that high levels of Vascular Endothelial Growth Factor (VEGF) trigger uncontrolled angiogenesis, whereas inadequate VEGF can lead to decreased tissue perfusion and oxygenation. The aim of this study was to investigate effects of VEGF in testicular and ocular tissues in both non-diabetic and diabetic rats treated by statin. Atorvastatin (10 mg/kg daily given by orally gavage) was administered for two weeks. Diabetes was induced by streptozotocin, (STZ, 45 mg/kg/ip) in diabetic group's rats. Two weeks later from STZ injection, atorvastatin treatment was initiated in diabetic group. VEGF levels were measured by using ELISA. The VEGF levels were decreased in vitrous, ocular and testicular tissues of all statin-administered rats. In diabetic group VEGF levels were found to be decreased in testicular tissue and increased in ocular tissues.

*Conclusion:* Statin use decreased in VEGF levels of testicular and ocular tissues in diabetic and non-diabetic rats. Statin treatment (anti-VEGF effect) had a protective effect in the development of diabetic retinopathy, yet statins may have a negative impact on tissues that depend on microcirculation by reducing VEGF levels. Further research is needed for statins' microcellular effects.

## 1. Introduction

Angiogenesis is a physiological process of development of new blood vessels from pre-existing vessels and has critical importance for tissue regeneration and wound healing. Vascular Endothelial Growth Factor (VEGF) mediates angiogenesis by inducing vascular innervation and resulting in enlargement of new blood vessels in induced angiogenesis (Carmeliet and Jain, 2011; Leung et al., 1989). Angiogenesis has crucial importance for particular organs such as ocular and testicular tissue. The angiogenesis seen in wound healing or as a physiological adaptation to exercise is regular but can be irregular during pathological processes such as diabetic retinopathy and solid organ tumours (Dewhirst et al., 2008).

Inadequate oxygenations at the cellular level can up regulate VEGF production. VEGF can result in improper angiogenesis if not controlled (Sene et al., 2015) therefore keeping VEGF levels within appropriate range is of importance in tissues where microcirculation is particularly crucial such as in retina and testis. VEGF is needed for endothelial cell proliferation and needed for adequate blood supply. Vascular defects linked with low VEGF levels were observed in experimental animal models (Stalmans et al., 2002).

The microcirculation of retina is structurally unique to allow the tissue to be translucent. The capillary density in the retina should not be too high or too low, it should be at the proper density for passing light from the retina to stimulate the retinal neurons (Shepro, 2006). VEGF signaling is very important in preserving corneal transparency and avascularity (Sene et al., 2015). It is known that VEGF-A upregulated irregular vascularization in diabetic eyes, due to the incomplete vascular integrity; risk of microaneurysms, rupture and hemorrhage is increased (Hammes, 2005; Ved et al., 2017). Anti-VEGF-A agents are considered standard of care treatment in retinal vascular diseases (Cheung et al., 2014).

Microcirculation in mammalian testis has critical role in primary testicular functions such as spermatogenesis, hormonal and paracrine

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control (Damber and Bergh, 1992; Reddy et al., 2012). Sertoli and Leydig cells secrete VEGF and VEGF receptors exist in the testicular tissue (Reddy et al., 2012).

Statins are potent 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, which interfere with cholesterol biosynthesis and are widely used to lower serum cholesterol levels in diabetic patients (Armitage, 2007). Statins are believed to regulate angiogenesis by lowering serum VEGF levels in the cardiovascular patients and reducing retinal VEGF levels in the diabetic retinopathy (Sahebkar et al., 2015; Ved et al., 2017). Statins are improved age-related macular degeneration via reducing VEGF levels (Miller, 2016). Recently, it was shown that preoperative statin treatment improved outcomes after vitrectomy in diabetic patients (Tuuminen et al., 2016a). The effects of statins on VEGF levels in the testis and retina are not fully known. The aim of this study is to investigate the effect of statins on VEGF levels in ocular, vitreous and testicular tissue of the diabetic and non-diabetic rats.

## 2. Materials and methods

Adult male Wistar Albino rats (Dokuz Eylul University, Experimental Animal Laboratory, Izmir, Turkey) were housed in individual cages with free access to water and laboratory chow. They were kept in a 12 h-light/12 h-dark cycle at constant room temperature (22 ± 1 °C), humidity (60%). Dokuz Eylul University School of Medicine Animal Care Committee approved all experimental procedures. Tissues materials used in current protocol were also used in another protocol IRB /Ethical Review Board Number: 29/2012 and title "Effects of statin in diabetic rats on hemoreological parameters and diabetic nephropathy".

