Association of paraoxonase 1 activity and insulin resistance models in type 2 diabetes mellitus: Cross-sectional study

Usha Adiga[a,*], Nandit Banawalikar[b], Desy Thayyil Menambath[c]

[a]Department of Biochemistry, KS Hegde Medical Academy, Nitte-DU, India; [b]Department of Pharmacology, KS Hegde Medical Academy, Nitte-DU, India; [c]Department of Biochemistry, KS Hegde Medical Academy, Nitte-DU, India

Abstract

Background: Diabetes mellitus is a metabolic disorder characterized by hyperglycemia due to defects in insulin secretion, insulin action, or both. Chronic hyperglycemia induces reactive oxygen species and increases oxidative stress. Human serum paraoxonase-1 (PON-1) is an enzyme synthesized in the liver, and it is an antioxidant enzyme with a beneficial role in fighting oxidative stress. The objective of the study was to compare PON-1 activity in type 2 diabetes mellitus (T2DM) and nondiabetics, as well as to find the association between PON-1 activity and different insulin resistance (IR) models in diabetics.

Methods: The cross-sectional study recruited 100 diabetic and 100 age and gender-matched controls. Fasting blood glucose, insulin, and C-peptide, were assayed. PON-1 activity was measured by the spectrophotometric method. Various insulin resistance models based on insulin and C-peptide were constructed using appropriate formulae. Receiver operating characteristic was constructed to find if PON-1 can be a good marker for diabetes.

Results: PON-1 activity was found to be significantly higher ($p < 0.0001$) in diabetics compared to controls. Highly significant hyperinsulinemia ($p < 0.0001$) was noted in diabetics. C-peptide levels were significantly lower in cases ($p = 0.0215$) as compared to controls. Homeostasis model assessment (HOMA)-IR C was insignificantly higher in cases. HOMA B cell, HOMA 1% B cell, and C-peptide–based IR (CIR) were significantly lower in cases ($p < 0.0001$ and $p = 0.002$), respectively, as compared to controls. An odds ratio of 3.15 was obtained, which suggests that the risk of T2DM is 3 times higher in subjects with elevated PON-1 levels. Chi-square showed a significant association ($p = 0.0001$) between DM and PON-1 levels; the chi-square statistic value (with Yates correction) was 14.49. Correlation data showed that PON-1 activity had a significant negative correlation with quantitative insulin sensitivity check index ($r = –0.265$, $p = 0.019$). A significant negative correlation ($r = –0.22$, $p = 0.016$) was also seen between PON-1 and CIR (HOMA-IR C). There was no significant correlation seen between PON-1 and other IR models.

Conclusion: It can be concluded from our study that PON-1 activity is elevated in T2DM patients, which can be a beneficial marker.

Keywords: Aryldialkylphosphatase; Diabetes mellitus; Insulin resistance

1. INTRODUCTION

Diabetes mellitus is a systemic disorder where the body cannot synthesize enough insulin or can't use insulin due to organ resistance. The progressive loss of β-cells is caused by increased glucotoxicity, lipotoxicity, endoplasmic reticulum–induced stress, and apoptosis seen in type 2 diabetes mellitus (T2DM).[1–5] The pathogenesis of T2DM is implicated by both peripheral insulin resistance (IR) and dysfunctional insulin secretion by pancreatic β-cells.[1–3]

IR has traditionally been associated with T2DM. IR leading to decreased glucose uptake and utilization and in the liver leads to increased hepatic glucose production. Worsening IR causes the β-cell to fail to compensate, leading to impaired glucose tolerance and frank diabetes. The exact pathophysiology of IR is not clear, but it possibly involves a combination of genetic and environmental factors. These factors may result in changes in the number of insulin receptors, their affinity for insulin, or a defect in post-receptor signaling or a combination of these.

Paraoxonase-1 (PON-1) is a member of a family of proteins, the genes for which are clustered in tandem on the long arms of human chromosome-7 and is synthesized mainly in the liver, and a fraction is secreted into the plasma, where it is coupled with high-density lipoproteins (HDLs). It has antioxidant functions when it binds to the HDL particles and prevents the oxidation of low-density lipoprotein (LDL), which possibly plays a role in the hindrance of atherosclerosis and coronary artery disease.[6]

It appears to be a bidirectional relationship between PON-1 and diabetes, suggesting diabetes reduces PON-1 levels, and the PON-1 gene polymorphisms causing a risk of diabetes development.[7] In T2DM, glycation has a major impact on PON-1 activity contributing to the typical inflammatory process leading to its manifestations and complications. PON-1 also protects the plasma membrane from free radical injury.[8] Reduction in PON-1 activity is considered to be a major cause of dysfunctional HDL in

*Address correspondence. Dr. Usha Adiga, Department of Biochemistry, KS Hegde Medical Academy, Derlakatte, Mangalore, Karnataka, India.

