Blood ALDH1 and GST Activity in Diabetes Type 2 and its Correlation with Glycated Hemoglobin

Authors
J. Giebułtowicz[1], S. Sołobodowska[1], D. Bobilewicz[2], P. Wroczyński[1]

Affiliations
[1] Department of Bioanalysis and Drugs Analysis, Warsaw Medical University, Warsaw, Poland
[2] Department of Laboratory Diagnostics, Medical University of Warsaw, Warsaw, Poland

Key words
- diabetes
- oxidative stress
- aldehyde dehydrogenase
- glutathione S-transferase

Abstract

There is increasing evidence that oxidative stress (OS) plays a major role in the pathogenesis of diabetes mellitus (DM) and the development of its complications. As one of the consequences of OS is increased lipid peroxidation (LP), the aim of our studies was to check, how the activity of 2 enzymes involved in the detoxification of aldehydes formed during LP, glutathione S-transferase (GST) and aldehyde dehydrogenase 1 (ALDH 1) is changed in patients suffering from DM.

GST and ALDH1A1 activities were determined in whole blood samples of DM type 2 patients (n = 64) and healthy controls (n = 60) using spectrophotometer (for GST activity) and fluorometer (for ALDH1 activity) and they were found to be significantly increased in diabetics when compared with healthy control ($p < 0.05$). Intriguingly, grouping the DM patients on the basis of the glucose level and HbA1c revealed unusually low ALDH activity in the group of patients (n = 16) with a relatively high level of these 2 parameters. The increase of ALDH1A1 and GST activity in DM seems to be associated with the severity of the disease and might be a compensatory effect against oxidative stress. Surprisingly low ALDH activity in DM patients with relatively high glucose and HbA1c levels can be a factor predisposing to the development of diabetic complications.

Introduction

The number of patients with diabetes mellitus (DM), a metabolic disorder, is rapidly increasing worldwide due to several factors: population growth, aging, urbanization, increasing prevalence of obesity and reduced physical activity [1]. Increased oxidative stress (OS) may have an important causal role in β-cell failure and the development of insulin resistance, it is believed to be related with the onset and progression of diabetes. Moreover, OS also results from hyperglycemia associated with diabetes. Therefore, it is considered as a factor related to diabetes complications [2]. It is highly probable that not only reactive oxygen species (ROS) themselves but also secondary by-products of lipid peroxidation (LP) contribute to the deleterious consequences of OS e.g. insulin resistance [3]. Lipid peroxidation-derived aldehydes (LDAs) may also increase the incidence of DM complications by the modification of the collagen of the cardiovascular system causing its stiffening and changes in the charge profile [4]. This emphasizes the importance of the cellular protective mechanisms against LADs as they can prevent the development of some oxidative stress-associated pathologies. Excessive levels of LDAs are decreased by several enzyme systems that are able to oxidize them to acids like aldehyde dehydrogenase (ALDH) or catalyze their conjugation with endogenous ligands like glutathione-S-transferase (GST) [5].

There are only a few studies on erythrocyte glutathione S-transferase (GST) activity in DM, the results of which are ambiguous. Jerntorp et al. tried to find an association between ALDH activity and DM and its complications such as large vessel disease, but the method they used did not lead to any valid conclusions [6,7].

Therefore, the aim of our studies was to compare the ALDH1 and GST activity in whole blood of DM patients and healthy control and analyze the relation of those enzymes activity with HbA1c.

Materials and Methods

Patients

64 DM type 2 patients of the Warsaw Medical University Hospital (Group 1) and 60 healthy controls from the out-patients clinic (Control group) were enrolled in this study.

received 07.07.2013
first decision 14.11.2013
accepted 20.11.2013

Bibliography
DOI http://dx.doi.org/10.1055/s-0033-1361177
Exp Clin Endocrinol Diabetes 2014; 122: 55–59

ISSN 0947-7349

Correspondence
Dr. J. Giebułtowicz
Medical University of Warsaw
Banacha 1 Street
02-097,Warsaw
Poland
Tel.: +48/225/720 949
Fax: +48/225/720 976
joanna.giebultowicz@wum.edu.pl

Table 1 Average values and dispersion (standard deviation and interquartile range) of age, RBC and HCT in the examined groups.

