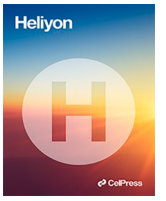
Heliyon 9 (2023) e18887

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Dysregulation of adipokines levels among healthy first-degree relatives of type 2 diabetes patients

Dyah Purnamasari [a,b,*], Cindya Klarisa Simanjuntak [c], Christian Tricaesario [b], Dicky Levenus Tahapary [a,b], Dante Saksono Harbuwono [a,b], Em Yunir [a,b]

[a] *Division of Endocrinology Metabolism and Diabetes, Department of Internal Medicine, Dr. Cipto Mangunkusumo National Referral Hospital, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia*
[b] *Metabolic Disorder, Cardiovascular and Aging Research Center, The Indonesian Medical Education and Research Institute, Faculty of Medicine Universitas Indonesia, Jakarta, Indonesia*
[c] *Department of Internal Medicine, Dr. Cipto Mangunkusumo National Referral Hospital, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia*

ARTICLE INFO

Keywords:
Family history
Insulin resistance
Leptin
Adiponectin
Adipokines
Type 2 diabetes mellitus

ABSTRACT

Background: Leptin, adiponectin and its ratio (L/A), as well as adipocyte fatty acid binding protein (A-FABP) have shown association to type 2 diabetes and atherosclerosis. Since first degree relatives (FDR) of type 2 diabetes are known to have higher risks of developing aforementioned diseases, this study aimed to see differences in adipokines profiles between FDR of type 2 diabetes and non-FDR counterpart.

Methods: Age, sex and body mass index (BMI)-matched normotensive-normoglycemic subjects, aged 19–39 years with BMI<30 kg/m^2, were included in this cross-sectional study. Serum adiponectin, leptin, and A-FABP levels were measured by sandwich ELISA while HOMA-IR was calculated from fasting blood glucose and insulin levels.

Results: Of 116 subjects recruited, there were significant difference of insulin level (6.00 vs 5.00 μIU/mL, $P = 0.029$) and HOMA-IR (1.27 vs 1.10, $P = 0.028$). Adiponectin, leptin, L/A ratio, and A-FABP levels were not statistically different between FDR and non-FDR groups. Stratified by BMI, non-obese FDR had higher L/A ratio (0.83 vs 0.49, $P = 0.020$) compared to those of corresponding non-FDR. In multivariate analysis, after adjusting for age, sex, waist circumference, BMI, and metabolic profiles (HbA1C, HOMA-IR, LDL-C, HDL-C, and triglyceride levels), FDR status became significantly associated with adiponectin level, and in non-obese subgroup, remained its significance with L/A ratio.

Conclusion: The FDR status was independently associated with adiponectin level. Furthermore, higher L/A ratio was more pronounced in non-obese FDR than those of non-FDR subjects, suggesting that FDR status may already contribute to the development of adipokines dysregulation before obesity occurs.

* Corresponding author. Division of Endocrinology Metabolism and Diabetes, Department of Internal Medicine, Dr. Cipto Mangunkusumo National Referral Hospital, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia.
E-mail address: dyah_p_irawan@yahoo.com (D. Purnamasari).

https://doi.org/10.1016/j.heliyon.2023.e18887
Received 16 October 2022; Received in revised form 29 July 2023; Accepted 1 August 2023
Available online 2 August 2023

1. Introduction

First-degree relatives (FDR) of type 2 diabetes patients are at a higher risk of developing type 2 diabetes compared to those without family history of type 2 diabetes. The annual conversion rate into type 2 diabetes is 3% for individuals with a family history of young-onset diabetes (before 40 years old), 1.6% in those with a family history of elderly-onset type 2 diabetes, and 0.7% in those without diabetes family history [1]. Therefore, it is of importance to understand the basic mechanism underlying this increased risk.

The main mechanism in the development of type 2 diabetes is insulin resistance [2]. Previous studies have shown that adipocytokines or adipokines, cytokines secreted by adipose tissue, contribute to the development of insulin resistance and chronic low-grade inflammation in type 2 diabetes. Among others, adiponectin and leptin are two adipokines that are mostly studied in relation to insulin resistance. Adiponectin has anti-inflammatory, antidiabetic and anti-atherogenic roles. Its low concentration is associated with obesity, insulin resistance, metabolic syndrome, essential hypertension, coronary artery disease, dyslipidemia, type 2 diabetes, and is also found in diabetes FDR [3]. Unlike adiponectin, leptin has inflammatory properties. Leptin depicts fat reserves, where in line with an increase in fat mass, leptin levels will increase [4]. Due to the contrast effect between adiponectin and leptin, some studies have suggested to use leptin to adiponectin ratio (L/A) as a better marker for metabolic syndrome compared to adiponectin or leptin alone [5]. L/A ratio also had been reported to have a greater contribution to metabolic syndrome compared to HOMA-IR [6]. In addition, adipocyte fatty acid binding protein (A-FABP), initially known as an intracellular protein, plays a role both in the development of insulin resistance and atherosclerosis [7,8].