E-mail address: ushachidu@yahoo.com (U. Adiga).

Conflicts of interest: The authors declare that they have no conflicts of interest related to the subject matter or materials discussed in this article.

Journal of Chinese Medical Association. (2022) 85: 77-80.

Received April 22, 2021; accepted June 15, 2021.

doi: 10.1097/JCMA.0000000000000665.

patients with T2DM, which has been suggested to lead to accelerated atherosclerosis and thereby amplified mortality due to CAD.[9] Hyperglycemia induces oxidative stress directly by depleting natural antioxidants and facilitating the production of reactive oxygen species; the caring effects of PON-1 activity in opposition to the peroxidation of LDL particles have been guessed to be more important in T2DM patients than in nondiabetic subjects.[10]

Altered PON-1 levels in metabolic syndrome and IR may be contributing factors for the development of T2DM. Estimation of PON-1 levels may be a useful diagnostic marker for the prediction of T2DM in subjects with IR and metabolic syndrome.[11] We hypothesize that PON-1 is an antioxidant enzyme, the activity of which may be elevated or decreased depending on the oxidative stress status. The alterations in PON-1 levels are mainly to balance the preoxidant-antioxidant balance.

The study was planned with the main aim of finding the association between PON-1 and IR and also to assess if PON-1 can be a useful marker to predict diabetes mellitus in insulin-resistant subjects.

The study was designed with the following objectives:

To compare PON-1 levels in patients with T2DM and nondiabetics.
To compare different IR models in patients with T2DM and nondiabetics.
To find the association between PON-1 and IR.

2. METHODS

2.1. Study design

The cross-sectional study was conducted in the Department of Biochemistry. Institutional ethics committee approval was sought before starting the study. Informed consent was obtained from the subjects.

2.2. Inclusion and exclusion criteria

Hundred T2DM patients (18–65 years), diagnosed as per American Diabetics Association 2016 guidelines were included as cases. Hundred age and gender-matched nondiabetics, healthy volunteers, or those having health packages undergoing surgery were considered as controls.

Alcoholics, diagnosed cases of acute and chronic hepatitis, other liver disorders, or any other systemic illness were excluded from the study.

2.3. Sample collection and analysis

Two milliliters of fasting venous blood sample was collected using aseptic precaution. The blood sample was centrifuged at 3000 rpm for 10 minutes, and serum was separated. Fasting blood glucose was estimated using a fully automated chemistry analyzer, Cobas C-311. Fasting insulin and C-peptide levels were assayed by ELISA. Various IR models with serum insulin and C-peptide values were constructed using suitable formulae (Table 1).[12] IR was calculated by the homeostasis model assessment (HOMA).

Table 1

IR models[12]

HOMA-IR	(Fasting glucose × fasting insulin)/22.5; insulin expressed in μU/L, glucose in mmol/L
HOMA B cell	20× insulin/(fasting blood glucose−3); FBS in mmol/L
HOMA B 1%	20× insulin/fasting plasma glucose−3.5; FBS in mmol/L
QUICKI	1/(log G + log I)
CIR	20/(glucose × C-peptide); glucose and C-peptide in mmol/L

CIR = C-peptide based insulin resistance; FBS = fasting blood sugar; HOMA = homeostasis model assessment; IR = insulin resistance; QUICKI = quantitative insulin sensitivity check index.

PON-1 activity was assayed using a spectrophotometric method.[10]

2.4. Statistical analysis

Statistical analysis was carried out using SPSS 23. Comparison of fasting blood sugar, PON-1, insulin, C-peptide levels, and various IR models was done using the Mann-Whitney *U* test. Spearman's correlation was used to find the correlation between PON-1 and various IR models. The receiver operating characteristic curve was used to evaluate the performance of PON-1 as a marker of T2DM. A Chi-square test was done to find the association between PON-1 and IR. Odds ratio was calculated to assess the risk of T2DM in patients with elevated PON-1 levels.