Rats were divided into four groups: (1) Control group (n = 7), (2) Diabetic group (n = 7), (3) Statin group (n = 7), (4) Diabetic group treated with statin (n = 7). Diabetes was induced by a single intraperitoneal injection of streptozotocin (Sigma, St. Louis, MO; 45 mg/kg). Twenty-four hours after streptozotocin treatment, induction of diabetes in the diabetic groups were confirmed by blood glucose levels over 250 mg/dl (Aksu et al., 2013; Uysal et al., 2005). Two weeks after streptozotocin injection, statin was given by orogastric gavage for two weeks (10 mg/kg/day). Experimental protocol ended in four weeks. Euthanasia was performed with exsanguination under ether anaesthesia. The vitreous was taken from the eyeball through the injector; the eyeball and testicular tissues were extracted for biochemical examination.

### 2.1. Biochemical investigation

Testicular and eye VEGF levels were measured using commercially available ELISA kits specific for rats (VEGF Catalog Number EK0308, Boster Immunoleader, Wuhan, China with assay sensitivity < 1 pg/ml and range 15.6–1000 pg/ml).

### 2.2. Statistical analysis

Statistical analysis was performed by SPSS 15.0. One-way ANOVA and post hoc Bonforroni comparison were used to determine the statistical differences between groups. Correlations between groups were calculated by Pearson correlation analysis. Results are presented as mean ± S.E.M., where $p < 0.05$ was considered statistically significant.

## 3. Results

VEGF levels were found to be increased in ocular tissues of the diabetic group (compared with all other groups, $p < 0.0001$). Statin use reduced VEGF in all groups (Statin group compared with control, $p < 0.001$; statin using diabetic group compared with diabetic group, $p < 0.0001$) (Fig. 1).

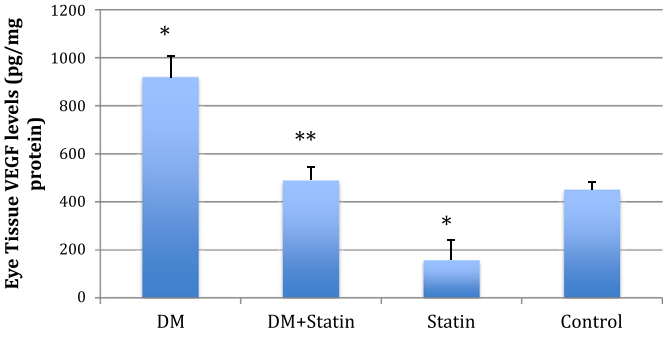



**Fig. 1.** Ocular tissue Vascular Endothelial Growth Factor (VEGF) levels. *p < 0.0001 compared with control; **p < 0.001 compared with diabetic group.

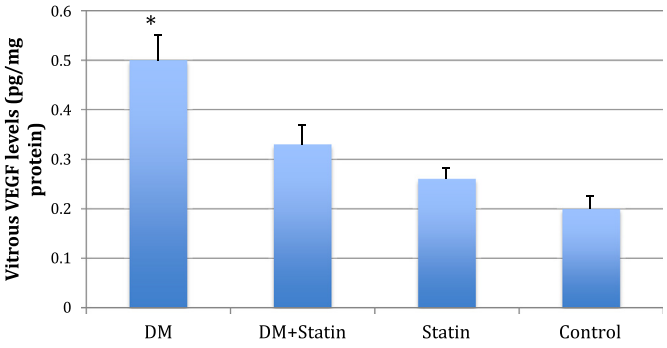



**Fig. 2.** Vitreous fluid Vascular Endothelial Growth Factor (VEGF) levels. *p < 0.0001 compared with control group.

VEGF levels in vitreous tissue was found to be increased in diabetic group (compared with statin using diabetics, $p < 0.01$; compared with other groups, $p < 0.0001$). Statin use reduced VEGF levels in diabetics ($p < 0.01$) (Fig. 2).

In testicular tissue, VEGF levels significantly decreased in diabetic and all statin using groups compared to control group (all groups, $p < 0.0001$) (Fig. 3).

A strong positive correlation was found between ocular tissue and vitreous tissue VEGF levels ($r = 0.522$, $p = 0.001$).

## 4. Discussion

Atorvastatin decreased VEGF in ocular and testicular tissues, in both diabetic and non-diabetic rats which, to our knowledge, has never been yet reported in the literature.

VEGF is known to have angiotrophic and neurotrophic effects, which promotes endothelial cell and neuron growth (Ferrara, 2000).