	Group 1 all DM patients (n=64)		Group 2 HbA1c≤7.5% (n=47)		Group 3 HbA1c>9.0% (n=12)		Control (n=60)	
	Mean (SD)	Median (IQR)	Mean (SD)	Median (IQR)	Mean (SD)	Median (IQR)	Mean (SD)	Median (IQR)
RBC [mln/µl]	4.32 (0.58)	4.39* (3.88–4.66)	4.34 (0.60)	4.38* (3.96–4.67)#	4.26 (0.50)	4.17* (3.91–4.43)#	4.67 (0.54)	4.64 (4.31–5.03)#
HCT [%]	38.5 (4.4)	38.4* (35.9–41.6)	38.7 (4.4)	38.7* (36.4–41.7)#	37.5 (3.2)	36.3* (35.4–39.0)#	41.2 (4.1)	41.7 (39.6–44.9)#
Age [years]	69 (15)	70* (59–80)#	70 (14)	70* (61–81)#	65 (14)	65 (56–75)	62 (16)	65 (51–77)

#Shapiro-Wilk normality test rejected the hypothesis that variables in analysed group were normally distributed; therefore, medians are better estimators of expected values

*statistically significant comparing to control group ($p<0.05$)

Blood samples

The blood samples used in the experiment was the blood that remained from the routine diagnostic tests in the Public Central Teaching Hospital in Warsaw. The material was provided in unmarked test tubes and therefore was anonymous. Samples were stored in a refrigerator and enzyme activity was measured within the same day of collection.

Methods

The blood for enzymatic measurements was hemolysed using 3 mM phosphate buffer pH = 7.4 with 1 mM EDTA and in the case of ALDH1A1 assay 0.5 mM DTT, additionally. Test tubes with $EDTAK_3$ were used for enzymatic assays, CBC and HbA1c determination. Those for the determination of glucose concentration contained also sodium fluoride.

ALDH1 activity was determined fluorometrically. Fluorometric assays were run in a 50 mM pyrophosphate buffer, pH 8.1, at 25 °C, in the presence of 1 mM EDTA and 0.5 mM DTT. The assays utilized a highly fluorogenic naphthaldehyde substrate, 6-methoxy-2-naphthaldehyde, MONAL (5 µM) (Sigma-Aldrich), reacting with NAD^+ (100 µM) (Sigma-Aldrich) as a co-substrate. All assays were run in the presence of 5 mM 4-methylpyrazole, 4 MP. 6-Methoxy-2-naphthoic acid, MONCO (1.5 µM) (Sigma-Aldrich) was added as an internal standard. The assay was also performed without NAD^+ addition to eliminate non-specific MONAL oxidation.

GST activity was determined photometrically by measuring an increase in the absorbance of GST-1-chloro-2,4-dinitrobenzene conjugates. Hemoglobin concentration was measured photometrically using Drabkin's reagent (Humana). HbA1c was measured using turbidimetric inhibition immunoassay (TINA) on Cobas Integra Roche 800 and the results were expressed as % of total hemoglobin. Serum glucose levels were performed using the hexokinase method and Cobas Integra Roche 800 auto analyser. Complete blood count (CBC) was performed using haematological analyzer (Sysmex, XT-2000i, Toa. Co., Japan).

Since the methodology was based on lysed whole blood not red blood cells (shorter preparation and higher reproducibility of the assay), the enzyme activities were expressed not only as U/l but also U/g of hemoglobin. GST and ALDH1 are located mainly in RBC therefore, this adjustment allowed to minimize the influence of HCT variation on the enzymes activities.