3. RESULTS

PON-1 activity was found to be significantly higher ($p < 0.0001$) in T2DM compared to controls (Table 2). Highly significant hyperinsulinemia ($p < 0.0001$) was noted in T2DM. Insulin-based HOMA-IR was also higher in T2DM ($p = 0.015$). HOMA B cell, HOMA 1% B cell, and quantitative insulin sensitivity check index (QUICKI) were very significantly low ($p < 0.0001$) in cases (Table 2).

C-peptide levels were significantly lower in cases ($p = 0.021$) as compared to controls. IR models based on C-peptides were also calculated. HOMA-IR C was insignificantly higher in cases. HOMA B cell C, HOMA 1% B cell C, and C-peptide IR (CIR) were significantly lower in cases ($p < 0.0001$ and $p = 0.002$), respectively, as compared to controls (Table 2).

A correlation study showed that PON-1 activity had a significant negative correlation with QUICKI ($r = -0.265$, $p = 0.019$). A significant negative correlation ($r = -0.22$, $p = 0.016$) was also seen between PON-1 and CIR (HOMA-IR C). There was no significant correlation seen between PON-1 and other IR models.

On analyzing receiver operating characteristic (ROC), the area under the curve (AUC) was found to be 0.688 with a sensitivity and specificity of 70.8% and 56.5%, respectively, with a cutoff PON-1 value of 0.745 nmol/mL/min (Fig. 1). ROC was constructed between PON-1 and HOMA-IR, considering HOMA-IR >2.4 as IR. AUC was 0.562, sensitivity was 56.4%, and specificity was 50% (Fig. 2).

An odds ratio of 3.15 was obtained, which suggests that the risk of T2DM is 3 times higher in subjects with elevated PON-1 levels. Chi-square showed a significant association ($p = 0.0001$) between DM and PON-1 levels; the chi-square statistic (with Yates correction) was 14.49.

Table 2

Comparing PON-1 activity and insulin-based IR models of type 2 diabetes mellitus and nondiabetics

Parameters, unit	Case	Controls	*p*
FBS, mg/dL	192.32 ± 8.72	101.6 ± 1.503	<0.0001
Insulin, mIU/L	25.93 ± 5.43	11.67 ± 1.46	0.0002
HOMA-IR	6.72 ± 1.07	3.05 ± 0.39	0.015
HOMA B cell	49.54 ± 12.9	87.17 ± 10.7	<0.0001
HOMA 1% B cell	59.05 ± 14.19	108.62 ± 13.69	<0.0001
QUICKI	0.305 ± 0.004	0.36 ± 0.008	<0.0001
HOMA-IR C	0.93 ± 0.11	0.31 ± 0.02	0.14
HOMA B cell C	4.77 ± 1.27	28.96 ± 2.204	<0.0001
HOMA 1% B cell C	5.95 ± 1.27	10.70 ± 1.83	<0.0001
CIR	4.99 ± 0.65	7.60 ± 1.51	0.042
PON-1, U/mL	0.919 ± 0.03	0.66 ± 0.04	<0.0001

CIR = C-peptide based insulin resistance; FBS = fasting blood sugar; HOMA = homeostasis model assessment; IR = insulin resistance; PON-1 = human serum paraoxonase-1; QUICKI = quantitative insulin sensitivity check index.

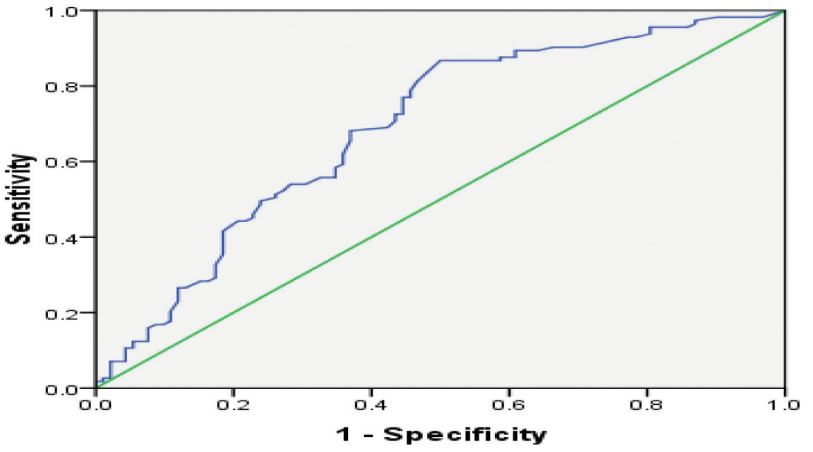

Fig. 1 Receiver operating characteristic for human serum paraoxonase-1 as a marker of diabetes mellitus.

