PHARMACIA

Pharmacia 68(2): 313–319
DOI 10.3897/pharmacia.68.e63449

Research Article

Assessment the effects of insulin on adiponectin, nitric oxide, myeloperoxidase and lipid profile in type 1 diabetic patients

Jehan A. Mohammad[1], Zainab H. Fathi[1], Thikra Ali Allwash[2]

1 *Department of Pharmacognosy and Medicinal Plants, College of Pharmacy, University of Mosul, Mosul-Iraq*
2 *Department of chemistry, Collage of Science, University of Mosul, Mosul-Iraq*

Corresponding author: Zainab H. Fathi (zainabh@uomosul.edu.iq)

Received 21 January 2021 ♦ **Accepted** 7 March 2021 ♦ **Published** 1 April 2021

Citation: Mohammad JA, Fathi ZH, Allwash TA (2021) Assessment the effects of insulin on adiponectin, nitric oxide, myeloperoxidase and lipid profile in type 1 diabetic patients. Pharmacia 68(2): 313–319. https://doi.org/10.3897/pharmacia.68.e63449

Abstract

Type 1 diabetes (T1DM) is well recognized risk factor cardiovascular disease (CVD). Insulin therapy is recommended for all patients with type 1 diabetes. Previous findings showed that diabetes impairs endothelial function and increased glucose level reduces nitric oxide (NO) output and increases myeloperoxidase (MPO) activity. However, adiponectin (APN) decreases serum glucose levels. The current study evaluated effects of insulin therapy on circulating levels of oxidative stress and CVD biomarkers like NO, APN, MPO, AIP and lipid profile in type 1 diabetic patients. Fifty patients with T1DM and 18 healthy people were enrolled in this study. The recruited people with T1DM were classified into two groups: 22 newly diagnosed (untreated) type 1 diabetic patients and 28 insulin treated patients. In all groups, circulating NO, APN, MPO, AIP and lipids levels were measured. Compared to control, untreated diabetes revealed a significant increase in the serum levels of APN, MPO, TG, VLDL, TC, LDL and AIP, with a marked reduction in NO and HDL levels. However, insulin therapy significantly lowered MPO, TC and LDL, with no significant changes in the other biochemical parameters. As expected, oxidative stress and CVD-associated markers were significantly increased in untreated diabetes. Insulin therapy exhibited a relatively positive effect on oxidative stress and CVD biomarkers. Accordingly, insulin plus antioxidant supplementation required to normalize these parameters.

Keywords

Adiponectin, Insulin, Lipid, Myeloperoxidase, Nitric oxide

Introduction

Type 1 diabetes is well-established risk factor for CVD with 3 to 4 fold higher risk of mortality in comparison to those without diabetes (Lind et al. 2014; Lee et al. 2019). All patients with T1DM required insulin therapy (Silver et al. 2018). NO is a critical modulator with various biological effects, including potent vasodilation activity (Chen et al. 2008). As well, NO plays a major role in vascular homeostasis by blocking tubular sodium reabsorption, thus increases natriuresis (Majid and Navar 1997). Accordingly, the therapeutic inhibition of NO synthesis leads to arterial hypertension and vascular injury (Qiu et al. 1998). In addition, inhibition of endothelial NO synthase

induce hypertension in mice (Komers and Cooper 1996), supporting for the significance of NO in the regulation of blood pressure. Because these actions are closely related to pathogenesis of atherosclerosis, inhibiting them by NO protects cardiovascular function. Among the biologically active adipocytokines, APN protein hormone which decreased in type 2 diabetes, obesity and after myocardial infarction (Su et al. 2011; Wang et al. 2017). In contrast, higher circulating APN levels have been shown in type 1 diabetic patients (Lindström et al. 2006).

MPO is a lysosomal enzyme activated and released by neutrophils (Khan et al. 2018). Previous studies have been reported that MPO may play a key role in the pathogenesis of CVD that may result from atherosclerotic plaque formation and endothelial dysfunction (Baldus et al. 2003; Heinecke 2003). In healthy people, high circulating MPO concentration is associated with increased risk of future CVD (Meuwese et al. 2007).

Increased circulating total cholesterol (RD&I Christchurch) and LDL levels are well-established predictors for CVD (Anand et al. 2011). The CVD occurring in around 2% per year in young age in patients with T1DM (Soedamah-Muthu et al. 2006). In addition, type 1 diabetic patients have 10-fold higher risk of cardiovascular mortality in comparison to general population (Lind et al. 2014). AIP is a critical marker for predicting CVD risk, because of the strong association between AIP and atherosclerosis (Begum et al. 2015).