While the role of adipokines in the development of type 2 diabetes has been widely studied, its specific role in FDR of type 2 diabetes has rarely been assessed. Our previous study among Indonesian FDR of type 2 diabetes population showed that despite there was no significant difference in body mass index between FDR and non-FDR, we observed a higher risk for atherogenic dyslipidemia [9], suggesting different metabolic effect despite having similar body fat, which might be mediated by adipokines. In the present study, we aimed to compare the levels of adiponectin, leptin, L/A ratio, and A-FABP between FDR of type 2 diabetes and non-FDR groups.

2. Materials and methods

2.1. Study design and participants

This study was conducted according to Declaration of Helsinki 1964 and was approved by the Ethics Committee of Faculty of Medicine Universitas Indonesia in 2018 and renewed in 2020 (No. 0242/UN2.F1/ETIK/2018 and No. KET-404/UN2.F1/ETIK/PPM.00.02/2020). All participants gave written informed consent.

This study was a cross-sectional study conducted in 2018. Healthy normotensive and normoglycemic subjects aged 19–39 years old were included. Subjects with body mass index (BMI) of $\geq$30 kg/m2, cardiovascular disorder, malignancy, autoimmune disorder, and subjects taking drugs that interfere with glucose metabolism such as steroid and statin, were excluded. First-degree relatives were defined as subjects with one or both parents with type 2 diabetes. The biological children of type 2 diabetes patients visiting outpatient diabetes clinic of Cipto Mangunkusumo Hospital in Jakarta, Indonesia, were recruited to the FDR group. Age, sex, and BMI-matched

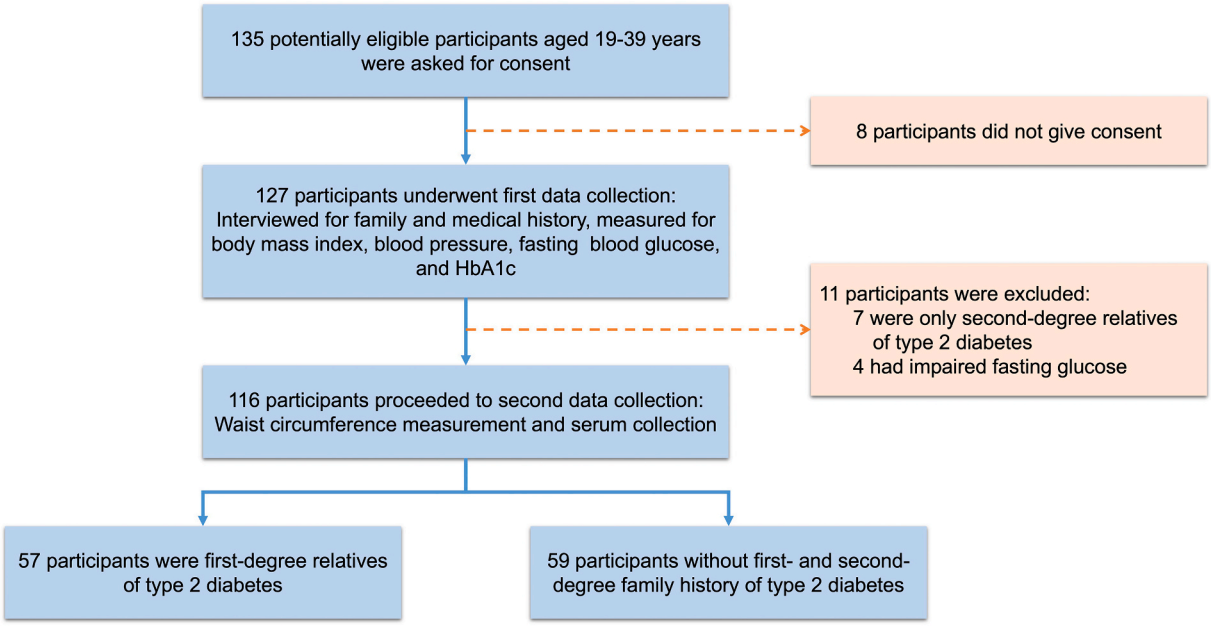


Fig. 1. Flowchart of the study participants recruitment process.

healthy subjects were recruited from hospital employees without type 2 diabetes first- and second-degree family history and assigned as non-FDR group.

2.2. Measurements and laboratory assay

All subjects who agreed to participate in this study underwent first data collection, including interview regarding medical and family history, body mass index (BMI), blood pressure, HbA1c, and fasting blood glucose level measurements. HbA1c was measured using A1c Glycohemoglobin Analyzer EZ 2.0 system (BioHermes Biomedical Technology Co., Ltd., Wuxi, China), and fasting blood glucose was examined after an overnight fasting of 8–10 h using Accu Check Performa tool (Roche Diabetes Care, Inc., Indianapolis, IN, US). Subjects who met the inclusion criteria proceeded with the second data collection in different day (Fig. 1). Abdominal circumference and blood sample were collected after an overnight fasting of 10–12 h for laboratory measurements including total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglycerides, fasting insulin, and fasting blood glucose. Remaining serum was centrifuged and stored at −80 °C freezer for ELISA analysis.

