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Aldehyde dehydrogenase 2 polymorphism is an important gene for insulin resistance in Japanese patients with type 2 diabetes

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ABSTRACT

Background: Aldehyde dehydrogenase 2 (ALDH2) is an important enzyme involved in alcohol metabolism. ALDH2 polymorphism has been reported as a risk factor for type 2 diabetes mellitus (T2DM) and is associated with liver insulin resistance due to alcohol consumption in non-diabetic individuals. Herein, we investigated the association between ALDH2 polymorphisms and insulin resistance in patients with T2DM.

Methods: We performed a meal tolerance test and the hyperinsulinemic-euglycemic clamp on 71 Japanese participants: 34 patients with T2DM, and 37 non-diabetic participants. We analyzed the ALDH2 polymorphism (ALDH2 rs67); GG type was defined as the T2DM high-risk group, compared with the low-risk AG and AA groups.

Results: Glucose levels were similar in the high- and low-risk T2DM groups. The high-risk group for T2DM showed a significantly higher BMI ($p < 0.005$), insulin resistance in HOMA-IR ($p < 0.05$), and Insulin sensitivity index ($p < 0.05$); however, there were no significant differences in insulin resistance in the clamp test ($p = 0.10$). Alcohol consumption did not differ significantly between groups ($p = 0.66$). Non-diabetic participants also showed higher HOMA-IR insulin resistance in the high-risk group ($p < 0.05$), but insulin resistance levels in the glucose clamp tests ($p = 0.56$) and insulin secretion were not significant.

Conclusion: The results suggest that ALDH2 is an important gene associated with insulin resistance and obesity in Japanese patients with type 2 diabetes.

1. Introduction

The main pathophysiology of type 2 diabetes mellitus (T2DM) involves increased insulin resistance in various tissues and decreased insulin secretion [1]. A recent meta-analysis of genome-wide association studies (GWAS) showed that aldehyde dehydrogenase-2 (ALDH2) polymorphism is a risk factor for T2DM in 433,540 East Asian individuals [2]. To investigate the mechanism by which ALDH2 is a risk factor for T2DM, a recent study showed that ALDH2 polymorphism is

associated with alcohol consumption and liver insulin resistance in Japanese men but not with muscle insulin resistance [3]. However, the previous study was performed in non-obese, non-diabetic men, and only few have reported on the role of ALDH2 polymorphisms in overt T2DM and obesity conditions.

Human ALDH2 is a 517-amino acid polypeptide encoded by a nuclear gene located on chromosome 12q24 [4]. The ALDH2 rs671 G allele encodes Glu at amino acid 504, resulting in an enzymatically active form, while the ALDH2 rs671 A allele encodes Lys at amino acid 504,

yielding an enzymatically inactive form. Heterozygous individuals are expected to have dramatically lower enzymatic activity than 50% of the wild-type [3–5]. Furthermore, genetic background and ethnic differences are important for T2DM; Asians exhibit low insulin secretion ability and insulin resistance despite mild obesity [6]. East Asians exhibit a higher rate of ALDH2 mutations [7]. In addition, there are few reports on the relationship between ALDH2 and insulin secretion.

ALDH2 polymorphism might be important for alcohol consumption and liver insulin resistance in Asian populations; however, the effects of ALDH2 on insulin resistance and insulin secretion in patients with overt T2DM are not well known. Therefore, we performed meal tolerance and glucose clamp tests in patients with overt T2DM and non-diabetic healthy Japanese volunteers. We investigated whether ALDH2 polymorphisms are associated with insulin resistance in Japanese patients with overt T2DM.

2. Materials and methods

2.1. Participants

A total of 34 patients with T2DM and 37 participants without diabetes (non-diabetes) were included in this study at the Tottori University Hospital between 2011 and 2022. The World Health Organization criteria [8] was used to diagnose T2DM. Participants with cancer, hepatitis B and C, liver cirrhosis, pancreatitis, renal failure, and those taking diabetogenic medications were excluded. All participants were of Japanese ethnicity and received diet therapy alone. This study employed a cross-sectional design. A previous Japanese study reported that the ALDH2 polymorphism showed a ratio of risk GG: low-risk AG of almost 1:1 [3]; the sample size was intended to be over 20 participants so that 10 individuals can be obtained for each group.

