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Evaluation of potential gene expression as early markers of insulin resistance and non-alcoholic fatty liver disease in the Indonesian population

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ABSTRACT Early detection of insulin resistance (IR) or non-alcoholic fatty liver disease (NAFLD) is crucial to preventing future risks of developing chronic diseases. The Homeostatic Model Assessment of Insulin Resistance (HOMA-IR), Liver Fat Score (LFS), and Fatty Liver Index (FLI) are generally employed to measure severity stages of IR and NAFLD. The study of gene expressions could explain the molecular mechanisms that occur early on in IR and NAFLD; thus providing potential early markers for both diseases. This study was conducted to evaluate the gene expressions that could potentially be early markers of IR and NAFLD. All participants (n = 21) had normal blood glucose and were categorized as without hepatosteatosis (n = 10), at higher risk of hepatosteatosis (n = 6), and hepatosteatosis (n = 5). Gene expression analysis was performed using the 2-ΔΔCT relative quantification method. There were significant differences in *galnt2* ($p < 0.002$) and *sirt1* ($p < 0.010$) expression between the first and the third tertiles of HOMA-IR; and in *ptpn1* ($p < 0.012$) expression between the first and the second tertiles of LFS. In conclusion, the expressions of *galnt2* and *sirt1* could be used as early markers of IR, while the expression of *ptpn1* could be employed as an early marker of NAFLD.

KEYWORDS *galnt2*; insulin resistance; NAFLD; *ptpn1*; *sirt1*

1. Introduction

Metabolic syndrome is a disorder of energy use and storage. People with metabolic syndrome have an increased risk of developing chronic diseases—such as type-2 diabetes mellitus (T2DM) and non-alcoholic fatty liver disease (NAFLD) (Mendrick et al. 2017), that lead to morbidity and mortality (IDF 2005). According to the eighth edition of Diabetes Atlas, a higher prevalence of diabetes was observed in developing countries than in developed countries. In 2017, there were 7.6 million people with undiagnosed diabetes, 10.3 million people (6.2% of the population) with diabetes, and 27.7 million people with impaired glucose tolerance (IGT) in Indonesia (IDF 2017). In 2002, it was reported that about 30% of the population in Indonesia was developing NAFLD (Hasan et al. 2002).

Type-2 diabetes mellitus and NAFLD do not develop suddenly but are more likely caused by a prolonged unhealthy lifestyle. These diseases can be prevented by adopting a healthier lifestyle. Dietary patterns that are high in glucose could eventually lead to hyperinsulinemia and insulin resistance (Olokoba et al. 2012). Insulin resistance is considered to play a role in the formation of NAFLD due to abnormal fat metabolism in which hyperinsulinemia triggers triglyceride synthesis and accumulation in the liver. Non-alcoholic fatty liver disease is defined as the accumulation of triglycerides >5% (steatosis) in the liver of individuals who rarely consume alcoholic beverages (Gaggini et al. 2013). In people with a normal BMI, NAFLD is more commonly found in Asian populations and is referred to as a "metabolically obese" condition (Wong and Ahmed 2014). Therefore, the early detection of insulin resistance and NAFLD prior to diagnosis is important to prevent the future risk of developing chronic diseases (Preethi et al. 2011).

Some markers have been commonly used as clinical measurement standards, such as the homeostatic model assessment for insulin resistance (HOMA-IR) (Singh and Saxena 2010) and the Liver Fat Score (LFS) (Kahl et al. 2014) and the Fatty Liver Index (FLI) for fatty liver disease (Du et al. 2014). These markers are widely employed to diagnose and categorize developed stages of insulin resistance or NAFLD. For example, tertile analysis of HOMA-IR shows an association with glycemic control in the lean, non-diabetic Asian population (Hirata et al. 2015). However, these markers could not explain the molecular mechanism underlying the early conditions of insulin resistance and NAFLD.

TABLE 1 Formulas to calculate the indices.