Insulin therapy used to restore the absolute insulin deficiency caused by β-cell destruction in type 1 diabetic patients (American Diabetes 2009). Several findings suggested that insulin therapy increases the cardiovascular risk and mortality (Boyne and Saudek 1999; Herman et al. 2017; Braffett et al. 2019), independently of its notable glucose-lowering effects. These findings propose that insulin therapy could increase the cardiovascular risk by pleiotropic effects on inflammation, atherosclerosis, dyslipidaemia, heart failure, and arrhythmias (Herman et al. 2017). In T1DM children, two findings revealed that APN levels have been increased significantly after intensive insulin therapy (Hecht Baldauff et al. 2016; Martos-Moreno et al. 2006). Furthermore, two conflicting findings concerning the effect of neutral protamine hagedorn (NPH) insulin therapy on NO levels in alloxan-induced diabetic rats (Cerchiaro et al. 2001; Martins et al. 2010).

Although of being commonly used in treatment of T1DM, published data concerning the effect of conventional insulin therapy (NPH and regular insulin) on the concentrations of NO, APN and MPO in type 1 diabetic patients are not available. Therefore, the correlation between NO, APN, MPO, AIP and lipid profile in T1D on insulin therapy were not assessed simultaneously. Hence, the current study aims to determine levels of NO, APN, MPO, AIP and lipids in type 1 diabetic patients on insulin therapy, in addition to evaluate the correlations among these parameters. The evaluation for these parameters aid to identify patients with cardiovascular risk and thus prevention of development of CVD.

Material and methods

Patients

Our comparative cross-sectional study included fifty patients with type 1 diabetes and eighteen healthy subjects aged between 12 and 31 years, between December 2019 and April 2020. Subjects were classified into three experimental groups; 22 type 1 diabetic patients (newly diagnosed); 28 type 1 diabetic patients treated with insulin (short-acting insulin with intermediate-acting insulin) twice a day for a period of 3–16 months.; and 18 healthy subjects as a control group.

Pregnant or lactating women, patients receiving any other drugs, patients with diabetes complications or other clinical conditions were excluded from the study. Height and weight were directly measured for all participants to calculate the body mass index (BMI).

Laboratory analysis

After an overnight fasting, blood samples were collected from patients and incubated immediately in water bath for 10 min, then centrifuged at approximately 3500 rpms for 12 mins. After direct estimation of fasting serum glucose, samples were stored at -20 °C for later analysis of NO, APN, MPO, TG, HDL and cholesterol levels. LDL and VLDL were calculated by Friedewald's equation. Then, AIP was estimated as log (TG/HDL) (Dobiásová and Frohlich 2001).

Serum insulin and glucose levels were determined by enzyme linked immunosorbent assay (ELISA) and enzymatic colorimetric method, respectively. Then, glucose and insulin values were used for determination of insulin resistance using the following equation (Matthews et al. 1985):

$$\text{HOMA-IR} = \text{Insulin } (\mu\text{U/mL}) \times \text{Glucose (mmol/L)} / 22.5$$

Serum NO levels were estimated by Greiss reagent (Miranda et al. 2001). Briefly, we mixed equal volumes (200 µl) of supernatant and Griess reagent, and then absorbance was measured at 540 nm by ELISA. NO concentration was determined according to the standard curve of sodium nitrite. Circulating APN levels were estimated by ELISA, using a kit supplied by USBIOLOGICAL (USA) (Ouchi et al. 2004).

Enzymatic activity of MPO was determined by a method (Kumar et al. 2002) based on enzymatic oxidation of $_0$-dianisidin (Inoue et al.) (reducing substrate) which catalysed by hydrogen peroxide (H_2O_2) to produce a coloured substance measured at 450 nm. Serum lipid levels were measured by a colorimetric method that depends on sulfo-phospho-vanillin reaction (Chabrol and Charonnat 1937).

Data analysis

All data are presented as mean ± SD. Mann Whitney test and Kruskal-Wallis test followed by a Dunn's multiple comparisons test were used in statistical analysis of two

and multiple datasets, respectively, using GraphPad Prism version 8.0 (San Diego, California, USA). Data values $p < 0.05$ were represented statistically significant.

Results

Clinical characteristics of control, untreated and insulin-treated groups.

Baseline characteristics of the study groups (age, body mass index and duration of therapy) are given in Table 1. No significant variations have been found among control, newly diagnosed and insulin-treated groups.

Table 1. Comparison of baseline characteristics of the study groups.

Parameters	Control (n = 18)	Untreated (n = 22)	Insulin (n = 28)
Age (years)	18.11 ± 5.989	18.09 ± 4.790	19.64 ± 6.314
BMI (kg/m^2)	21.97 ± 2.831	21.84 ± 1.782	21.07 ± 1.894

Validation of serum glucose, insulin and HOMA-IR

As compared to healthy control, untreated patients revealed a significant increase in serum glucose with concomitant decrease in insulin level. As expected, insulin therapy exhibited a significant increase in serum insulin and HOMA-IR with a marked reduction in glucose level. (Table 2).