This study was conducted in accordance with the principles of the Declaration of Helsinki. This study was approved by the Ethics Committee of the Faculty of Medicine of Tottori University (approval number: G161). Informed consent was obtained from all participants using a procedure approved by the Ethics Committee.

2.2. Meal tolerance test

We conducted the meal tolerance test (MTT) assay using a test meal developed by the Japan Diabetes Society, as reported in previous studies [9,10]. The participants ate a test meal (460 kcal, 50% carbohydrates, 15% protein, 35% fat, and 1.6 g salt) after overnight fasting. Plasma glucose and serum insulin levels were measured at 0, 30, 60, 120, and 180 min after the test meal. We measured plasma glucose using the glucose oxidase method, and serum insulin levels using chemiluminescent immunoassays (human insulin immunoassay kits; Kyowa Medix, Tokyo, Japan). HbA1c levels were measured by high-performance liquid chromatography. We converted HbA1c percentage values to the International Federation of Clinical Chemistry values (mmol/mol) using an HbA1c converter from the National Institutes of Diabetes and Digestive and Kidney Diseases [11].

Insulin secretion index was calculated as follows: Homeostasis model assessment of beta cell function (HOMA-beta) = {20 × [fasting plasma insulin (pmol/L)]}/{[fasting plasma glucose (mmol/L)] − 3.5} [12].

Insulinogenic index = {[insulin (pmol/L) at 30 min] − [insulin (pmol/L) at 0 min]}/{[glucose (mmol/L) at 30 min] − [glucose (mmol/L) at 0 min]} [13].

Insulin resistance was calculated as follows: Homeostatic model assessment insulin resistance (HOMA-IR) = [fasting plasma glucose (mmol/L)] × [fasting plasma insulin (pmol/L)]/135 [12].

Insulin sensitivity index (ISI) = 10,000/square root [{fasting plasma glucose (mmol/L) × fasting plasma insulin (pmol/L)} × {mean glucose (mmol/L) × mean insulin (pmol/L) during MTT}] [14].

The Area Under the Curve (AUC) was calculated by the horizontal method.

2.3. Hyperinsulinemic–euglycemic clamp

We performed the glucose clamp test as previously reported [9,10]. Briefly, we performed a hyperinsulinemic-euglycemic clamp test using an artificial endocrine pancreas (STG 55; Nikkiso, Shizuoka, Japan) to evaluate insulin sensitivity. We used the protocol involving a primed constant infusion of insulin (100 mU/m^2/min) and maintained the plasma glucose levels at 5.2 mmol/L (95 mg/dL). According to previous studies, a steady-state plasma insulin level of 1200 pmol/L (200uU/ml) was achieved in patients with T2DM [15]. The glucose infusion rate (GIR) was measured during the steady-state 90–120 min. The mean GIR was defined as the glucose disposal rate (GDR), commonly used as a marker of peripheral insulin sensitivity. We also calculated the M/I ratio as a measure of the quantity of glucose metabolized per unit of plasma insulin concentration and defined the M and I values as the GDR and the steady-state insulin concentration, respectively [16].

2.4. ALDH2 gene analysis

The ALDH2 risk allele, ALDH2 rs67, was assessed using the polymerase chain reaction (PCR) single-strand conformation polymorphism method after DNA sequencing, as previously reported [10,17]. We divided the patients into high-risk (G/G) and low-risk genotypes (G/A) (A/A).

Genomic DNA was obtained from peripheral blood leukocytes using proteinase K digestion and phenol/chloroform extraction. The PCR mixture contained genomic DNA as a template, primers, dNTPs, AmpliTaq Gold (PE Biosystems, Tokyo, Japan), and supplementary buffer. The primer sequences were forward primer (Name: ALDH2-F):5′-CAAATTACAGGGTCAACTGCT-3,' reverse-G (Name: ALDH2-R-Normal):5′-CCACACTCACAGTTTTCTCTTC-3,' reverse-A (Name: ALDH2-R-Mutant):5′-CCACACTCACAGTTTTCTCTTT-3.' PCR was performed under the following conditions: 5 min at 95 °C and then amplification for 32 cycles of 1 min at 95 °C, 1 min at 60 °C, and 1 min at 72 °C in a thermal cycler. PCR products were electrophoresed on 2% agarose gel.