Indices	Formula	Citation
HOMA-IR	= (FPI [mIU/L] × FPG (mmol/L)/22.5	Singh and Saxena (2010)
LFS	= −2.89 + 1.18 × MetS (yes=1/no=0) + 0.45 × T2D (yes=2/no=0) + 0.15 × FPI (mIU/L) + 0.04 × AST (IU/L) 0.94 × AST/ALT	Kahl et al. (2014)
FLI	= ($e^{0.953 \times \log e (TG) + 0.139 \times BMI + 0.718 \times \log e (GGT) + 0.053 \times WC - 15.745}$) / (1 + $e^{0.953 \times \log e (TG) + 0.139 \times BMI + 0.718 \times \log e (GGT) + 0.053 \times WC - 15.745}$) × 100	Du et al. (2014)

All blood parameters used should be collected after a 10–12 h overnight fast. ALT: alanine aminotransferase; AST: aspartate aminotransferase; BMI: body mass index; FPG: fasting plasma glucose; FPI: fasting plasma insulin; GGT: gamma-glutamyl transferase; MetS: metabolic syndrome; TG: triglyceride; T2D: Type-2-Diabetes; WC: waist circumference; WHR: waist-hip ratio.

Several genes are known to be involved in the development of insulin resistance; thus, they could be employed as potential molecular markers for insulin resistance. The relevant genes are *galnt2*, *sirt1*, and *ptpn1*. The expression of *galnt2*, a gene that encodes N-acetylgalactosaminyltransferase, could decrease the expression of *enpp1*, which encodes the inhibitor protein involved in insulin receptor signaling (inhibits insulin and insulin receptor (IR) interaction) (Marucci et al. 2013a). The genes *sirt1* and *ptpn1* have been studied for their role in the development of insulin resistance. The *ptpn1* gene encodes protein tyrosine phosphatase 1B (PTP1B), a protein that could catalyze dephosphorilation of IR and insulin receptor substrates (IRS) tyrosine residues, and cause a disturbance of the insulin signaling pathway (Stull et al. 2012). Sirtuin 1 was involved in the deacetylation of PTP1B, which further deactivates PTP1B as a negative regulator of insulin, and was able to improve insulin sensitivity under conditions of insulin resistance (Sun et al. 2007). Thus, this study evaluates the gene expression of *galnt2*, *sirt1*, and *ptpn1*, which could potentially be early markers of insulin resistance and NAFLD in the Indonesian population.

2. Materials and methods

Study participants were selected from employees of PT Nutrifood Indonesia. A total of 21 participants consisting of 10 men and 11 women were involved in this study. Inclusion criteria included healthy adults aged 23–40 years, with a fasting blood glucose <100 mg/dL, no history of hepatitis, and not smoking, pregnant or breastfeeding. Subjects were asked to sign a medical action agreement, and they underwent anthropometric and blood biochemical parameters testing according to ethical clearance approved by Research Ethics Commission of UNIKA Atma Jaya. The anthropometric parameters measured were body mass index (BMI), visceral fat area, waist circumference, and hip circumference. Body composition was measured using InBody 230 (Biospace) according to the tool protocols. For fasting blood sampling, subjects were asked to fast for 10–12 h (overnight). The blood biochemical parameters (other than fasting insulin) were measured using a commercial laboratory service (Prodia). The values of HOMA-IR, LFS, and FLI were calculated using the formulas presented in Table 1. Fasting insulin was measured using Ultrasensitive Insulin ELISA (Mercodia).

The isolation of RNA from fasting blood samples was performed using a QIAamp® RNA Blood Mini Kit (Qiagen). The concentration and purity of RNA were measured with a NanoDrop2000 (Thermo Fisher Scientific). The cDNA synthesis was performed using a RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific) on PCR GS482 (G-Storm). The cDNA synthesis results were then used to measure gene expression. The gene expression was quantified by using a StepReePlus Real-Time PCR System (Thermo Fisher Scientific). The reaction was carried out under the following conditions: pre-denaturation at 95°C for 2 min; denaturation at 95°C for 20 s; primer annealing for 40 s with the suitable temperatures: 55°C (for *actb* and *galnt2*), 60°C (for *sirt1*), and 62°C (for *ptpn1*); and fluorescent acquisition at 72°C for 30 s; with a total of 40× qPCR cycles. The expression level analysis was performed using the 2-ΔΔCT relative quantification method, using the expression of *actb* as a reference to normalize the gene expression level of target genes (Livak and Schmittgen 2001). The forward and reverse primers used for qPCR were: *actb* (5'-TCCCTGGAGAAGAGCTACGA-3' and 5'-ATCTGCT-

TABLE 2 Study participants' characteristics as mean (± SD).