4. DISCUSSION

Our results suggest that PON-1 levels were increased significantly in T2DM as compared to controls. The study by Suvarna et al reported that T2DM patients with complications have significantly decreased HDL-C and PON-1 activity.[13] A study by Ferretti et al showed a significantly lower PON-1 activity in type 1 diabetes mellitus (T1DM).[14] Lowered PON-1 activity was reported by Abbott et al in both T1DM and T2DM patients.[15] Contradictory results are also available, as Beer et al found no significant difference in PON 1 activity and concentration in the diabetic group when compared to impaired fasting glucose and controls.[16]

PON-1 hydrolyses proinflammatory oxidized lipids, which are present in oxidized low-density lipoprotein, and destroys their atherogenic characteristics, and it also decreases the accumulation of lipid peroxidation products.[17,18] The enzyme plays a role in decreasing oxidative stress. PON-1 is an important endogenous free radical scavenging system in the human body.[18]

In T2DM, due to hyperglycemia, free radicals are generated, increasing oxidative stress. This is proven to be one of the mechanisms for the development of complications in T2DM.[19] T2DM is associated with elevated pro-oxidants and hence increased oxidative stress. As PON-1 is an antioxidant and has a protective role, its elevation in our cases could be a defensive mechanism to combat oxidative stress.

Kota et al have reviewed several studies reporting low PON activity in T2DM contributing to a greater risk of atherosclerosis and cardiovascular disease.[20] However, some of these studies reported no difference in PON activity between T2DM and nondiabetics.[15,19,21]

Other studies have also found a significant decrease in PON activity in T2DM patients with microvascular complications when compared to patients with no complications.[15,22]

Hyperinsulinemia observed in T2DM patients is an important marker of IR. This could be due to the exaggerated response of the pancreas to insulin insufficiency or IR. Homeostatic model assessment for IR (HOMA-IR), which describes glucose-insulin homeostasis, has been elevated in diabetics. Markers of basal insulin secretion by β-cells of the pancreas, HOMA B cell, and HOMA1% B cell were decreased in cases, suggesting the lowered insulin-secreting capacity by β-cells. Lowered QUICKI indicates low insulin sensitivity in cases.

A negative correlation was observed between PON-1 and QUICKI, as well as C-peptide–based HOMA-IR (HOMA-IR C) in our study.

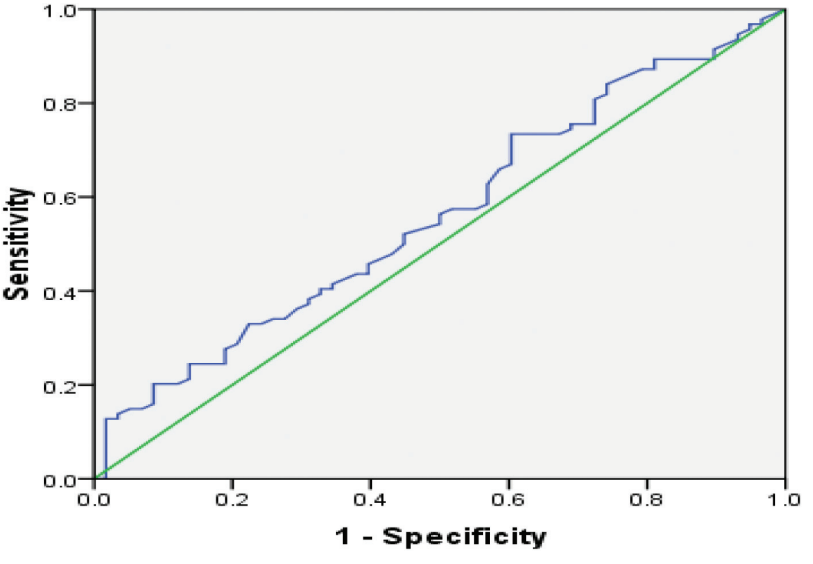

Fig. 2 Receiver operating characteristic for human serum paraoxonase-1 as a marker of insulin resistance.