2.5. Statistical analysis

Data are expressed as the mean ± standard deviation. We assessed differences in the mean values of clinical parameters between ALDH2 high-risk and low-risk participants using the Mann–Whitney U test. The chi-square test was used for categorical comparisons of sex data. We conducted a power analysis to compare the HOMA-IR between the ALDH2 gene high-risk and low-risk groups using an EZR calculator (EZR version 1.61, Jichi Medical University, Saitama, Japan) [18]. Additionally, we analyzed the correlation between alcohol consumption and HOMA-IR using Spearman analysis.

Statistical significance was set at $P < 0.05$. PRISM9 software (GraphPad Software, San Diego, CA, USA) was used for all analyses.

3. Results

The participants' characteristics are shown in Tables 1 and 2. Participants with T2DM had 15 GG, 18 GA, 1 AA, and non-diabetic participants had 23 GG, 12 GA, and 2 AA genotypes. Fig. 1 shows the glucose and insulin responses during the MTT assay. There were no significant differences in the glucose levels between the high- and low-risk groups in either the T2DM or non-diabetes groups (Fig. 1a and b).

Among patients T2DM, the high-risk group showed significantly higher body mass index (BMI), waist circumference, HOMA-IR, and ISI than the low-risk group (Table 1). However, the clamp test showed no significant differences in the GDR and M/I. The high-risk group showed significantly higher HOMA-beta values than the low-risk group in T2DM. However, there were no significant differences in the insulinogenic index or insulin AUC. Alcohol consumption and liver function did not differ significantly between the groups.

Table 1
Clinical data comparison between ALDH2 genotype high- and low-risk in Type 2 diabetes mellitus.

Parameters	High-risk	Low-risk	
	(n = 15)	(n = 19)	P value
Age (years)	57.0 ± 11.1	56.9 ± 12.8	0.99
Sex (Male/Female)	8/7	10/9	0.99
BMI (kg/m²)	29.2 ± 3.4	24.9 ± 3.9	<0.005
Waist circumference (cm)	99.1 ± 8.1	89.3 ± 11.9	<0.05
Duration of Diabetes (years)	3.9 ± 4.8	3.0 ± 3.6	0.60
Ethanol consumption (g/day)	6.68 ± 11.7	8.9 ± 17.9	0.66
Fasting Plasma Glucose (mmol/L)	6.97 ± 0.98	7.17 ± 1.20	0.60
Fasting Plasma Insulin (pmol/L)	102.0 ± 52.9	58.6 ± 39.4	<0.05
HbA1c (%)	7.69 ± 1.78	7.63 ± 0.95	0.91
HbA1c (mmol/mol)	60 ± 19	59 ± 11	
AST	35.3 ± 20.8	30.9 ± 19.1	0.54
ALT	53.0 ± 30.5	47.9 ± 40.3	0.68
GGTP (IU/L)	51.3 ± 45.4	56.1 ± 52.2	0.77
HOMA-beta (%)	104.9 ± 59.0	56.7 ± 39.2	<0.05
Insulinogenic Index	0.61 ± 0.37	0.65 ± 0.98	0.84
Glucose AUC	19.3 ± 3.3	20.0 ± 4.0	0.57
Insulin AUC	808.8 ± 379.3	640.7 ± 398.1	0.16
HOMA-IR	5.29 ± 2.88	3.15 ± 2.17	<0.05
Insulin Sensitivity Index	2.95 ± 1.34	5.92 ± 5.36	<0.05
GDR (mg/kg/min)	5.80 ± 2.10	6.26 ± 1.71	0.49
M/I	5.58 ± 3.79	8.83 ± 7.25	0.10

Data are presented as the mean ± SD values.
The comparison of parameters between high-risk and low-risk groups was performed using the Mann-Whitney *U* test.
ALDH2, Aldehyde dehydrogenase 2; AUC, area under the curve; BMI, body mass index; GDR, glucose disposal rate; HbA1c, glycated hemoglobin; HOMA-beta, homeostatic model assessment beta-cell function; HOMA-IR, homeostasis model assessment for insulin resistance.