Characteristics	All (n = 21)	SEM	Kolgomorov-Smirnov
Age (years)	30.52 (±5.32)	1.16	ns
Triglycerides (mg/dL)	93.48 (±43.22)	9.43	ns
Glucose (mg/dL)	82.29 (±9.71)	2.12	*
Insulin (mIU/L)	8.50 (±6.91)	1.51	‡
AST (IU/L)	28.31 (±11.62)	2.54	*
ALT (IU/L)	33.28 (±28.48)	6.21	†
GGT (IU/L)	25.00 (±16.26)	3.55	*
WC (cm)	89.90 (±9.09)	1.98	ns
WHR	0.89 (±0.05)	0.01	ns
BMI	26.10 (±3.51)	0.76	ns
HDL (mg/dL)	46.95 (±7.50)	1.63	ns

* $p < 0.05$; † $p < 0.01$; ‡ $p < 0.001$ SEM: Standard error means. All blood parameters were collected after a 10–12 h overnight fast. ALT: alanine aminotransferase; AST: aspartate aminotransferase; BMI: body mass index; GGT: gamma-glutamyl transferase; HDL: high-density lipoprotein; WC: waist circumference; WHR: waist-hip ratio.

GGAAGGTGGACAG-3'); *galnt2* (5'-AAGGAGAAGTCGGTGAAGCA-3' and 5'-TTGAGCGTGAACTTCCACTG-3'); *sirt1* (5'-TGAGGCACTTCATGGGGTATGG-3' and 5'-TCCTAGGtTGCCCAGCTGATGAA-3'); and *ptpn1* (5'-TGGGTGAAGGAAGAGACCCA-3' and 5'-CCCACGACCCGACTTCTAAC-3').

The collected data were statistically analyzed using SPSS Statistics 22 (IBM, Armonk, New York, United States). Normality, outlier, and descriptive analyses were performed for each fasting blood biochemical parameter and index and for gene expression. For the gene expression analysis, HOMA-IR, LFS, and FLI were sorted from the lowest value to the highest value and were divided into tertiles. Statistical analysis was performed among tertiles of referred indices, in which this method of tertile analysis could be used to evaluate associations of markers (Hirata et al. 2015). An independent T-Test mean difference was performed on the expression of *galnt2*, *sirt1*, and *ptpn1* between tertiles of HOMA-IR, LFS, and FLI.

3. Results and discussion

Characteristics of all study participants are presented in Table 2. All study participants were normoglycemic (FPG <100 mg/dL). Normality and outlier analysis resulted in valid HOMA-IR values for further analysis (n = 18) ranged from 0.45–2.20, LFS (n = 20) ranged from −3.99–0.71, and FLI (n = 21) ranged from 3.40–83.21. Two participants were categorized as insulin resistant based on the cut-off value of HOMA-IR ≥ 2.04 for the diagnosis of insulin resistance in Indonesia (Purnamasari et al. 2010). Thirteen participants were categorized as non-NAFLD, three participants were estimated to have elevated liver fat, four participants were predicted to have NAFLD, and no participants were diagnosed with NAFLD, based on the cut-off values of LFS < −1.413 to exclude NAFLD, LFS > −0.640 to predict NAFLD, and LFS > 1,257 to diagnose NAFLD (Kotronen et al. 2009). Ten participants were categorized without hepatosteatosis, six participants were identified as having a higher risk of hepatosteatosis, and five participants were characterized as having hepatosteatosis, based on the cut-off values of FLI < 30 to exclude NAFLD and FLI ≥ 60 to diagnose hepatosteatosis (Bedogni et al. 2006).

Based on the U.S. National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III), the triglyceride concentrations of our study population (52-242 mg/dL) were classified as normal to high (NCEP 2001). The study population levels of GGT and HDL were categorized as normal. Increased ALT levels in some participants might occur once in a while, intermittently, or be caused by steatohepatitis (Aragon and Younossi 2010).

The HOMA-IR, LFS, and FLI values were divided into tertiles. The lowest values are the first tertile (T1), and the highest values are the third tertile (T3). Analyses of study participants' characteristics relative to HOMA-IR, LFS, and FLI are presented in Table 3, 4, and 5, respectively. An analysis of gene expression among each tertile group is presented in Table 6.