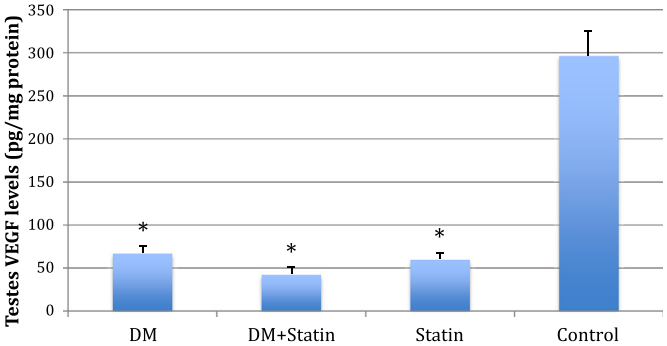



**Fig. 3.** Ocular tissue Vascular Endothelial Growth Factor (VEGF) levels. *p < 0.0001 compared with control group.

VEGF is also known to be critical for spermatogenesis, as it stimulates proliferation and, in turn, contributes to the survival of germ cells, to the microcirculation of testicular tissue by increasing endothelial permeability (Anand et al., 2003; Caires et al., 2009). Testicular artery has high resistance and capillary pressure is much lower which results in high vascular permeability (Sweeney et al., 1991). VEGF supports to transport of nutritional components and oxygen (Kilic et al., 2009; Rudolfsson et al., 2004). In this study, we showed that statin administration reduces VEGF in testicular tissue of healthy rats, while it had not effect in diabetic testes. Testicular effects of statins have been studies in the literature (Cui et al., 2017; Heeba and Hamza, 2015; Yang et al., 2010; Pons-Rejraji et al., 2014; Tada et al., 2015; Dostal et al., 2001).

It is a known fact that diabetes mellitus causes testicular dysfunction in both humans and rats (Guneli et al., 2008; Sexton and Jarow, 1997; Sisman et al., 2014). In our previous study, we demonstrated that testicular VEGF was found to be low in diabetic rats and the angiotropic/neurotropic reduction was linked to an increased rate of apoptosis and testicular damage (Sisman et al., 2014). which is known to be secondary to increased oxidative stress that facilitates the apoptotic cell death. (Amaral et al., 2008; Uysal et al., 2011). VEGF is an important neurotrophic factor for the male reproductive system, hence we suggested that our previous study could serve as a biomarker for diabetic testicular damage (Sisman et al., 2014). In the current study, we once again confirmed that VEGF levels decreased in diabetic rat's testicular tissue which is consistent with our previos study.

The VEGF plays an essential role in the angiogenesis of retina (Shepro, 2006) Statin effects on VEGF has been studied; macular degeneration and diabetic retinopathy have been the main focus. (Al-Shabrawey et al., 2008; Bartoli et al., 2009; Caldwell et al., 2005; Gehlbach et al., 2015; Guymer et al., 2013; Li et al., 2010; Miyahara et al., 2004; Robinson et al., 2011; Yokota et al., 2007; Zhang and Yan, 2012; Zheng et al., 2010; Chou et al., 2014; Sene et al., 2015). (Al-Shabrawey et al., 2008; Bartoli et al., 2009; Caldwell et al., 2005; Gehlbach et al., 2015; Guymer et al., 2013; Li et al., 2010; Miyahara et al., 2004; Robinson et al., 2011; Yokota et al., 2007; Zhang and Yan, 2012; Zheng et al., 2010). Statin treatment is known to have decreased VEGF levels in vitro environment (Tuuminen et al., 2016b). Anti-VEGF treatment has been effectively used in diabetic retinopathy. In our study, we demonstrated that increased tissue VEGF due to diabetes was found to be decreased with statin use. However, surprisingly statin lowered the level of VEGF in the ocular tissue of the non diabetic healthy subjects. Boucher et al. showed that statin is induced apoptosis in healthy pericyte cell line (Boucher et al., 2006). Pericytes have a critical role in vascular development and function. And also, the interaction between pericytes and endothelium is vital to the development, maturation and maintenance of the vascular system (Hirschi and D'Amore, 1996).

## 5. Conclusion

In our study we demonstrated that atorvastatin reduced VEGF in testicular and ocular tissue of both diabetic and non-diabetic rats. As a side note, we were not able to note any protecting effect of VEGF on testicular damage in the setting of diabetes. It appears that statins are more useful if retinopathy exists, if it does not, statins would reduce VEGF. Our findings indicate the potential adverse affects of statin use on microvascular circulation prompts further investigation. Further studies are needed to unfold the complex effects of statins on body.

## Conflict of interest

The authors confirm that this article content has no conflict of interest.

## Acknowledgements

Declared none.