Statistical analyses

Normal distribution and homogeneity of variance of the data were assessed using the Shapiro-Wilk test and Levene's test, respectively. In case of a lack of normal distribution or homogeneity of variances ($p<0.05$), a subsequent statistical evaluation of significance was performed using the Mann-Whitney U Test (for 2 groups) or the Kruskal-Wallis test (followed by the post-hoc Dunn test; for more than 2 groups). Otherwise, the t-Student test or the analysis of variance (ANOVA; the post-hoc LSD Fisher test) was used. The strength of the relationship between variables was measured by the Spearman's rank correlation coefficient. The Chi-squared test was used to analyze the association between nominal (categorical) variables. Ward's hierarchical clustering method (agglomerative method) was used for grouping the individuals characterized by 2 dimensional data (glucose concentration; HbA1c). Calculations were performed using STATISTICA version 10 software.

For the following parameters: ALDH and GST activity, age, RBC, glucose concentration and HCT not only mean values but also medians were calculated, which are better measures of the centre for a skewed distribution.

Results

HbA1c and plasma glucose level

The DM patients (group 1) were divided on the basis of HbA1c into 2 groups: those with controlled diabetes mellitus (DM-controlled, group 2, HbA1c ≤ 7.5%, n = 47) and uncontrolled diabetes mellitus (DM-uncontrolled, group 3, HbA1c > 9.0%, n = 12). The average (median) HbA1c of group 1–3 was: 6.2 (5.6–7.0), 5.7 (5.5–6.3) and 11.0 (9.9–11.4), respectively. Glucose levels in the plasma of examined groups were higher than those in the control group ($p<0.0001$ for each comparison) with medians 113 (98–139) mg/dl, in group 1, 113 (98–124) mg/dl, in group 2, 119 (98–188) mg/dl, in group 3 and 88 (86–100) mg/dl, in the control group. The experimental groups did not differ significantly from each other or from the control group regarding gender distribution. The M:F gender distribution of the groups 1–3 were 1:1 and of the control group 8:6 ($p>0.05$).

Age, RBC and HCT values are summarized in **Table 1**, whereas ALDH1 and GST activity in **Table 2**.

Enzymes activities and their correlations

Our results revealed that in comparison to the control group the ALDH1 activity [mU/g of hemoglobin] was higher in group 1 ($p=0.0483$), group 2 ($p=0.0206$) and group 3 ($p=0.0017$). Moreover, the enzyme activity was higher in group 3 than group 2 ($p=0.0055$). What is more, when enzyme activity was expressed

Table 2 Average values and dispersion (standard deviation and interquartile range) of ALDH1 and GST activity in the examined groups.

	Group 1 all DM patients (n = 64)		Group 2 HbA1c ≤ 7.5% (n = 47)		Group 3 HbA1c > 9.0% (n = 12)		Control (n = 60)	
	Mean (SD)	Median (IQR)	Mean (SD)	Median (IQR)	Mean (SD)	Median (IQR)	Mean (SD)	Median (IQR)
ALDH1 [mU/g]	9.7 (6.3)	7.8* (5.3–12.3)#	9.5 (6.0)	7.5* (5.6–12.5)#	13.7 (7.5)	12.7* (7.9–19.3)#	9.8 (9.9)	7.3 (3.9–10.0)#
ALDH1 [U/l]	1.3 (1.0)	0.9* (0.6–1.6)#	1.3 (1.1)	0.9* (0.7–1.5)#	1.5 (0.9)	1.5* (0.9–1.7)#	0.9 (0.7)	0.77 (0.4–1.1)#
GST [U/g]	7.1 (4.6)	6.1 (4.7–7.9)#	6.7 (4.2)	6.1 (4.2–7.7)#	10.7 (6.8)	8.4* (6.1–11.8)#	7.2 (2.9)	6.9 (4.8–8.0)#
GST [U/ml]	0.28 (0.12)	0.26 (0.21–0.35)#	0.28 (0.12)	0.26 (0.21–0.35)#	0.33 (0.09)	0.28* (0.26–0.44)	0.26 (0.12)	0.26 (0.16–0.33)

Shapiro-Wilk normality test rejected the hypothesis that variables in analysed group were normally distributed; therefore, medians are better estimators of expected values

* statistically significant comparing to control group ($p < 0.05$)

Table 3 Average values and dispersion (standard deviation and interquartile range) of blood glucose concentration, HbA1c and activity of ALDH1 and GST in the clusters.