There were significant mean differences between tertiles of HOMA-IR for TG (T1–T2), insulin (T1–T2, T2–T3, T1–T3), WC (T2–T3, T1–T3), WHR (T2–T3, T1–T3), and BMI (T2–T3) (Table 3). These suggest that the anthropometric parameters of WC, WHR, and BMI strongly influence the glycemic response and might be interpreted as early signs of insulin resistance in normoglycemic subjects. There were significant mean differences between tertiles of LFS for fasting glucose (T1–T3), insulin (T1–T2, T1–T3), ALT (T2–T3, T1–T3), GGT (T1–T2, T2–T3, T1–T3), WC (T1–T2, T1–T3), WHR (T1–T3), and HDL (T2–T3) (Table 4). There were significant mean differ-

TABLE 3 Tertiles of HOMA-IR.

Characteristics	T1 (n = 6)	T2 (n = 6)	T3 (n = 6)	p		
				T1 vs. T2	T2 vs. T3	T1 vs. T3
Age (years)	30.17 (±5.46)	34.00 (±4.90)	29.00 (±5.80)	ns	ns	ns
Triglycerides (mg/dL)	65.17 (±12.25)	93.00 (±25.67)	94.33 (±30.61)	∗	ns	ns
Glucose (mg/dL)	77.83 (±2.93)	78.67 (±4.84)	84.84 (±7.19)	ns	ns	ns
Insulin (mIU/L)	3.54 (±0.84)	5.56 (±0.62)	8.81 (±1.19)	†	‡	‡
AST (IU/L)	23.83 (±4.26)	31.00 (±12.33)	32.50 (±17.57)	ns	ns	ns
ALT (IU/L)	20.00 (±10.73)	38.33 (±34.89)	43.83 (±38.32)	ns	ns	ns
GGT (IU/L)	14.67 (±5.20)	21.67 (±15.91)	32.50 (±18.31)	ns	ns	ns
WC (cm)	82.17 (±5.49)	85.67 (±4.68)	97.00 (±3.85)	ns	†	‡
WHR	0.86 (±0.04)	0.87 (±0.04)	0.93 (±0.04)	ns	∗	†
BMI	24.03 (±3.79)	24.87 (±2.11)	28.30 (±3.06)	ns	∗	ns
HDL (mg/dL)	47.00 (±5.51)	50.17 (±8.40)	47.33 (±6.80)	ns	ns	ns
HOMA-IR	0.68 (±0.17)	1.08 (±0.16)	1.85 (±0.33)	†	†	‡

∗ p < 0.05; † p < 0.01; ‡ p < 0.001. All blood parameters were collected after a 10-12 h overnight fast. ALT: alanine aminotransferase; AST: aspartate aminotransferase; BMI: body mass index; GGT: gamma-glutamyl transferase; HDL: high-density lipoprotein; WC: waist circumference; WHR: waist-hip ratio.

TABLE 4 Tertiles of LFS.

Characteristics	T1 (n = 7)	T2 (n = 6)	T3 (n = 7)	p		
				T1 vs. T2	T2 vs. T3	T1 vs. T3
Age (years)	33.14 (±5.55)	30.00 (±7.16)	29.00 (±2.52)	ns	ns	ns
Triglycerides (mg/dL)	64.86 (±12.75)	96.33 (±31.85)	114.29 (±59.28)	ns	ns	ns
Glucose (mg/dL)	76.29 (±3.25)	81.33 (±5.61)	89.71 (±12.93)	ns	ns	*
Insulin (mIU/L)	3.93 (±1.15)	6.53 (±2.16)	11.75 (±6.33)	*	ns	*
AST (IU/L)	23.57 (±5.74)	24.83 (±5.15)	38.00 (±15.70)	ns	ns	ns
ALT (IU/L)	16.43 (±7.76)	24.33 (±9.05)	59.71 (±36.32)	ns	*	*
GGT (IU/L)	12.00 (±4.51)	18.00 (±3.74)	43.57 (±14.11)	*	†	†
WC (cm)	82.29 (±5.12)	92.67 (±8.66)	92.71 (±8.81)	*	ns	*
WHR	0.85 (±0.03)	0.90 (±0.04)	0.91 (±0.05)	ns	ns	*
BMI	24.07 (±3.48)	26.90 (±3.87)	27.10 (±2.96)	ns	ns	ns
HDL (mg/dL)	51.71 (±6.90)	47.50 (±6.92)	41.29 (±5.77)	ns	ns	*
LFS	−2.92 (±0.60)	−1.95 (±0.26)	−0.27 (±0.84)	†	†	‡