Table 2
Clinical data comparison between ALDH2 genotype high- and low-risk in Non-Diabetes.

Parameters	High-risk	Low-risk	
	(n = 23)	(n = 14)	P value
Age (years)	33.0 ± 8.6	30.2 ± 5.8	0.30
Sex (Male/Female)	15/8	8/6	0.73
BMI (kg/m²)	21.8 ± 2.9	21.7 ± 3.5	0.95
Waist circumference (cm)	78.0 ± 9.7	75.0 ± 11.7	0.46
Duration of Diabetes (years)	–	–	–
Ethanol consumption (g/day)	4.80 ± 12.0	2.04 ± 5.65	0.35
Fasting Plasma Glucose (mmol/L)	4.90 ± 0.44	4.80 ± 0.37	0.44
Fasting Plasma Insulin (pmol/L)	52.5 ± 29.4	37.9 ± 13.0	<0.05
HbA1c (%)	5.29 ± 0.29	5.52 ± 0.21	<0.05
HbA1c (mmol/mol)	34 ± 3	36 ± 3	<0.05
AST	19.8 ± 4.8	23.4 ± 13.4	0.34
ALT	22.0 ± 14.0	31.0 ± 37.0	0.40
GGTP (IU/L)	22.0 ± 10.9	24.1 ± 15.4	0.66
HOMA-beta (%)	128.2 ± 69.1	101.4 ± 39.0	0.14
Insulinogenic Index	1.66 ± 1.35	1.08 ± 0.86	0.12
Glucose AUC	11.5 ± 1.3	11.8 ± 1.3	0.41
Insulin AUC	570.1 ± 285.9	596.8 ± 213.8	0.84
HOMA-IR	1.95 ± 1.22	1.36 ± 0.50	<0.05
Insulin Sensitivity Index	7.26 ± 3.53	7.84 ± 4.13	0.66
GDR (mg/kg/min)	9.93 ± 2.93	10.1 ± 3.05	0.82
M/I	11.3 ± 5.1	12.4 ± 5.5	0.56

Data are presented as the mean ± SD values.
The comparison of parameters between high-risk and low-risk groups was performed using the Mann-Whitney *U* test.
ALDH2, Aldehyde dehydrogenase 2; AUC, area under the curve; BMI, body mass index; GDR, glucose disposal rate; HbA1c, glycated hemoglobin; HOMA-beta, homeostatic model assessment beta-cell function; HOMA-IR, homeostasis model assessment for insulin resistance.

We conducted a power analysis of the statistical test used to compare the HOMA-IR between the high- and low-risk groups for T2DM. The difference between the mean HOMA-IR in the high and low-risk groups

was 2.14 ± 0.71, and the estimated power was >99%.

The non-diabetic group also showed significant differences in HOMA-IR between the high- and low-risk groups (Table 2). However, there were no significant differences in GDR and M/I in the clamp test. The non-diabetic group with high-risk ALDH2 polymorphisms exhibited significantly lower HbA1c levels than the low-risk group.

We also analyzed the correlation between alcohol consumption and HOMA-IR. The *r* value was 0.42 (Drinker, n = 12, p = 0.08) in patients with T2DM, and 0.15 (n = 6, p = 0.41) in non-diabetic individuals.

4. Discussion

Participants with the ALDH2 high-risk genotype in T2DM showed significantly higher insulin resistance in the MTT; however, there were no significant differences in glucose levels during the meal tolerance test or insulin resistance in the glucose clamp test. A previous study showed that ALDH2 polymorphism was associated with liver insulin resistance in non-diabetic men; however, muscle insulin resistance was not associated with ALDH2 [3]. The GDR mainly reflects muscle insulin resistance, HOMA-IR mainly reflects liver insulin resistance, and ISI reflects whole-body insulin resistance [19]. Our study also showed no significant muscle insulin resistance; however, patients with T2DM and are ALDH2 high-risk had significantly higher BMI and liver insulin resistance, despite the absence of significant ethanol consumption. These results suggest that ALDH2 is important in liver insulin resistance and obesity in the overt T2DM conditions. However, alcohol consumption in patients with T2DM and are ALDH2 high-risk tended to correlate with HOMA-IR and alcohol consumption, which is also important for insulin resistance. The non-diabetic group with high-risk ALDH2 polymorphisms exhibited significantly reduced HbA1c levels. Moreover, they showed significantly high fasting insulin levels and HOMA-IR, and tended to have high HOMA-beta and insulinogenic indices. These results imply that the non-diabetic group with high-risk ALDH2 polymorphisms might have a markedly high insulin secretion ability, which may explain why they showed relatively low HbA1c.