Values represent as mean ± SD. [a] represent differences between untreated and insulin-treated patients in contrast to healthy control; [b] represents differences between insulin and untreated patients. ***$p < 0.001$; ****$p < 0.0001$ represents statistically significant differences, as set by Kruskal-Wallis test followed by a Dunn's multiple comparison post-hoc test.

Table 2. Metabolic parameters of study groups.

Parameters	Control	Untreated	Insulin
Serum glucose (mmol/l)	4.621± 0.5767	12.98 ± 0.9856 [a****]	10.68 ± 0.9562 [a*** b***]
Serum insulin (µu/L)	10.33 ± 0.9423	3.596 ± 0.9118 [a****]	6.414 ± 0.6916 [a*** b***]
HOMA-IR	2.126 ± 0.4945	2.061 ± 0.4983	3.045 ± 0.4362 [a**** b****]

Validation of serum NO, APN, MPO, lipids and AIP

Newly diagnosed patients had significantly higher levels of circulating APN, MPO, TG, VLDL, TC, LDL and AIP, and significantly lower levels of serum NO and HDL as compared to healthy control. In insulin-treated patients, levels of circulating MPO, TC and LDL decreased significantly, with a relatively positive effect on the other biochemical parameters (Tables 3 and 4). MPO is negatively correlated with NO in type 1 diabetic group (r = -0.67) (Table 4).

Values represent as mean ± SD. [a] represent differences between untreated and insulin-treated patients in contrast to healthy control; [b] represents differences between insulin and untreated patients. **$p < 0.01$; ****$p < 0.0001$ represents statistically significant differences, as set by Kruskal-Wallis test followed by a Dunn's multiple comparison post-hoc test.

Table 3. Levels of NO, APN and MPO in control, untreated and insulin-treated groups.

Parameters	Control	Untreated	Insulin
Nitric oxide (µmol/L)	13.52 ± 0.9837	11.33 ± 0.7312 [a****]	11.51 ± 0.736 [a****]
Adiponectin (µg/ml)	12.92 ± 0.7566	16.86 ± 0.8661 [a****]	16.38 ± 0.6784 [a****]
Myeloperoxidase (U/ml)	15.63 ± 1.39	24.81 ± 1.618 [a****]	22.45 ± 1.654 [a**** b**]

Table 4. Correlation between MPO and NO in T1DM.

Parameters	T1DM
MPO / NO	r=-6744***

(***$p < 0.001$) representing statistically significant relation, were calculated by Spearman correlation analysis. r = correlation coefficient.

Table 5. Lipid levels and AIP in control, untreated and insulin-treated groups.

Parameters	Control	Untreated	Insulin
TG (mmol/L)	1.213 ± 0.1891	2.091 ± 0.2136 [a****]	1.971 ± 0.2566 [a****]
VLDL (mmol/L)	0.5513 ± 0.08597	0.9504 ± 0.0971 [a****]	0.8961 ± 0.1166 [a****]
TC (mmol/L)	4.433 ± 0.6259	5.668 ± 0.2191 [a****]	5.039 ± 0.4219 [a* b****]
LDL (mmol/L)	2.507 ± 0.6167	3.632 ± 0.2531 [a****]	2.978 ± 0.5029 [b****]
HDL (mmol/L)	1.375 ± 0.1000	1.086 ± 0.1151 [a****]	1.165 ± 0.1986 [a***]
AIP	-0.05798 ± 0.07048	0.2845 ± 0.08406 [a****]	0.2311 ± 0.1172 [a****]

Values represent as mean ± SD. [a] represent differences between untreated and insulin-treated patients in contrast to healthy control; [b] represents differences between insulin and untreated patients. *$p < 0.05$; ***$p < 0.001$; ****$p < 0.0001$ represents statistically significant differences, as set by Kruskal-Wallis test followed by a Dunn's multiple comparison post-hoc test.

Discussion

The present study was conducted to evaluate the impact of insulin on oxidative stress and CVD biomarkers in type 1 diabetic patients.

Alteration of NO level in diabetic patients has been reported by various studies; however, results are discrepant. Some findings revealed reduced serum NO in type 1 and type 2 diabetes (Daimon et al. 2000; Ayub et al. 2011), while the opposite effect has been found by others (Hoeldtke et al. 2003; Mylona-Karayanni et al. 2006; Adela et al. 2015). In addition, NO bioavailability was reported as being reduced in diet-induced obese and diabetic mice (Kim et al. 2008). Our findings revealed that serum NO level is reduced in diabetic patients as compared to control. Previous findings have revealed racial variations in NO levels across various ethnicities (Mata-Greenwood and Chen 2008). Insulin therapy revealed a non-significant increase in NO level. Ding et al. (2000) reported that insulin increased NO production in cultured endothelial cells incubated with high-glucose conc., but this stimulatory effect of insulin is inhibited by hyperglycaemia.