Cluster	1 (n = 16)		2 (n = 19)		3 (n = 20)		4 (n = 9)	
	Mean (SD)	Median (IQR)	Mean (SD)	Median (IQR)	Mean (SD)	Median (IQR)	Mean (SD)	Median (IQR)
glucose [mg%]	124 (13)#	120 (114–132)	99 (10)#	98 (95–105)*	83 (5)#	86 (79–86)*	219 (48) #	197 (188–225)*
Hb1Ac [%]	6.5 (1.1)#	6.3 (5.6–6.9)	14 (2.5)#	5.9 (5.6–10.8)	5.5 (0.2)#	5.5 (5.5–5.6)*	7.3 (2.1) #	6.2 (5.7–8.9)
ALDH1 [mU/g]	7.5 (4.4)#	6.4 (4.2–11.1)*	10.0 (6.6)#	7.5 (5.6–12.2)*	12 (15)#	8.2 (5.6–12.9)*	12.3 (8.0) #	11 (4.9–15)*
ALDH1 [U/l]	0.13 (0.11)#	0.11 (0.07–0.14)*	0.17 (0.13)#	0.13 (0.09–0.20)*	0.16 (0.10)#	0.14 (0.09–0.19)*	0.25 (0.23) #	0.16 (0.09–0.28)*
GST [U/g]	6.8 (3.9)#	5.9 (3.7–7.2)	7.6 (5.4)#	6.2 (4.7–7.7)	6.8 (2.1)	6.5 (5.5–8.1)	6.9 (2.5)	6.1 (5.4–8.6)
GST [U/ml]	0.28 (0.14)	0.28 (0.21–0.33)	0.26 (0.12)	0.26 (0.19–0.33)	0.28 (0.12)	0.26 (0.21–0.38)	0.28 (0.12) #	0.26 (0.21–0.45)

Shapiro-Wilk normality test rejected the hypothesis that variables in analysed group were normally distributed; therefore, medians are better estimators of expected values

* statistically significant comparing to cluster 1 ($p < 0.05$)

in U/l units an even more prominent difference was observed for groups 1–3 vs. control ($p < 0.0001$). However, post-hoc Dunn test revealed no difference ($p = 0.17$) between average ALDH1 activities expressed in U/l between groups 2 and 3 ($p = 0.04988$ according to Mann-Whitney U Test).

Since ALDH1 activity is in 98% located in erythrocytes, we aimed to check whether high activity in group 3 [U/l] is not a result of a high erythrocyte level in blood. The results showed that in group 3 the RBC and HCT was comparable to those in group 2 (NS, $p = 0.0421$) and lower than in the control group ($p < 0.0001$ in both cases). The RBC and HTC in the control group were also higher than in groups 1 and 2 ($p < 0.0001$ in both cases).

In group 3 there was a significant correlation between HCT and ALDH ($r_s = -0.86$, $p < 0.0001$; $r_s = -0.76$, $p < 0.0001$) and GST ($r_s = -0.60$, $p = 0.0067$; $r_s = -0.80$, $p < 0.0001$) activity for U/l and U/g activity, respectively. Significant correlations were also observed between ALDH and GST activity. The highest was observed for group 3 ($r_s = 0.78$, $p < 0.0001$; $r_s = 0.50$, $p < 0.0001$), then for group 1 ($r_s = 0.50$, $p < 0.0001$; $r_s = 0.50$, $p < 0.0001$), group 2 ($r_s = 0.38$, $p < 0.0001$; $r_s = 0.50$, $p < 0.0001$) and the lowest for the control group ($r_s = 0.31$, $p = 0.0013$; $r_s = 0.41$, $p < 0.0001$) U/g and U/l, respectively.

GST activity was significantly higher in group 3 than group 2 ($p = 0.0008$, $p = 0.0762$) and the control group ($p < 0.0001$, $p = 0.002$) for the activity in U/g and U/ml, respectively.