Oxidative stress has been recognized as a key mechanism in inducing IR. There are various mechanisms of oxidative stress in T2DM. NADPH oxidase 4 (NOX4) is a powerful oxidizing enzyme that produces reactive oxygen species (ROS). Hyperinsulinemia results in a shift in the signaling pathway at PI3-kinase. PI3-kinase phosphorylates Rac instead of phosphatidylinositol 4,5-bisphosphate, which amplifies the activity of NOX4.[23] As a result, ROS levels increase, further activate pathways that divert glucose transporter type 4 to lysosomes, instead of the cell membrane. This hinders glucose transport, and hyperglycemia persists. This explains the negative correlation between PON-1, QUICKI, and HOMA-IR C. Lesser the insulin sensitivity, the more is the oxidative stress and hence higher is the PON-1 activity to combat free radicals.

A significant association between PON-1 activity and diabetes mellitus, as well as three times higher risk of T2DM in subjects with elevated PON-1 levels, supports the mutual or bidirectional association between PON-1 and T2DM.

A reciprocal relationship between PON-1 and diabetes, so that diabetes reduces PON-1 levels, and the PON-1 genotype may additionally have a role in the risk of T2DM development.[7] PON-1 may have a protective effect on the β-cells against high-concentration glucose cytotoxicity and may play a role in the secretion of insulin from these cells.[24] In this context, Lee and colleagues[25] indicated that the cell-permeable fusion proteins PEP-1–PON-1 play a beneficial role in protecting β-cells from cytokine-induced cytotoxicity and restoring insulin secretion through alleviating oxidative/nitrosative stress, inflammation, and ER stress, and it might be an effective method to decrease the destruction and dysfunction of pancreatic β-cells in autoimmune diabetes.

A study by Gluzer et al has demonstrated that PON-1 decreases fasting blood glucose levels, improves glucose tolerance, as well as the HOMA estimation of IR, and increases the sensitivity of peripheral tissue to insulin.[26]

ROC analysis in our study showed low sensitivity, specificity, as well as AUC for PON-1 as a marker of T2DM, as well as IR.

However, PON-1 can be a good marker to predict T2DM in insulin-resistant individuals.

In conclusion, it can be concluded from our study that PON-1 activity is elevated in T2DM patients, which might be beneficial. A strong association between PON-1 activity and T2DM suggested that PON-1 might be a marker to predict T2DM.

ACKNOWLEDGMENTS

We sincerely thank Dr Suchetha Kumari, Central Research Laboratory, for supporting us to utilize the infrastructure and Dr. Katyayani for helping in the standardization of the technique.