Several studies have been previously demonstrated that adiponectin level is higher in type 1 diabetic patients as compared to healthy control (Galler et al. 2007; Maahs et al. 2007; LeCaire and Palta 2015), which are in line of our findings. Again, insulin therapy reported a non-significant reduction in APN level. Skovso et al. (2015) revealed that insulin therapy has a relatively positive effect on APN in rats. Recently, it has been found that insulin works via two main molecular mechanisms: by reducing the inflammatory effect that produced by elevated free fatty acid in adipose tissue and by reducing the production of reactive oxygen species (ROS) caused by increased blood glucose level (Kadowaki and Yamauchi 2005). As a result of these metabolic effects, insulin maintains the function of mitochondria, increases APN production and thus activates AMP-activated protein kinase enzyme, resulting in depleting fat stores in adipose tissues and restoring glucose utilization and oxidation (Yamauchi et al. 2001). Moreover, by reducing the expression and effect of nitric oxide synthase in the endothelium, insulin can prevent microcirculatory changes and thereby, reducing cellular hypoxia (Langouche et al. 2005). However, the favourable effects of insulin therapy are dose-dependent.

Our findings revealed that serum MPO level were significantly increased in type 1 diabetic patients as compared to control. Heilman et al. (2009) reported that the serum level of MPO was significantly higher in type 1 diabetic children than that in healthy control, which is explain the elevated risk for CVD in type 1 diabetic patients. In contrast, another study by Saeed and Castle (1998) showed that MPO activity can be diminished in diabetic patients. Increased glucose levels are associated with modulation of cellular expression of adhesion molecules and cytokines that involved in the pathogenesis of atherosclerosis. Although the exact mechanism underlying is not clear, one possible mechanism is associated MPO's role as a mediator of vascular injury (Zhang et al. 2004). Our findings revealed a significant increase in MPO levels in type 1 diabetic patients in comparison to control group, resulting in increased risk for future CVD. In the present study, a strong inverse correlation was observed between plasma NO and MPO levels in diabetic patients, which is in accordance with previous study (Eiserich et al. 1998). Recently, NO was suggested as modulator for peroxidase activity of MPO and acts as a substrate for MPO (Davies 2011). Therefore, increased MPO activity resulting in higher NO consumption and subsequent endothelial dysfunction. MPO can generate reactive nitrating oxidant species from NO (Eiserich et al. 1998), which have been identified as an important proinflammatory mediator in CVD (Shishehbor et al. 2003). As a result, MPO pathway may connect between inflammatory process and vascular complications in diabetes.

CVD is the main cause of mortality in type 1 diabetic patients (Libby et al. 2005). Dyslipidaemia has been found to be closely associated with T1DM (Grauslund et al. 2010). Therefore, it looks important to focus on dyslipidaemia in type 1 diabetic patients to reduce CVD. Several studies confirmed that circulating levels of CVD risk markers were higher type 1 diabetic patients, including increased TG, VLDA, TC, LDL and AIP, as well as reduced plasma HDL (Guy et al. 2009; Zachurzok et al. 2016; Zabeen et al. 2018). Interestingly, these studies are in line with our findings. The pathogenesis of this dyslipidaemia is not fully understood, but it is likely related to hyperglycaemia and defect in insulin actions (Vergès 2009; Fathi et al. 2020). Moreover, Goldberg (2001) suggested that insulin therapy may normalize or improve these abnormal lipid profile, and good blood glucose control may have enhanced HDL and reduced TG levels. In our study, insulin treated patients revealed a significant reduction in TC and LDL, as well as a non-significant improvement in circulating levels of TG, VLDL, HDL and AIP. Insulin plays a pivotal role in regulating metabolism of lipids, where insulin inhibits lipase in adipose tissue. Therefore, insulin increasing TG in the adipocytes and lowering the circulating levels of free fatty acids (Vergès 2001). Moreover, insulin suppresses hepatic production of VLDL and increases LDL clearance by up-regulation of LDL receptor expression (Mazzone et al. 1984). In addition, insulin regulates HDL metabolism by acting on hepatic activities of lipase (Ruotolo et al. 1994). Therefore, the low dose of insulin is probably the possible explanation for the relatively little effect of insulin on oxidative stress and CVD risk parameters.

Conclusion

Type 1 diabetes mellitus is closely linked with increased biomarkers of oxidative stress and dyslipidaemia. Insulin therapy produced relatively positive effects on oxidative stress and CVD risk factors. Thus, type 1 diabetic patients required a combined therapy of insulin and antioxidant (vitamin C and vitamin E) to normalize these parameters.

Acknowledgements

The authors are grateful to the University of Mosul for its continuous support and encouragement to all researchers.