A recent study reported that ALDH2 mutant mice showed obesity-related insulin resistance, which affected the diversity of gut microbiota, decreased the abundance of Actinobacteria, and increased Deferribacteres [20]. These results suggest that ALDH2 affects obesity and insulin resistance. Although further studies are needed, these findings might explain why East Asians show a higher rate of diabetes and insulin resistance with mild obesity than other populations [6] as they exhibit a higher rate of ALDH2 mutations [7]. Another study reported that an activator of ALDH2 not only enhanced glucose-stimulated insulin secretion but also ameliorated the glucolipotoxicity of beta cells by reducing both mitochondrial and intracellular ROS levels, thereby improving mitochondrial function, restoring beta cell function, and protecting beta cells from apoptosis and death [21]. Our results also showed that patients with an active ALDH2 genotype and T2DM had higher HOMA-beta values than those with a low active ALDH2 genotype. These results imply that active ALDH2 genotype patients with T2DM have higher insulin levels and insulin resistance. Another basic study reported that ALDH2 activation enhances adipogenesis and signaling pathways involving PPARγ [22]. Our results also showed that patients with active ALDH2 genotype and T2DM had higher BMI and waist circumference than those with low active ALDH2 genotype. These results imply that patients with active ALDH2 genotype T2DM are more obese than those with a low active ALDH2 genotype.

Our study had a few limitations. The few participants included in this study indicated that our results require confirmation in a larger study. However, the glucose clamp test is a complicated technique, and recruiting patients with overt diabetes who are not taking medication is difficult. We are currently conducting a large study and will publish our results in the future. We also did not evaluate hepatic insulin resistance using tritium glucose because tritium glucose cannot be used in Japan. The ALDH2 risk in patients with T2DM showed a significantly higher
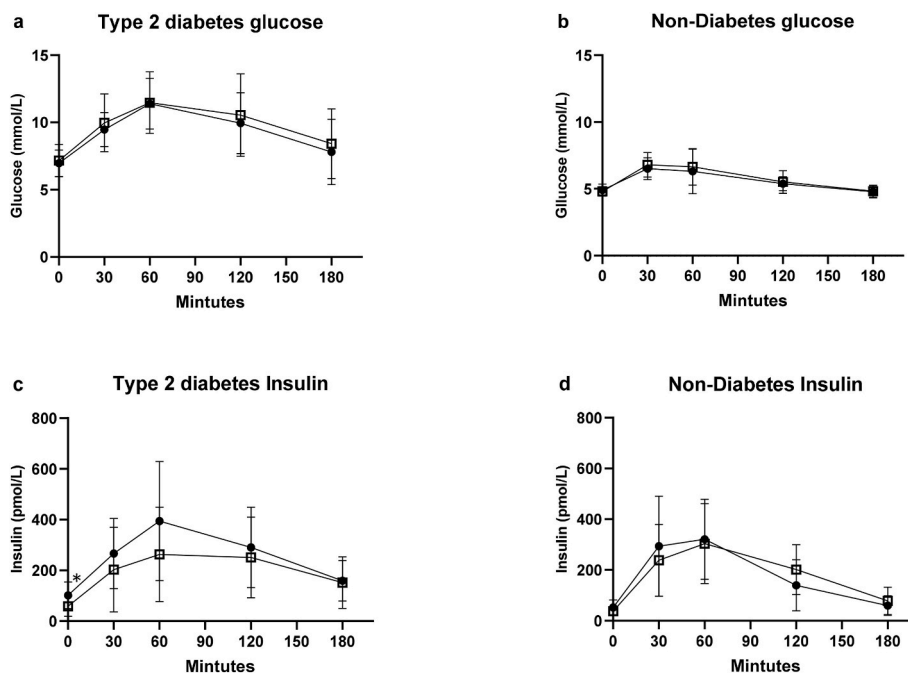
Fig. 1. Glucose and insulin response at Meal tolerance test

Fig. 1. Meal tolerance test (MTT) results. Fig. 1(a) shows glucose levels of type 2 diabetes after the MTT, (b) glucose of Non-diabetes, (c) insulin of type 2 diabetes (d) insulin of Non-diabetes. Black circles represent the data for high-risk participants, and white squares indicate low-risk participants. *; P < 0.05.