Moreover, there was a positive correlation between glucose concentration and activity of GST [U/l] ($r_s = 0.34$; $p = 0.0028$), ALDH [U/l] ($r_s = 0.28$, $p = 0.0044$) and ALDH [U/g] ($r_s = 0.24$, $p = 0.0191$) in group 2. The ALDH1 activity [U/l] was correlated with HbA1c ($r_s = 0.53$; $p = 0.0080$) in group 3.

Cluster analysis

Cluster analysis was also performed to identify the subgroups of individuals on the basis of glucose concentration and HbA1c. It revealed that among the 4 created clusters, one ($n = 16$) was characterized by surprisingly low ALDH activity despite relatively high glucose and Hb1Ac concentrations (**Table 3**). The activity in [mU/g] and [U/l] in this cluster was lower than in the cluster 2 ($p = 0.0355$; $p = 0.0198$), cluster 3 ($p = 0.0303$; $p = 0.0197$) and cluster 4 ($p = 0.0076$; $p = 0.0026$).

Discussion

There is emerging evidence that oxidative stress (OS) significantly contributes to the progression of diabetes and its complications.

Oxidative stress is defined as an imbalance between the production of reactive species – reactive oxygen intermediates (ROI), reactive nitrogen species (RNS), and reactive chlorine species (RCS), and antioxidant defense, leading to tissue damage. In both types of diabetes, hyperglycemia results in the extensive production of reactive species during such processes as glucose oxidation, nonenzymaticglycation of proteins, and the subsequent oxidative degradation of glycated proteins [2].

The results of OS in the blood of diabetes patients are noticeable not only in serum, but also in erythrocytes, where higher MDA and lower GSH and membrane –SH group concentrations compared to normal erythrocytes were detected [8,9]. Moreover, evidence from several studies suggests that antioxidant enzymes in erythrocytes are altered. However, those concerning GST activity are sparse and inconclusive [10].

Our results revealed that GST activity was significantly higher in uncontrolled DM than in controlled DM and in the control group. No significant differences between the whole DM group (group 1) and the control group were detected.

There are no reports concerning ALDH1 activity in diabetes. Jerntorp et al. found altered acetaldehyde metabolism in diabetics with macroangiopathy and concluded that ALDH1 is responsible for lowering the half-life of this aldehyde in patients suffering from this particular diabetes complication. However, in subsequent reports it was suggested that the cause of the rapid acetaldehyde elimination in angiopathy patients is the low capacity of blood proteins for acetaldehyde binding. Therefore no conclusion on ADLH activity was made [6,7,11].

This study has shown that ALDH1 activity was higher in DM than in the control group. Moreover, the activity in the uncontrolled DM was higher than in controlled DM. Since ALDH1 in blood is located mainly in erythrocyte, we confirmed that higher activity of ALDH1 in DM is not simply a result of higher erythrocyte levels. The association of ALDH activity with the severity of the diseases was also considered [12,13]. In the uncontrolled DM negative correlation between ALDH activity and HCT was found. This can be explained, at least in part, by the fact that increased osmolality of blood, caused by elevated blood glucose levels in those patients, may result in the interstitial fluid transfer from tissue into circulation, decreasing hematocrit. There was also a positive relationship between ALDH activity and HbA1c in this group of patients. In the case of controlled DM, ALDH activity was positively correlated with glucose levels.

Higher activity of ALDH1 and GST may be a result of a compensatory mechanism against oxidative stress. The expression of these proteins is regulated via, Nrf2 and Nf-κB signaling pathways activated in oxidative stress [14–16]. Since correlation coefficients between ALDH1 and GST activity increases as follows: control group, controlled DM, whole DM, uncontrolled DM, we suggest that the contribution of those pathways to the expression of these enzymes is increasing in the same order.