References

Adela R, Nethi SK, Bagul PK, Barui AK, Mattapally S, Kuncha M, Patra CR, Reddy PNC, Banerjee SK (2015) Hyperglycaemia enhances nitric oxide production in diabetes: a study from South Indian patients. PLoS ONE 10: e0125270. https://doi.org/10.1371/journal.pone.0125270

American Diabetes A (2009) Diagnosis and classification of diabetes mellitus. Diabetes Care 32 Suppl 1: S62–67. https://doi.org/10.2337/dc09-S062

Anand AV, Muneeb M, Divya N, Senthil R, Kapoor MMA, Gowri J, Begum TN (2011) Clinical significance of hypertension, diabetes and inflammation, as predictor of cardiovascular disease.

Ayub T, Khan SN, Ayub SG, Dar R, Andrabi KI (2011) Reduced nitrate level in individuals with hypertension and diabetes. Journal of Cardiovascular Disease Research 2: 172–176. https://doi.org/10.4103/0975-3583.85264

Baldus S, Heeschen C, Meinertz T, Zeiher AM, Eiserich JP, Munzel T, Simoons ML, Hamm CW, Investigators C (2003) Myeloperoxidase serum levels predict risk in patients with acute coronary syndromes. Circulation 108: 1440–1445. https://doi.org/10.1161/01.CIR.0000090690.67322.51

Begum M, Kumar JA, D'Souza HP, Sushith S, Prathima MB, Shridhar R, Dasegowda SM, Anil M, Nair SS, Kumar KA (2015) Myeloperoxidase, Malondialdehyde and serum Lipids in type 2 diabetes mellitus. Journal of Investigational Biochemistry 4: 1–13. https://doi.org/10.5455/jib.20150331115356

Boyne MS, Saudek CD (1999) Effect of insulin therapy on macrovascular risk factors in type 2 diabetes. Diabetes Care 22 Suppl 3: C45–53.

Braffett BH, Dagogo-Jack S, Bebu I, Sivitz WI, Larkin M, Kolterman O, Lachin JM, Group DER (2019) Association of Insulin Dose, Cardiometabolic Risk Factors, and Cardiovascular Disease in Type 1 Diabetes During 30 Years of Follow-up in the DCCT/EDIC Study. Diabetes Care 42: 657–664. https://doi.org/10.2337/dc18-1574

Cerchiaro GA, Scavone C, Texeira S, Sannomiya P (2001) Inducible nitric oxide synthase in rat neutrophils: role of insulin. Biochem Pharmacol 62: 357–362. https://doi.org/10.1016/S0006-2952(01)00672-4

Chabrol E, Charonnat R (1937) Une nouvelle réaction pour l'étude des lipides: L'oleidemie. La Presse Médicale 45: e1713.

Chen K, Pittman RN, Popel AS (2008) Nitric oxide in the vasculature: where does it come from and where does it go? A quantitative perspective. Antioxid Redox Signal 10: 1185–1198. https://doi.org/10.1089/ars.2007.1959

Daimon M, Sugiyama K, Saitoh T, Yamaguchi H (2000) Increase in serum ceruloplasmin levels is correlated with a decrease of serum nitric oxide levels in type 2 diabetes. Diabetes Care 23: e559. https://doi.org/10.2337/diacare.23.4.559

Davies MJ (2011) Myeloperoxidase-derived oxidation: mechanisms of biological damage and its prevention. Journal of clinical biochemistry and nutrition 48: 8–19. https://doi.org/10.3164/jcbn.11-006FR

Ding Y, Vaziri ND, Coulson R, Kamanna VS, Roh DD (2000) Effects of simulated hyperglycemia, insulin, and glucagon on endothelial nitric oxide synthase expression. American Journal of Physiology-Endocrinology and Metabolism 279: E11–17. https://doi.org/10.1152/ajpendo.2000.279.1.E11

Dobiásová M, Frohlich J (2001) The plasma parameter log (TG/HDL-C) as an atherogenic index: correlation with lipoprotein particle size and esterification rate in apoB-lipoprotein-depleted plasma (FER(HDL)). Clinical Biochemistry 34: 583–588. https://doi.org/10.1016/S0009-9120(01)00263-6

Eiserich JP, Hristova M, Cross CE, Jones AD, Freeman BA, Halliwell B, van der Vliet A (1998) Formation of nitric oxide-derived inflammatory oxidants by myeloperoxidase in neutrophils. Nature 391: 393–397. https://doi.org/10.1038/34923

Fathi Z, Mohammed J, Mohammed M (2020) Levels of Myeloperoxidase, Malondialdehyde and Lipid Profile in Type 2 Diabetic Patients on Metformin Versus Glibenclamide Therapy. Systematic Reviews in Pharmacy 11: 1777–1782.