BMI than those in the low-risk group; therefore, a BMI-adjusted study is needed. Non-diabetes participants also had younger ages and lower BMI than that of the participants with T2DM; age, and BMI-adjusted studies are also needed. Despite these limitations, we believe that our study will contribute to daily clinical practice. In public health setting, alcoholic drinker who do not manifest flushing might be considered to have an active ALDH2 genotype, and thus are at risk of high insulin resistance and obesity; attention should be given to reduce the risk of this specific population. In addition, an ethanol patch test for screening ALDH2 genetic polymorphisms may be a convenient tool for estimating high insulin resistance and obesity in patients with T2DM.

In conclusion, ALDH2 is an important gene associated with insulin resistance and obesity in Japanese patients with T2DM.

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CRediT authorship contribution statement

Tsuyoshi Okura: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Roles, Writing – original draft, All the authors have read and approved the final version of the manuscript. **Risa Nakamura:** Data curation, All the authors have read and approved the final version of the manuscript. **Mari Anno:** Data curation, All the authors have read and approved the final version of the manuscript. **Yuichi Ito:** Data curation, All the authors have read and approved the final version of the manuscript. **Sonoko Kitao:** All the authors have read and approved the final version of the manuscript, Data curation, All the authors have read and approved the final version of the manuscript. **Satomi Endo:** Data curation, Methodology, All the authors have read and approved the final version of the manuscript. **Natsuka Taneda:** Data curation, All the authors have read and approved the final version of the manuscript. **Kazuhisa Matsumoto:** All the authors have read and approved the final version of the manuscript, Data curation. **Kyoko Shoji:** Data curation, All the authors have read and approved the final version of the manuscript. **Hiroko Okura:** Data curation, All the authors have read and approved the final version of the manuscript. **Kazuhiko Matsuzawa:** Data curation, All the authors have read and approved the final version of the manuscript. **Shoichiro Izawa:** Data curation, All the authors have read and approved the final version of the manuscript. **Etsuko Ueta:** Methodology, All the authors have read and approved the final version of the manuscript. **Masahiko Kato:** Supervision, All the authors have read and approved the final version of the manuscript. **Takeshi Imamura:** Supervision, Writing – review & editing, All the authors have read and approved the final version of the manuscript. **Shin-ichi Taniguchi:** Supervision, All the authors have read and approved the final version of the manuscript. **Kazuhiro Yamamoto:** Funding acquisition, Supervision, All the authors have read and approved the final version of the manuscript.

Declaration of competing interest

The authors declare that they have no competing interests.

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References

[1] Kahn SE, Cooper ME, Del Prato S. Pathophysiology and treatment of type 2 diabetes: perspectives on the past, present, and future. Lancet 2014;383:1068–83.

[2] Spracklen CN, Horikoshi M, Kim YJ, et al. Identification of type 2 diabetes loci in 433,540 East Asian individuals. Nature 2020;582(7811):240–5.

[3] Takeno K, Tamura Y, Kakehi S, Kaga H, Kawamori R, Watada H. ALDH2 rs671 is associated with Elevated FPG, reduced glucose clearance and hepatic insulin resistance in Japanese men. J Clin Endocrinol Metab 2021;106:e3573–81.

[4] Chen CH, Ferreira JC, Gross ER, Mochly-Rosen D. Targeting aldehyde dehydrogenase 2: new therapeutic opportunities. Physiol Rev 2014;94:1–34.

[5] Eng MY, Luczak SE, Wall TL. ALDH2, ADH1B, and ADH1C genotypes in Asians: a literature review. Alcohol Res Health 2007;30:22–7.