REFERENCES

1. Mishra M, Ndisang JF. A critical and comprehensive insight on heme oxygenase and related products including carbon monoxide, bilirubin, biliverdin and ferritin in type-1 and type-2 diabetes. *Curr Pharm Des* 2014;**20**:1370–91.
2. Ndisang JF, Jadhav A. Hemin therapy improves kidney function in male streptozotocin-induced diabetic rats: role of the heme oxygenase/atrial natriuretic peptide/adiponectin axis. *Endocrinology* 2014;**155**:215–29.
3. Petersen MC, Vatner DF, Shulman GI. Regulation of hepatic glucose metabolism in health and disease. *Nat Rev Endocrinol* 2017;**13**:572–87.
4. Ndisang JF, Jadhav A, Mishra M. The heme oxygenase system suppresses perirenal visceral adiposity, abates renal inflammation and ameliorates diabetic nephropathy in Zucker diabetic fatty rats. *PLoS One* 2014;**9**:e87936.
5. Hossain P, Kawar B, El Nahas M. Obesity and diabetes in the developing world–a growing challenge. *N Engl J Med* 2007;**356**:213–5.
6. El-said NH, Nasr-Allah MM, Sadik NA, Sharaf SA. Paraoxonase-1 activity in type 2 diabetes mellitus with and without nephropathy. *EJIM* 2015;**27**:63-8.
7. Farbstein D, Levy AP. The genetics of vascular complications in diabetes mellitus. *Cardiol Clin* 2010;**28**:477–96.
8. Aviram M, Hardak E, Vaya J, Mahmood S, Milo S, Hoffman A, et al. Human serum paraoxonases (PON1) Q and R selectively decrease lipid peroxides in human coronary and carotid atherosclerotic lesions: PON1 esterase and peroxidase-like activities. *Circulation* 2000;**101**:2510–7.
9. Haffner SM, Lehto S, Rönnemaa T, Pyörälä K, Laakso M. Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction. *N Engl J Med* 1998;**339**:229–34.
10. Richter RJ, Jarvik GP, Furlong CE. Determination of paraoxonase 1 status without the use of toxic organophosphate substrates. *Circ Cardiovasc Genet* 2008;**1**:147–52.
11. Ferré N, Feliu A, García-Heredia A, Marsillach J, París N, Zaragoza-Jordana M, et al. Impaired paraoxonase-1 status in obese children. Relationships with insulin resistance and metabolic syndrome. *Clin Biochem* 2013;**46**:1830–6.
12. Arora S, Singh B, Saxena A. Surrogate markers of insulin resistance: a review. *World J Diabetes* 2010;**1**:36-47.
13. Suvarna R, Rao SS, Joshi C, Kedage V, Muttigi MS, Shetty JK, et al. Paraoxonase activity in type 2 diabetes mellitus patients with and without complications. *J Clin Diagnostic Res* 2011;**5**:63-5.
14. Ferretti G, Bacchetti T, Masciangelo S, Bicchiega V. HDL-paraoxonase and membrane lipid peroxidation: a comparison between healthy and obese subjects. *Obesity* 2010;**18**:1079-84.
15. Abbott CA, Mackness MI, Kumar S, Boulton AJ, Durrington PN. Serum paraoxonase activity, concentration, and phenotype distribution in diabetes mellitus and its relationship to serum lipids and lipoproteins. *Arterioscler Thromb Vasc Biol* 1995;**15**:1812–8.
16. Beer S, Moren X, Ruiz J, James RW. Postprandial modulation of serum paraoxonase activity and concentration in diabetic and non-diabetic subjects. *Nutr Metab Cardiovasc Dis* 2006;**16**:457–65.
17. Toker A, Kadi M, Yildirim AK, Aksoy H, Akçay F. Serum lipid profile paraoxonase and arylesterase activities in psoriasis. *Cell Biochem Funct* 2009;**27**:176–80.
18. Erdem FH, Karatay S, Yildirim K, Kiziltunc A. Evaluation of serum paraoxonase and arylesterase activities in ankylosing spondylitis patients. *Clinics (Sao Paulo)* 2010;**65**:175–9.
19. Jamuna Rani A, Mythili SV, Nagarajan S. Study on paraoxonase 1 in type 2 diabetes mellitus. *Indian J Physiol Pharmacol* 2014;**58**:13–6.
20. Kota SK, Meher LK, Kota SK, Jammula S, Krishna SV, Modi KD. Implications of serum paraoxonase activity in obesity, diabetes mellitus, and dyslipidemia. *Indian J Endocrinol Metab* 2013;**17**:402–12.
21. Patil Asmita B, Ganu Jayashree V. Paraoxonase-1, total cholesterol and HDL cholesterol in diabetes mellitus. *Indian J Basic Appl Med Res* 2013;**2**:998-1001.
22. Mackness B, Durrington PN, Abuashia B, Boulton AJ, Mackness MI. Low paraoxonase activity in type II diabetes mellitus complicated by retinopathy. *Clin Sci (Lond)* 2000;**98**:355–63.
23. Campa CC, Ciraolo E, Ghigo A, Germena G, Hirsch E. Crossroads of PI3K and Rac pathways. *Small Gtpases* 2015;**6**:71–80.
24. Koren-Gluzer M, Aviram M, Meilin E, Hayek T. The antioxidant HDL-associated paraoxonase-1 (PON1) attenuates diabetes development and stimulates β-cell insulin release. *Atherosclerosis* 2011;**219**:510–8.
25. Lee SJ, Kang HK, Choi YJ, Eum WS, Park J, Choi SY, et al. PEP-1-paraoxonase 1 fusion protein prevents cytokine-induced cell destruction and impaired insulin secretion in rat insulinoma cells. *BMB Rep* 2018;**51**:538–43.
26. Koren-Gluzer M, Aviram M, Hayek T. Paraoxonase1 (PON1) reduces insulin resistance in mice fed a high-fat diet, and promotes GLUT4 overexpression in myocytes, via the IRS-1/Akt pathway. *Atherosclerosis* 2013;**229**:71–8.