Galler A, Gelbrich G, Kratzsch J, Noack N, Kapellen T, Kiess W (2007) Elevated serum levels of adiponectin in children, adolescents and young adults with type 1 diabetes and the impact of age, gender, body mass index and metabolic control: a longitudinal study. European Journal of Endocrinology 157: 481–489. https://doi.org/10.1530/EJE-07-0250

Goldberg IJ (2001) Clinical review 124: Diabetic dyslipidemia: causes and consequences. The Journal of Clinical Endocrinology and Metabolism 86: 965–971. https://doi.org/10.1210/jcem.86.3.7304

Grauslund J, Jorgensen TM, Nybo M, Green A, Rasmussen LM, Sjolie AK (2010) Risk factors for mortality and ischemic heart disease in patients with long-term type 1 diabetes. Journal of Diabetes and its Complications 24: 223–228. https://doi.org/10.1016/j.jdiacomp.2009.05.003

Guy J, Ogden L, Wadwa RP, Hamman RF, Mayer-Davis EJ, Liese AD, D'Agostino Jr R, Marcovina S, Dabelea D (2009) Lipid and lipoprotein profiles in youth with and without type 1 diabetes: the SEARCH for Diabetes in Youth case-control study. Diabetes Care 32: 416–420. https://doi.org/10.2337/dc08-1775

Hecht Baldauff N, Tfayli H, Dong W, Arena VC, Gurtunca N, Pietropaolo M, Becker DJ, Libman IM (2016) Relationship of adiponectin and leptin with autoimmunity in children with new-onset type 1 diabetes: a pilot study. Pediatr Diabetes 17: 249–256. https://doi.org/10.1111/pedi.12267

Heilman K, Zilmer M, Zilmer K, Lintrop M, Kampus P, Kals J, Tillmann V (2009) Arterial stiffness, carotid artery intima-media thickness and plasma myeloperoxidase level in children with type 1 diabetes. Diabetes Research and Clinical Practice 84: 168–173. https://doi.org/10.1016/j.diabres.2009.01.014

Heinecke JW (2003) Oxidative stress: new approaches to diagnosis and prognosis in atherosclerosis. The American Journal of Cardiology 91: 12a–16a. https://doi.org/10.1016/S0002-9149(02)03145-4

Herman ME, O'Keefe JH, Bell DSH, Schwartz SS (2017) Insulin Therapy Increases Cardiovascular Risk in Type 2 Diabetes. Progress in Cardiovascular Diseases 60: 422–434. https://doi.org/10.1016/j.pcad.2017.09.001

Hoeldtke RD, Bryner KD, McNeill DR, Warehime SS, Van Dyke K, Hobbs G (2003) Oxidative stress and insulin requirements in patients with recent-onset type 1 diabetes. The Journal of Clinical Endocrinology and Metabolism 88: 1624–1628. https://doi.org/10.1210/jc.2002-021525

Inoue T, Kotooka N, Morooka T, Komoda H, Uchida T, Aso Y, Inukai T, Okuno T, Node K (2007) High molecular weight adiponectin as a predictor of long-term clinical outcome in patients with coronary artery disease. The American Journal of Cardiology 100: 569–574. https://doi.org/10.1016/j.amjcard.2007.03.062

Kadowaki T, Yamauchi T (2005) Adiponectin and adiponectin receptors. Endocrine Reviews 26: 439–451. https://doi.org/10.1210/er.2005-0005

Khan AA, Alsahli MA, Rahmani AH (2018) Myeloperoxidase as an Active Disease Biomarker: Recent Biochemical and Pathological Perspectives. Medical Sciences (Basel) 6: 1–33. https://doi.org/10.3390/medsci6020033

Kim F, Pham M, Maloney E, Rizzo NO, Morton GJ, Wisse BE, Kirk EA, Chait A, Schwartz MW (2008) Vascular inflammation, insulin resistance, and reduced nitric oxide production precede the onset of peripheral insulin resistance. Arteriosclerosis, Thrombosis, and Vascular Biology 28: 1982–1988. https://doi.org/10.1161/ATVBAHA.108.169722

Komers R, Cooper ME (1996) Renal sodium handling in experimental diabetes: role of NO. Nephrology Dialysis Transplantation 11: 2170–2177. https://doi.org/10.1093/oxfordjournals.ndt.a027133

Kumar P, Pai K, Pandey HP, Sundar S (2002) NADH-oxidase, NADPH-oxidase and myeloperoxidase activity of visceral leishmaniasis patients. Journal of Medical Microbiology 51: 832–836. https://doi.org/10.1099/0022-1317-51-10-832

Langouche L, Vanhorebeek I, Vlasselaers D, Vander Perre S, Wouters PJ, Skogstrand K, Hansen TK, Van den Berghe G (2005) Intensive insulin therapy protects the endothelium of critically ill patients. The Journal of clinical investigation 115: 2277–2286. https://doi.org/10.1172/JCI25385

LeCaire TJ, Palta M (2015) Longitudinal Analysis of Adiponectin through 20-Year Type 1 Diabetes Duration. Journal of Diabetes Research 2015: e730407. https://doi.org/10.1155/2015/730407