OS may result in hemoglobin denaturation, which leads to the release of hemin into the RBC membrane and the released hemin is capable of oxidizing membrane proteins. GST can bind free hemin, presumably reducing damage to the RBC membrane [4,6,7]. ALDH1 is indirectly involved in the protection against the effects of oxidative stress, oxidizing 4-HNE and MDA, the concentration of which increases as a result of lipid peroxidation [17]. The metastable aldehydes react with macromolecules such as proteins, lipids, phospholipids and DNA, which results in cytotoxic effects [18]. Therefore, insufficient activity of erythrocyte enzymes, such as ALDH1 or GST, participating directly or indirectly in protection against OS, can result in changes of membrane protein conformation as well as in the increase of membrane lipid peroxidation. Membrane properties and cytoplasmic content influence erythrocyte functionality, e.g., deformability. Therefore, overstraining the cell protective system might increase the risk of diabetes complications, such as those caused by a decrease of erythrocyte deformability i.e., chronic vascular complications of diabetes mellitus, nephropathy [19].

Surprisingly low activity of ALDH in the cluster composed of patients with relatively high blood glucose concentration and HbA1c may be explained as follows: a) despite the high glucose and Hb1Ac levels this group of patients experiences lower oxidative stress because of a high level of nonenzymatic antioxidants. Therefore this group could be less vulnerable to developing DM complications. b) this group could present single nucleotide polymorphism (SNP) of Nrf2/ARE protein pathway or ALDH, which would result in lower than expected (considering glucose and Hb1Ac levels) protein expression and/or enzyme activity. Therefore, this low enzyme activity could be a risk factor for DM and predispose to DM complication occurrence.

Therefore we recommend further studies to explain the observed phenomenon. The additional analyses at the molecular level would allow for assessing whether the observed changes in enzymes activity were associated with structural modifications of these proteins or differences in their synthesis levels. In particular it would be worth examining polymorphisms in Nrf2/ARE pathways responsible for the induction of antioxidant enzymes as a factor predisposing to the occurrence of diabetic complications.

Conclusion

Conclusively, higher erythrocyte ALDH1 activity was observed in diabetes mellitus and it seems to be associated with the severity of the disease. Differences of GST activity were less prominent. The higher activity of ALDH1 and GST in DM might be a compensatory effect against oxidative stress. Further research on peculiarly low ALDH activity in one subgroup of DM patients should be performed since it could be a risk factor of DM or/and its complications.

Acknowledgement

We would like to acknowledge the support provided by the students within the Students' Scientific Group, especially A. Szamałek, M. Dujka, P. Siek and S. Kosmala.

Conflict of interest: The authors declared that there is no conflict of interest.

References

1 *Rathmann W, Giani G.* Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes care 2004; 27: 2568–2569 author reply 9. PubMed PMID: 15451946. Epub 2004/09/29. eng

2 *Rosen P, Nawroth PP, King G et al.* The role of oxidative stress in the onset and progression of diabetes and its complications: a summary of a Congress Series sponsored by UNESCO-MCBN, the American Diabetes Association and the German Diabetes Society. Diabetes/metabolism research and reviews 2001; 17: 189–212 PubMed PMID: 11424232. Epub 2001/06/26. eng