[6] Hsu WC, Araneta MR, Kanaya AM, Chiang JL, Fujimoto W. BMI cut points to identify at-risk Asian Americans for type 2 diabetes screening. Diabetes Care 2015; 38:150–8.

[7] Lai CL, Yao CT, Chau GY, Yang LF, Kuo TY, Chiang CP, Yin SJ. Dominance of the inactive Asian variant over activity and protein contents of mitochondrial aldehyde dehydrogenase 2 in human liver. Alcohol Clin Exp Res 2014;38:44–50.

[8] Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabet Med 1998;15:539–53.

[9] Ohkura T, Shiochi H, Fujioka Y, Sumi K, Yamamoto N, Matsuzawa K, Izawa S, Kinoshita H, Ohkura H, Kato M, Taniguchi SI, Yamamoto K. 20/(fasting C-peptide x fasting plasma glucose) is a simple and effective index of insulin resistance in patients with type 2 diabetes mellitus: a preliminary report. Cardiovasc Diabetol 2013;12:21.

[10] Okura T, Fujioka Y, Nakamura R, Anno M, Ito Y, Kitao S, Matsumoto K, Shoji K, Sumi K, Matsuzawa K, Izawa S, Okura H, Ueta E, Noma H, Kato M, Imamura T, Taniguchi SI, Yamamoto K. Hepatic insulin clearance is increased in patients with high HbA1c type 2 diabetes: a preliminary report. BMJ Open Diabetes Res Care 2020;8:e001149.

[11] National Institutes of diabetes and digestive and Kidney Diseases. the HbA1c converter, [article online], http://www.ngsp.org/convert1.asp; 1999.

[12] Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28: 412–9.

[13] Uwaifo GI, Fallon EM, Chin J, Elberg J, Parikh SJ, Yanovski JA. Indices of insulin action, disposal, and secretion derived from fasting samples and clamps in normal glucose-tolerant black and white children. Diabetes Care 2002;25:2081–7.

[14] Matsuda M, DeFronzo RA. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. Diabetes Care 1999;22:1462–70.

[15] Tamura Y, Tanaka Y, Sato F, Choi JB, Watada H, Niwa M, Kinoshita J, Ooka A, Kumashiro N, Igarashi Y, Kyogoku S, Maehara T, Kawasumi M, Hirose T, Kawamori R. Effects of diet and exercise on muscle and liver intracellular lipid contents and insulin sensitivity in type 2 diabetic patients. J Clin Endocrinol Metab 2005;90:3191–6.

[16] DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. Am J Physiol 1979;237:214–23.

[17] Okura T, Nakamura R, Ito Y, Kitao S, Anno M, Endo S, Taneda N, Matsumoto K, Shoji K, Okura H, Matsuzawa K, Izawa S, Ueta E, Kato M, Imamura M, Taniguchi SI, Yamamoto K. BMJ Open Diabetes Research and Care 2022;10: e002908.

[18] Kanda Y. Investigation of the freely available easy-to-use software 'EZR' for medical statistics. Bone Marrow Transplant 2013;48:452–8.

[19] Muniyappa R, Lee S, Chen H, Quon MJ. Current approaches for assessing insulin sensitivity and resistance in vivo: advantages, limitations, and appropriate usage. Am J Physiol Endocrin Metab 2008;294:E15–26.

[20] Yang SS, Chen YH, Hu JT, Chiu CF, Hung SW, Chang YC, Chiu CC, Chuang HL. Aldehyde dehydrogenase mutation Exacerbated high-fat-diet-Induced Nonalcoholic fatty liver Disease with gut microbiota Remodeling in Male mice. Biology 2021;10:737.

[21] Chen SM, Hee SW, Chou SY, Liu MW, Chen CH, Mochly-Rosen D, Chang TJ, Chuang LM. Activation of aldehyde dehydrogenase 2 Ameliorates glucolipotoxicity of pancreatic beta cells. Biomolecules 2021;11:1474.

[22] Yu YH, Liao PR, Guo CJ, Chen CH, Mochly-Rosen D, Chuang LM. PKC-ALDH2 pathway Plays a Novel role in Adipocyte Differentiation. PLoS One 2016;11: e0161993.