Lee YB, Han K, Kim B, Lee SE, Jun JE, Ahn J, Kim G, Jin SM, Kim JH (2019) Risk of early mortality and cardiovascular disease in type 1 diabetes: a comparison with type 2 diabetes, a nationwide study. Cardiovascular Diabetology 18: e157. https://doi.org/10.1186/s12933-019-0953-7

Libby P, Nathan DM, Abraham K, Brunzell JD, Fradkin JE, Haffner SM, Hsueh W, Rewers M, Roberts BT, Savage PJ, Skarlatos S, Wassef M, Rabadan-Diehl C, National Heart L, Blood I, National Institute of D, Digestive, Kidney Diseases Working Group on Cardiovascular Complications of Type 1 Diabetes M (2005) Report of the National Heart, Lung, and Blood Institute-National Institute of Diabetes and Digestive and Kidney Diseases Working Group on Cardiovascular Complications of Type 1 Diabetes Mellitus. Circulation 111: 3489–3493. https://doi.org/10.1161/CIRCULATIONAHA.104.529651

Lind M, Svensson AM, Kosiborod M, Gudbjornsdottir S, Pivodic A, Wedel H, Dahlqvist S, Clements M, Rosengren A (2014) Glycemic control and excess mortality in type 1 diabetes. The New England Journal of Medicine 371: 1972–1982. https://doi.org/10.1056/NEJMoa1408214

Lindström T, Frystyk J, Hedman CA, Flyvbjerg A, Arnqvist HJ (2006) Elevated circulating adiponectin in type 1 diabetes is associated with long diabetes duration. Clinical Endocrinology (Oxf) 65: 776–782. https://doi.org/10.1111/j.1365-2265.2006.02666.x

Maahs DM, Ogden LG, Snell-Bergeon JK, Kinney GL, Wadwa RP, Hokanson JE, Dabelea D, Kretowski A, Eckel RH, Rewers M (2007) Determinants of serum adiponectin in persons with and without type 1 diabetes. American Journal of Epidemiology 166: 731–740. https://doi.org/10.1093/aje/kwm125

Majid DS, Navar LG (1997) Nitric oxide in the mediation of pressure natriuresis. Clinical and Experimental Pharmacology and Physiology 24: 595–599. https://doi.org/10.1111/j.1440-1681.1997.tb02098.x

Martins JO, Wittlin BM, Anger DB, Martins DO, Sannomiya P, Jancar S (2010) Early phase of allergic airway inflammation in diabetic rats: role of insulin on the signaling pathways and mediators. Cellular Physiology and Biochemistry 26: 739–748. https://doi.org/10.1159/000322341

Martos-Moreno GA, Barrios V, Soriano-Guillén L, Argente J (2006) Relationship between adiponectin levels, acylated ghrelin levels, and short-term body mass index changes in children with diabetes mellitus type 1 at diagnosis and after insulin therapy. European Journal of Endocrinology 155: 757–761. https://doi.org/10.1530/eje.1.02273

Mata-Greenwood E, Chen DB (2008) Racial differences in nitric oxide-dependent vasorelaxation. Reproductive Sciences 15: 9–25. https://doi.org/10.1177/1933719107312160

Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC (1985) Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28: 412–419. https://doi.org/10.1007/BF00280883

Mazzone T, Foster D, Chait A (1984) In vivo stimulation of low-density lipoprotein degradation by insulin. Diabetes 33: 333–338. https://doi.org/10.2337/diab.33.4.333

Meuwese MC, Stroes ES, Hazen SL, van Miert JN, Kuivenhoven JA, Schaub RG, Wareham NJ, Luben R, Kastelein JJ, Khaw KT, Boekholdt SM (2007) Serum myeloperoxidase levels are associated with the future risk of coronary artery disease in apparently healthy individuals: the EPIC-Norfolk Prospective Population Study. Journal of the American College of Cardiology 50: 159–165. https://doi.org/10.1016/j.jacc.2007.03.033

Miranda KM, Espey MG, Wink DA (2001) A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. Nitric Oxide 5: 62–71. https://doi.org/10.1006/niox.2000.0319

Mylona-Karayanni C, Gourgiotis D, Bossios A, Kamper EF (2006) Oxidative stress and adhesion molecules in children with type 1 diabetes mellitus: a possible link. Pediatric Diabetes 7: 51–59. https://doi.org/10.1111/j.1399-543X.2006.00147.x

Ouchi N, Kobayashi H, Kihara S, Kumada M, Sato K, Inoue T, Funahashi T, Walsh K (2004) Adiponectin stimulates angiogenesis by promoting cross-talk between AMP-activated protein kinase and Akt signaling in endothelial cells. Journal of Biological Chemistry 279: 1304–1309. https://doi.org/10.1074/jbc.M310389200

Qiu C, Muchant D, Beierwaltes WH, Racusen L, Baylis C (1998) Evolution of chronic nitric oxide inhibition hypertension: relationship to renal function. Hypertension 31: 21–26. https://doi.org/10.1161/01.HYP.31.1.21

RD&I Christchurch (2005) Pekapeka / Bats. Department of Conservation, Te Papa Atawhai.