3 *Pillon NJ*, *Croze ML*, *Vella RE et al.* The lipid peroxidation by-product 4-hydroxy-2-nonenal (4-HNE) induces insulin resistance in skeletal muscle through both carbonyl and oxidative stress. Endocrinology 2012; 153: 2099–2111 PubMed PMID: 22396448. Epub 2012/03/08. eng
4 *Slatter DA*, *Bolton CH*, *Bailey AJ.* The importance of lipid-derived malondialdehyde in diabetes mellitus. Diabetologia 2000; 43: 550–557 PubMed PMID: 10855528. Epub 2000/06/16. eng
5 *Yadav UC*, *Ramana KV*. Regulation of NF-kappaB-Induced Inflammatory Signaling by Lipid Peroxidation-Derived Aldehydes. Oxidative medicine and cellular longevity 2013; 2013: 690545 PubMed PMID: 23710287. Pubmed Central PMCID: PMC3654319. Epub 2013/05/28. eng
6 *Jerntorp P*, *Ohlin H*, *Almer LO*. Aldehyde dehydrogenase activity and large vessel disease in diabetes mellitus. A preliminary study. Diabetes 1986; 35: 291–294 PubMed PMID: 3949078. Epub 1986/03/01. eng
7 *Ohlin H*, *Helander A*, *Tottmar O et al.* Aldehyde dehydrogenase activity and vascular disease in type II diabetes – a comparison between 2 different assays for activity. Diabetes research (Edinburgh, Scotland) 1987; 6: 91–94 PubMed PMID: 3427870. Epub 1987/10/01. eng
8 *Kedziora-Kornatowska KZ*, *Luciak M*, *Blaszczyk J et al.* Lipid peroxidation and activities of antioxidant enzymes in erythrocytes of patients with non-insulin dependent diabetes with or without diabetic nephropathy. Nephrology, dialysis, transplantation: official publication of the European Dialysis and Transplant Association – European Renal Association 1998; 13: 2829–2832 PubMed PMID: 9829486. Epub 1998/11/26. eng
9 *Nwose EU*, *Jelinek HF*, *Richards RS et al.* Changes in the erythrocyte glutathione concentration in the course of diabetes mellitus. Redox report: communications in free radical research 2006; 11: 99–104 PubMed PMID: 16805963. Epub 2006/06/30. eng
10 *Velladath SU*, *Kumar R.* Erythrocyte Glutathione-S-transferase Activity in Diabetics and its Association with HBA1c. WebmedCentral CLIN BIOCHEM 2011; 2
11 *Ohlin H*, *Jerntorp P*, *Brattstrom L et al.* Altered metabolism of acetaldehyde in blood is not a specific marker of diabetic macroangiopathy. Diabetes research (Edinburgh, Scotland) 1991; 16: 75–79 PubMed PMID: 1817808. Epub 1991/02/01. eng
12 *Salazar Vazquez BY*, *Salazar Vazquez MA*, *Venzor VC et al.* Increased hematocrit and reduced blood pressure following control of glycemia in diabetes. Clinical hemorheology and microcirculation 2008; 38: 57–64 PubMed PMID: 18094460. Epub 2007/12/21. eng
13 *Bray GA*, *Edelstein SL*, *Crandall JP et al.* Long-term safety, tolerability, and weight loss associated with metformin in the Diabetes Prevention Program Outcomes Study. Diabetes care 2012; 35: 731–737 PubMed PMID: 22442396. Pubmed Central PMCID: PMC3308305. Epub 2012/03/24. eng
14 *Wu KC*, *Cui JY*, *Klaassen CD*. Effect of graded Nrf2 activation on phase-I and -II drug metabolizing enzymes and transporters in mouse liver. PloS one 2012; 7: e39006 PubMed PMID: 22808024. Pubmed Central PMCID: PMC3395627. Epub 2012/07/19. eng
15 *Morceau F*, *Duvoix A*, *Delhalle S et al.* Regulation of glutathione S-transferase P1-1 gene expression by NF-kappaB in tumor necrosis factor alpha-treated K562 leukemia cells. Biochemical pharmacology 2004; 67: 1227–1238 PubMed PMID: 15013838. Epub 2004/03/12. eng
16 *Godbout R*, *Monckton EA*. Differential regulation of the aldehyde dehydrogenase 1 gene in embryonic chick retina and liver. The Journal of biological chemistry 2001; 276: 32896–32904 PubMed PMID: 11438538. Epub 2001/07/05. eng
17 *Marchitti SA*, *Brocker C*, *Stagos D et al.* Non-P450 aldehyde oxidizing enzymes: the aldehyde dehydrogenase superfamily. Expert opinion on drug metabolism & toxicology 2008; 4: 697–720 PubMed PMID: 18611112. Pubmed Central PMCID: PMC2658643. Epub 2008/07/10. eng.
18 *O'Brien PJ*, *Siraki AG*, *Shangari N*. Aldehyde sources, metabolism, molecular toxicity mechanisms, and possible effects on human health. Critical reviews in toxicology 2005; 35: 609–662 PubMed PMID: 16417045. Epub 2006/01/19. eng
19 *Singh M*, *Shin S*. Changes in erythrocyte aggregation and deformability in diabetes mellitus: a brief review. Indian journal of experimental biology 2009; 47: 7–15 PubMed PMID: 19317346. Epub 2009/03/26. eng