## References

Aksu, I., et al., 2013. Serum IGF-1 levels correlate negatively to liver damage in diabetic rats. Biotech. Histochem. 88, 194–201.
Al-Shabrawey, M., et al., 2008. Role of NADPH oxidase and Stat3 in statin-mediated protection against diabetic retinopathy. Invest. Ophthalmol. Vis. Sci. 49, 3231–3238.
Amaral, S., et al., 2008. Diabetes and the impairment of reproductive function: possible role of mitochondria and reactive oxygen species. Curr. Diabetes Rev. 4, 46–54.
Anand, R.J.K., et al., 2003. Regulation of vascular endothelial growth factor production by Leydig cells in vitro: the role of protein kinase A and mitogen-activated protein kinase cascade. Biol. Reprod. 68, 1663–1673.
Armitage, J., 2007. The safety of statins in clinical practice. Lancet 370, 1781–1790.
Bartoli, M., et al., 2009. HMG-CoA reductase inhibitors (statin) prevents retinal neovascularization in a model of oxygen-induced retinopathy. Invest. Ophthalmol. Vis. Sci. 50, 4934–4940.
Boucher, K., et al., 2006. HMG-CoA reductase inhibitors induce apoptosis in pericytes. Microvasc. Res. 71, 91–102.
Caires, K.C., et al., 2009. Vascular endothelial growth factor regulates germ cell survival during establishment of spermatogenesis in the bovine testis. Reproduction 138, 667–677.
Carmeliet, P., Jain, R.K., 2011. Molecular mechanisms and clinical applications of angiogenesis. Nature 473, 298–307.
Caldwell, R.B., et al., 2005. Vascular endothelial growth factor and diabetic retinopathy: role of oxidative stress. Curr. Drug Targets 6, 511–524.
Cheung, N., et al., 2014. Ocular anti-VEGF therapy for diabetic retinopathy: overview of clinical efficacy and evolving applications. Diabetes Care 37, 900–905.
Chou, J., et al., 2014. Role of endothelial cell and pericyte dysfunction in diabetic retinopathy: review of techniques in rodent models. Adv. Exp. Med. Biol. 801, 669–675.
Cui, X., et al., 2017. Protective effects of fluvastatin on reproductive function in obese male rats induced by high-fat diet through enhanced signaling of mTOR. Cell. Physiol. Biochem. 41, 598–608.
Damber, J.E., Bergh, A., 1992. Testicular microcirculation—a forgotten essential in andrology? Int. J. Androl. 15, 285–292.
Dewhirst, M.W., et al., 2008. Cycling hypoxia and free radicals regulate angiogenesis and radiotherapy response. Nat. Rev. Cancer 8, 425–437.
Dostal, L.A., et al., 2001. Repeated analysis of semen parameters in beagle dogs during a 2-year study with the HMG-CoA reductase inhibitor, atorvastatin. Toxicol. Sci. 61, 128–134.
Ferrara, N., 2000. VEGF: an update on biological and therapeutic aspects. Curr. Opin. Biotechnol. 11, 617–624.
Gehlbach, P., et al., 2015. Statins for age-related macular degeneration. Cochrane Database Syst. Rev. 4 (8), Cd006927.
Guneli, E., et al., 2008. Effect of melatonin on testicular damage in streptozotocin-induced diabetes rats. Eur. Surg. Res. 40, 354–360.
Guymer, R.H., et al., 2013. Proof of concept, randomized, placebo-controlled study of the effect of simvastatin on the course of age-related macular degeneration. PLoS One 8, e83759.
Hammes, H.P., 2005. Pericytes and the pathogenesis of diabetic retinopathy. Horm. Metab. Res. 37 (Suppl. 1), 39–43.
Heeba, G.H., Hamza, A.A., 2015. Rosuvastatin ameliorates diabetes-induced reproductive damage via suppression of oxidative stress, inflammatory and apoptotic pathways in male rats. Life Sci. 141, 13–19.
Hirschi, K.K., D'Amore, P.A., 1996. Pericytes in the microvasculature. Cardiovasc. Res. 32, 687–698.
Kilic, S., et al., 2009. Caspase-3 and VEGF immunopositivity in seminiferous tubule germ cells in cases of obstructive and non-obstructive azoospermia in smokers versus non-smokers. J. Assist. Reprod. Genet. 26, 57–63.
Leung, D.W., et al., 1989. Vascular endothelial growth factor is a secreted angiogenic mitogen. Science 246, 1306–1309.
Li, J., et al., 2010. Inhibition of reactive oxygen species by Lovastatin downregulates vascular endothelial growth factor expression and ameliorates blood-retinal barrier breakdown in db/db mice: role of NADPH oxidase 4. Diabetes 59, 1528–1538.
Miller, J.W., 2016. Beyond VEGF-the Weisenfeld lecture. Invest. Ophthalmol. Vis. Sci. 57, 6911–6918.
Miyahara, S., et al., 2004. Simvastatin inhibits leukocyte accumulation and vascular permeability in the retinas of rats with streptozotocin-induced diabetes. Am. J. Pathol. 164, 1697–1706.
Pons-Rejraji, H., et al., 2014. Evaluation of atorvastatin efficacy and toxicity on spermatozoa, accessory glands and gonadal hormones of healthy men: a pilot prospective clinical trial. Reprod. Biol. Endocrinol. 12, 65.
Reddy, N., et al., 2012. Expression of vascular endothelial growth factor (VEGF) transcript and protein in the testis of several vertebrates, including endangered species. Theriogenology 77, 608–614.
Robinson, R., et al., 2011. Fluvastatin downregulates VEGF-A expression in TNF-alpha-induced retinal vessel tortuosity. Invest. Ophthalmol. Vis. Sci. 52, 7423–7431.
Rudolfsson, S.H., et al., 2004. Hormonal regulation and functional role of vascular endothelial growth factor a in the rat testis. Biol. Reprod. 70, 340–347.
Sahebkar, A., et al., 2015. Does statin therapy reduce plasma VEGF levels in humans? A systematic review and meta-analysis of randomized controlled trials. Metabolism 64, 1466–1476.