* $p < 0.05$; † $p < 0.01$; ‡ $p < 0.001$. All blood parameters were collected after a 10–12 h overnight fast. ALT: alanine aminotransferase; AST: aspartate aminotransferase; BMI: body mass index; GGT: gamma-glutamyl transferase; HDL: high-density lipoprotein; WC: waist circumference; WHR: waist-hip ratio.

ences between tertiles of FLI for age (T2–T3), TG (T1–T3), insulin (T2–T3, T1–T3), GGT (T1–T3), WC (T2–T3, T1–T3), WHR (T1–T3), BMI (T1–T2, T1–T3), and HDL (T2–T3) (Table 5). The elevation of AST, ALT, and GGT, which are liver-produced enzymes, usually indicates liver damage, which can also be caused by NAFLD (Aragon and Younossi 2010). Increased insulin levels in participants with normal glucose levels, along with the increased risk of NAFLD, suggest an association between IR and NAFLD, even at its earliest stages. Significant differences observed for WC, WHR, and BMI also confirm that these anthropometric parameters play a role in developing early insulin resistance and further contribute to the development of NAFLD.

There were significant mean differences of *galnt2* ($p < 0.002$) and *sirt1*($p < 0.010$) expression between the first tertile (T1) and the third tertile (T3) of HOMA-IR. According to study participants' characteristics among tertiles of HOMA-IR, there was a significant difference in fasting insulin among tertiles, but there was no significant difference in fasting glucose (Table 3). This result indicates that although T3 of HOMA-IR was categorized as insulin sensitive due to its normal glucose level, it was somewhat closer to insulin resistance condition than T1. Therefore, T3 of HOMA-IR can be referred to as the early condition of insulin resistance (Preethi et al. 2011). These results suggest that *galnt2* and *sirt1* expression can be used as markers of the early condition of insulin resistance.

The significant difference in *galnt2* expression between the first and the third tertiles of HOMA-IR indicates that an increasing value of HOMA-IR in normoglycemic participants was followed by the increased expression of *galnt2* (Table 6). This result seems to be contradictory to the previous study by Marucci et al. (2013b), which demonstrated that *galnt2* mRNA expression levels in peripheral whole blood cells were significantly reduced from control to obese to diabetics. In-vitro study in human monocytes had identified hyperglycemia as a major cause of *galnt2* down-regulation in patients with T2D (Marucci et al. 2013b). Our study population had a wide range of fasting insulin levels, but they were still categorized as normoglycemic (fasting glucose level <100 mg/dL); thus, the expression of galnt2 was not expected to be heterogeneous among the subjects. Interestingly, in the T3 group, where insulin resistance levels were higher, the expression of *galnt2* was higher than in the T1 group. This condition was might be due to in the early condition of insulin resistance, *galnt2* would be expressed higher to inhibit *enpp1*, an inhibitor for insulin signaling (Marucci et al. 2013b), thus, enabling the insulin to work optimally. This result suggests that *galnt2* could be a molecular marker for the early development of insulin resistance.

The significant difference in *sirt1* expression between the first and the third tertiles of HOMA-IR also indicate that the increasing value of HOMA-IR is associated with a significantly higher expression of *sirt1* (Table 6). This result seems to be contradictory with the previous study by Song et al. (2011) that demonstrated that *sirt1* mRNA expression levels in the T2DM group were lower compared with the healthy group. SIRT1 deacetylase activity consumes NAD+, and a high blood sugar level in T2DM patients will decrease the NAD+/NADH ratio. The decrease of NAD+/NADH caused by altered homeostasis of glucose metabolism will be followed by a subsequent decrease of sirt1 expression (Kitada and Koya 2013). Therefore, as for *galnt2*, a hyperglycemia might also be the cause of *sirt1* down-regulation in patients with T2D. Our study population had a wide range of fasting insulin levels, but they were still categorized as normoglycemic (fasting glucose level <100 mg/dL); thus, the expression of *sirt1*

was not supposed to be heterogeneous among the subjects. Instead, our study illustrates that a higher fasting plasma insulin (T3) within the normoglycemic population is associated with increased *sirt1* expression. This result is in accordance with SIRT1's positive role in the insulin-signaling pathway by inducing insulin secretion, in which SIRT1 activation increases glucose uptake and insulin signaling (Song et al. 2011). In the early condition of insulin resistance, *sirt1* would be expressed higher to induce insulin secretion until the body was unable to maintain normal blood sugar homeostasis, after which the expression of *sirt1* would drop. Thus, *sirt1* could be a molecular marker of the early development of insulin resistance.