Ruotolo G, Parlavecchia M, Taskinen MR, Galimberti G, Zoppo A, Le NA, Ragogna F, Micossi P, Pozza G (1994) Normalization of lipoprotein composition by intraperitoneal insulin in IDDM. Role of increased hepatic lipase activity. Diabetes Care 17: 6–12. https://doi.org/10.2337/diacare.17.1.6

Saeed FA, Castle GE (1998) Neutrophil chemiluminescence during phagocytosis is inhibited by abnormally elevated levels of acetoacetate: implications for diabetic susceptibility to infections. Clinical and Vaccine Immunology 5: 740–743. https://doi.org/10.1128/CDLI.5.5.740-743.1998

Shishehbor MH, Aviles RJ, Brennan ML, Fu X, Goormastic M, Pearce GL, Gokce N, Keaney JF, Jr., Penn MS, Sprecher DL, Vita JA, Hazen SL (2003) Association of nitrotyrosine levels with cardiovascular disease and modulation by statin therapy. Jama 289: 1675–1680. https://doi.org/10.1001/jama.289.13.1675

Silver B, Ramaiya K, Andrew SB, Fredrick O, Bajaj S, Kalra S, Charlotte BM, Claudine K, Makhoba A (2018) EADSG Guidelines: Insulin Therapy in Diabetes. Diabetes Therapy 9: 449–492. https://doi.org/10.1007/s13300-018-0384-6

Skovso S, Damgaard J, Fels JJ, Olsen GS, Wolf XA, Rolin B, Holst JJ (2015) Effects of insulin therapy on weight gain and fat distribution in the HF/HS-STZ rat model of type 2 diabetes. International Journal of Obesity (Lond) 39: 1531–1538. https://doi.org/10.1038/ijo.2015.92

Soedamah-Muthu SS, Fuller JH, Mulnier HE, Raleigh VS, Lawrenson RA, Colhoun HM (2006) High risk of cardiovascular disease in patients with type 1 diabetes in the U.K.: a cohort study using the gener-

al practice research database. Diabetes Care 29: 798–804. https://doi.org/10.2337/diacare.29.04.06.dc05-1433

Su H, Lau WB, Ma XL (2011) Hypoadiponectinaemia in diabetes mellitus type 2: molecular mechanisms and clinical significance. Clinical and Experimental Pharmacology and Physiology 38: 897–904. https://doi.org/10.1111/j.1440-1681.2011.05606.x

Vergès B (2001) Insulin sensitiviy and lipids. Diabetes Metab 27:e 223.

Vergès B (2009) Lipid disorders in type 1 diabetes. Diabetes Metab 35: 353–360. https://doi.org/10.1016/j.diabet.2009.04.004

Wang Y, Ma XL, Lau WB (2017) Cardiovascular adiponectin resistance: the critical role of adiponectin receptor modification. Trends in Endocrinology & Metabolism 28: 519–530. https://doi.org/10.1016/j.tem.2017.03.004

Yamauchi T, Kamon J, Waki H, Terauchi Y, Kubota N, Hara K, Mori Y, Ide T, Murakami K, Tsuboyama-Kasaoka N, Ezaki O, Akanuma Y, Gavrilova O, Vinson C, Reitman ML, Kagechika H, Shudo K, Yoda M, Nakano Y, Tobe K, Nagai R, Kimura S, Tomita M, Froguel P, Kadowaki T (2001) The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. Nature Medicine 7: 941–946. https://doi.org/10.1038/90984

Zabeen B, Balsa AM, Islam N, Parveen M, Nahar J, Azad K (2018) Lipid Profile in Relation to Glycemic Control in Type 1 Diabetes Children and Adolescents in Bangladesh. Indian Journal of Endocrinology and Metabolism 22: 89–92. https://doi.org/10.4103/ijem.IJEM_217_17

Zachurzok A, Deja G, Gawlik A, Drosdzol-Cop A, Klimek K, Malecka-Tendera E (2016) Lipid Profile in Adolescent Girls with Type 1 Diabetes Mellitus and Hyperandrogenemia. International Journal of Endocrinology 2016: e9473158. https://doi.org/10.1155/2016/9473158

Zhang C, Yang J, Jennings LK (2004) Leukocyte-derived myeloperoxidase amplifies high-glucose–induced endothelial dysfunction through interaction with high-glucose–stimulated, vascular non--leukocyte-derived reactive oxygen species. Diabetes 53: 2950–2959. https://doi.org/10.2337/diabetes.53.11.2950