Sene, A., et al., 2015. Seeing through VEGF: innate and adaptive immunity in pathological angiogenesis in the eye. Trends Mol. Med. 21, 43–51.
Sexton, W.J., Jarow, J.P., 1997. Effect of diabetes mellitus upon male reproductive function. Urology 49, 508–513.
Shepro, D., 2006. Microvascular Research : Biology and Pathology. Elsevier Academic Press, Burlington, MA.
Sisman, A.R., et al., 2014. Potential Novel Biomarkers for Diabetic Testicular Damage in Streptozotocin-induced Diabetic Rats: Nerve Growth Factor Beta and Vascular Endothelial Growth Factor. 2014. pp. 108106.
Stalmans, I., et al., 2002. Arteriolar and venular patterning in retinas of mice selectively expressing VEGF isoforms. J. Clin. Invest. 109, 327–336.
Sweeney, T.E., et al., 1991. Microvascular pressure distribution in the hamster testis. Am. J. Phys. 260, H1581–9.
Tada, Y., et al., 2015. Transient azoospermia following rosuvastatin medication for hypercholesterolemia. Clin. Exp. Obstet. Gynecol. 42, 545–546.
Tuuminen, R., et al., 2016a. Improved outcome after primary vitrectomy in diabetic patients treated with statins. Eur. J. Ophthalmol. 26, 174–181.
Tuuminen, R., et al., 2016b. Simvastatin use associated with low intraocular ADP levels in patients with sight-threatening diabetic retinopathy. Graefes Arch. Clin. Exp. Ophthalmol. 254, 1643–1644.
Uysal, N., et al., 2005. Effect of L-carnitine on diabetogenic action of streptozotocin in rats. Neuro Endocrinol. Lett. 26, 419–422.
Uysal, N., et al., 2011. Maternal exercise decreases maternal deprivation induced anxiety of pups and correlates to increased prefrontal cortex BDNF and VEGF. Neurosci. Lett. 505, 273–278.
Ved, N., et al., 2017. Vascular endothelial growth factor-A165b ameliorates outer-retinal barrier and vascular dysfunction in the diabetic retina. Clin. Sci. (Lond.) 131, 1225–1243.
Yang, S., et al., 2010. Simvastatin attenuates testicular injury induced by torsion-detorsion. J. Urol. 184, 750–756.
Yokota, T., et al., 2007. Involvement of the Rho/Rho kinase signaling pathway in platelet-derived growth factor BB-induced vascular endothelial growth factor expression in diabetic rat retina. Jpn. J. Ophthalmol. 51, 424–430.
Zhang, W., Yan, H., 2012. Simvastatin increases circulating endothelial progenitor cells and reduces the formation and progression of diabetic retinopathy in rats. Exp. Eye Res. 105, 1–8.
Zheng, Z., et al., 2010. Improvement of retinal vascular injury in diabetic rats by statins is associated with the inhibition of mitochondrial reactive oxygen species pathway mediated by peroxisome proliferator-activated receptor gamma coactivator 1alpha. Diabetes 59, 2315–2325.