There was no significant mean difference in *ptpn1* expression across the tertiles of HOMA-IR. This result probably showed that *ptpn1* expression would be up-regulated in the later stage of insulin resistance (Stull et al. 2012), and not in the early condition of insulin resistance. In this study, a difference of *ptpn1* expression ($p < 0.012$) was only observed between the first and the second tertile of LFS, where the second tertile was significantly higher than the first tertile. Higher expression in T2 than in T1 in this study was in accordance with another report that found *ptpn1* to be up-regulated in liver biopsies of Non-Alcoholic SteatoHepatitis (NASH) patients (Sanderson and Smyrk 2005). Higher expression of *ptpn1* could also be caused by hepatocyte inflammation of TNF-α, as reported in the previous study of hepatocyte inflammation in vitro (Zabolotny et al. 2008). Therefore, these results suggest that changes in *ptpn1* expression (which is a protein tyrosine kinase) in blood might also be studied as molecular markers for the early stages of NAFLD. No

TABLE 5 Tertiles of FLI.

Characteristics	T1 (n = 7)	T2 (n = 7)	T3 (n = 7)	p		
				T1 vs. T2	T2 vs. T3	T1 vs. T3
Age (years)	32.29 (±6.34)	32.86 (±4.14)	26.43 (±2.70)	ns	†	ns
Triglycerides (mg/dL)	65.14 (±12.55)	89.00 (±25.87)	126.29 (±56.42)	ns	ns	∗
Glucose (mg/dL)	78.71 (±5.71)	81.43 (±6.16)	86.71 (±14.33)	ns	ns	ns
Insulin (mIU/L)	5.18 (±2.27)	5.32 (±2.19)	15.00 (±8.69)	ns	∗	∗
AST (IU/L)	24.43 (±6.37)	30.57 (±10.39)	31.43 (±16.36)	ns	ns	ns
ALT (IU/L)	17.00 (±9.59)	41.43 (±30.30)	41.43 (±35.06)	ns	ns	ns
GGT (IU/L)	13.29 (±5.28)	24.71 (±13.60)	37.00 (±13.44)	ns	ns	∗
WC (cm)	82.29 (±6.18)	88.29 (±4.07)	99.14 (±7.10)	ns	†	‡
WHR	0.85 (±0.03)	0.88 (±0.03)	0.92 (±0.05)	ns	ns	†
BMI	22.86 (±1.93)	26.34 (±2.62)	29.11 (±2.73)	∗	ns	‡
HDL (mg/dL)	53.29 (±7.85)	44.57 (±3.46)	43.00 (±6.43)	∗	ns	∗
FLI	9.28 (±5.00)	29.03 (±12.27)	63.97 (±11.40)	†	‡	‡

∗ $p < 0.05$; † $p < 0.01$; ‡ $p < 0.001$. All blood parameters were collected after a 10–12 h overnight fast. ALT: alanine aminotransferase; AST: aspartate aminotransferase; BMI: body mass index; GGT: gamma-glutamyl transferase; HDL: high-density lipoprotein; WC: waist circumference; WHR: waist-hip ratio.

TABLE 6 Expression of *galnt2*, *sirt1*, and *ptpn1* (mean ± SD) between tertiles of HOMA-IR, LFS, and FLI.

Index	Gene	T1	T2	T3	p		
					T1 vs. T2	T2 vs. T3	T1 vs. T3
HOMA-IR	galnt2	0.66 (±0.08)	0.71 (±0.32)	0.90 (±0.12)	ns	ns	†
	sirt1	0.74 (±0.05)	0.84 (±0.20)	0.92 (±0.12)	ns	ns	∗
	ptpn1	0.89 (±0.13)	0.84 (±0.29)	1.06 (±0.15)	ns	ns	ns
LFS	galnt2	0.68 (±0.16)	0.86 (±0.13)	0.78 (±0.32)	ns	ns	ns
	sirt1	0.78 (±0.12)	0.88 (±0.15)	0.85 (±0.24)	ns	ns	ns
	ptpn1	0.83 (±0.15)	1.07 (±0.13)	0.90 (±0.28)	∗	ns	ns
FLI	galnt2	0.74 (±0.16)	0.62 (±0.27)	0.88 (±0.17)	ns	ns	ns
	sirt1	0.80 (±0.15)	0.79 (±0.18)	0.91 (±0.18)	ns	ns	ns
	ptpn1	0.89 (±0.17)	0.84 (±0.27)	0.97 (±0.14)	ns	ns	ns

Independent sample t-test. ∗ $p < 0.05$; † $p < 0.01$; ‡ $p < 0.001$. The expression level analysis was performed using the expression of*actb* as a reference to normalize gene expression level of target genes.

significant mean difference in *galnt2* and *sirt1* expression among tertiles of LFS and FLI was found.

This study was designed as a preliminary study, in which researchers were aware of the limitations of using a relatively small number of participants. However, this study provides insight for further exploration of potential markers for the early stages of insulin resistance and NAFLD in healthy individuals.

4. Conclusions

Further study is needed for *galnt2* and *sirt1* expression as potential molecular markers for the early stages of insulin resistance in a larger population. And although *ptpn1* expression was not correlated with early insulin resistance, *ptpn1* expression could be studied further as a molecular marker for early non-alcoholic fatty liver disease.

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Authors' contributions

AS, FK designed the study. EL carried out the laboratory work and analyzed the data. EL, FK wrote the manuscript. All authors read and approved the final version of manuscript.

Competing interests

The authors confirm no competing interest in this study.

References

Aragon G, Younossi ZM. 2010. When and how to evaluate mildly elevated liver enzymes in apparently healthy patients. Cleve Clin J Med. 77(3):195–204. doi:10.3949/ccjm.77a.09064.

Bedogni G, Bellentani S, Miglioli L, Masutti F, Passalacqua M, Castiglione A, Tiribelli C. 2006. The Fatty Liver Index: a simple and accurate predictor of hepatic steatosis in the general population. BMC Gastroenterol. 6(1):33. doi:10.1186/1471-230x-6-33.

Du T, Yuan G, Zhang M, Zhou X, Sun X, Yu X. 2014. Clinical usefulness of lipid ratios, visceral adiposity indicators, and the triglycerides and glucose index as risk markers of insulin resistance. Cardiovasc Diabetol. 13(1):146. doi:10.1186/s12933-014-0146-3.

Gaggini M, Morelli M, Buzzigoli E, DeFronzo RA, Bugianesi E, Gastaldelli A. 2013. Non-alcoholic fatty liver disease (NAFLD) and its connection with insulin resistance, dyslipidemia, atherosclerosis and coronary heart disease. Nutrients. 5(5):1544–1560. doi:10.3390/nu5051544.

Hasan I, Gani R, Machmud R, et al. 2002. Prevalence and risk factors for nonalcoholic fatty liver in indonesia. J Gastroenterol Hepatol. 17(Suppl A):30.

Hirata T, Higashiyama A, Kubota Y, Nishimura K, Sugiyama D, Kadota A, Nishida Y, Imano H, Nishikawa T, Miyamatsu N, et al. 2015. HOMA-IR values are associated with glycemic control in japanese subjects without diabetes or obesity: the KOBE study. J Epidemiol. 25(6):407–414. doi:10.2188/jea.je20140172.

[IDF] International Diabetes Federation. 2005. The IDF consensus worldwide definition of the metabolic syndrome. Technical report. International Diabetes Federation. Brussels.

[IDF] International Diabetes Federation. 2017. IDF diabetes atlas. 8*th* ed. Technical report. International Diabetes Federation. Brussels.

Kahl S, Straßburger K, Nowotny B, Livingstone R, Klüppelholz B, Keßel K, Hwang JH, Giani G, Hoffmann B, Pacini G, et al. 2014. Comparison of liver fat indices for the diagnosis of hepatic steatosis and insulin resistance. PLoS ONE. 9(4):e94059. doi:10.1371/journal.pone.0094059.

Kitada M, Koya D. 2013. SIRT1 in type 2 diabetes: mechanisms and therapeutic potential. Diabetes Metab J. 37(5):315–325. doi:10.4093/dmj.2013.37.5.315.

Kotronen A, Peltonen M, Hakkarainen A, Sevastianova K, Bergholm R, Johansson LM, Lundbom N, Rissanen A, Ridderstråle M, Groop L, et al. 2009. Prediction of non-alcoholic fatty liver disease and liver fat using metabolic and genetic factors. Gastroenterology. 137(3):865–872. doi:10.1053/j.gastro.2009.06.005.

Livak KJ, Schmittgen TD. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2- $\delta\delta$CT method. Methods. 25(4):402–408. doi:10.1006/meth.2001.1262.

Marucci A, Cozzolino F, Dimatteo C, Monti M, Pucci P, Trischitta V, Di Paola R. 2013a. Role of GALNT2 in the modulation of ENPP1 expression, and insulin signaling and action: GALNT2: a novel modulator of insulin signaling. Biochim Biophys Acta Mol Cell Res. 1833(6):1388–1395. doi:10.1016/j.bbamcr.2013.02.032.

Marucci A, Di Mauro L, Menzaghi C, Prudente S, Mangiacotti D, Fini G, Lotti G, Trischitta V, Di Paola R. 2013b. GALNT2 expression is reduced in patients with type 2 diabetes: possible role of hyperglycemia. PLoS ONE. 8(7):e70159. doi:10.1371/journal.pone.0070159.

Mendrick DL, Diehl AM, Topor LS, Dietert RR, Will Y, La Merrill MA, Bouret S, Varma V, Hastings KL, Schug TT, et al. 2017. Metabolic syndrome and associated diseases: from the bench to the clinic. Toxicol Sci. 162(1):36–42. doi:10.1093/toxsci/kfx233.

[NCEP] National Cholesterol Education Program. 2001. ATP III guidelines at-a-glance quick desk reference. Bethesda: National Heart, Lung, and Blood Institute Bethesda.

Olokoba AB, Obateru OA, Olokoba LB. 2012. Type 2 diabetes mellitus: a review of current trends. Oman Med J. 27(4):269–273. doi:10.5001/omj.2012.68.

Preethi B, Jaisri G, Kumar KP, Sharma R. 2011. Assessment of insulin resistance in normoglycemic young adults. Hum Physiol. 37(1):105–112. doi:10.1134/s0362119711010154.

Purnamasari D, Soegondo S, Oemardi M, Gumiwang I. 2010. Insulin resistance profile among siblings of type 2 diabetes mellitus (preliminary study). Acta Med Indones. 42(4):204–208.

Sanderson SO, Smyrk TC. 2005. The use of protein tyrosine phosphatase 1B and insulin receptor immunostains to differentiate nonalcoholic from alcoholic steatohepatitis in liver biopsy specimens. Am J Clin Pathol. 123(4):503–509. doi:10.1309/1px2lmpquh1ee12u.

Singh B, Saxena A. 2010. Surrogate markers of insulin resistance: a review. World J Diabetes. 1(2):36. doi:10.4239/wjd.v1.i2.36.

Song R, Xu W, Chen Y, Li Z, Zeng Y, Fu Y. 2011. The expression of sirtuins 1 and 4 in peripheral blood leukocytes from patients with type 2 diabetes. Eur J Histochem. 55(1). doi:10.4081/ejh.2011.e10.

Stull AJ, Wang ZQ, Zhang XH, Yu Y, Johnson WD, Cefalu WT. 2012. Skeletal muscle protein tyrosine phosphatase 1B regulates insulin sensitivity in African Americans. Diabetes. 61(6):1415–1422. doi:10.2337/db11-0744.

Sun C, Zhang F, Ge X, Yan T, Chen X, Shi X, Zhai Q. 2007. SIRT1 improves insulin sensitivity under insulin-resistant conditions by repressing PTP1B. Cell Metab. 6(4):307–319. doi:10.1016/j.cmet.2007.08.014.

Wong RJ, Ahmed A. 2014. Obesity and non-alcoholic fatty liver disease: disparate associations among Asian populations. World J Hepatol. 6(5):263. doi:10.4254/wjh.v6.i5.263.

Zabolotny JM, Kim YB, Welsh LA, Kershaw EE, Neel BG, Kahn BB. 2008. Protein-tyrosine phosphatase 1B expression is induced by inflammation in vivo. J Biol Chem. 283(21):14230–14241. doi:10.1074/jbc.m800061200.