Adiponectin, leptin, and A-FABP levels were measured from stored blood serum using sandwich ELISA with human adiponectin/Acrp30 DY1065, human leptin DY398, and human A-FABP DY3150-05 ELISA kit, respectively, by R&D Systems (Minneapolis, MN, US). The inter-assay coefficients of variation were 2.03%, 2.01%, and 5.54% for leptin, adiponectin, and A-FABP assays, respectively. Since we did not conduct these assays in duplicate, intra-assay coefficient of variation could not be calculated.

2.3. Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics 25.0. Sample size was calculated with hypothesis test to see mean differences between two groups with standard deviation 1, type 1 error 5%, type 2 error 10%, with minimal sample size of 51 subjects for each group, hence the minimal total sample of 102 subjects. Normality of data distribution was analyzed using Kolgomorov-Smirnov for dataset of 50 or more and Shapiro-Wilk for dataset of less than 50. Normally distributed data were presented in mean ± standard deviation, while non-normally distributed data were presented in median (interquartile range). To compare the levels of adiponectin, leptin, L/A ratio and A-FABP between FDR and non-FDR groups, we used T-test for normally distributed data and Mann-Whitney test for abnormally distributed data with $P < 0.05$ was considered as statistically significant. Multiple linear regression was used for multivariate analysis.

3. Results

3.1. Characteristics of study population

A total of 116 subjects were recruited, 57 subjects in the FDR group and 59 participants in the non-FDR group. There were significant differences in fasting insulin level (6.00 [4.65–8.30] vs 5.00 [4.20–7.00], $p = 0.029$) and HOMA-IR (1.27 [0.96–1.84] vs 1.10 [0.78–1.54], $p = 0.028$), but no significant differences in demographic, anthropometric, fasting glucose, HbA1C, and lipid profile were

Table 1
Baseline characteristics.

Variable	All subjects (n = 116)	FDR (n = 57)	Non-FDR (n = 59)	*P* value
Age (years)	28 (25–34)	28 (25–34)	29.08 ± 5.65	0.870
Sex, n (%)				0.633
Female	80 (69.0%)	41 (71.9%)	39 (66.1%)	
Male	36 (31.0%)	16 (28.1%)	20 (33.9%)	
Waist circumference (cm)	78.40 ± 9.18	78.48 ± 8.67	78.31 ± 9.72	0.920
Female	76.42 ± 8.55	76.17 ± 7.57	76.68 ± 9.58	0.789
Male	82.79 ± 9.09	84.42 ± 8.68	81.48 ± 9.42	0.343
Body mass index (kg/m^2)	22.73 ± 3.39	22.86 ± 3.19	22.59 ± 3.60	0.671
BMI <25 kg/m^2, n	86 (74.1%)	42 (73.7%)	44 (74.6%)	1.000
BMI ≥25 kg/m^2, n	30 (25.9%)	15 (26.3%)	15 (25.4%)	
Total cholesterol (mg/dL)	187.74 ± 34.22	189.09 ± 34.23	186.44 ± 34.45	0.679
Triglyceride (mg/dl)	77 (62.00–103.75)	76 (58.50–112.50)	78 (62.00–101.00)	0.860
HDL-C (mg/dl)	51.11 ± 10.95	51.79 ± 10.35	50.46 ± 11.56	0.515
LDL-C (mg/dL)	127.27 ± 33.04	128.49 ± 33.22	126.08 ± 33.10	0.697
HbA1c (%)	5.1 ± 4.9–5.4	5.0 (4.70–5.35)	5.2 (4.9–5.4)	0.062
HbA1c (mmol/mol)	32 (30–36)	31 (28–35)	33 (30–36)	0.062
Fasting blood glucose (mg/dL)	85 (80–90)	85.21 ± 6.57	84.53 ± 7.06	0.590
Fasting insulin (μIUm/L)	5.55 (4.43–7.48)	6.00 (4.65–8.30)	5.00 (4.20–7.00)	**0.029**[a]
HOMA-IR	1.15 (0.89–1.58)	1.27 (0.96–1.84)	1.10 (0.78–1.54)	**0.028**[a]

Values are expressed as mean ± standard deviation or median (interquartile range).
BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment for insulin resistance.
[a]Values denote statistical significance at $p < 0.05$ with Mann-Whitney test.

found between FDR and non-FDR groups (Table 1). Dividing subjects based on sex showed that male FDR had higher mean value of waist circumference compared to non-FDR (84.42 ± 8.68 vs 81.48 ± 9.42, $p = 0.34$) but it didn't reach statistical significance. Whereas female FDR had similar waist circumference to that of non-FDR (76.17 ± 7.57 vs 76.68 ± 9.58, $p = 0.79$).

3.2. Adipokines level

From these 116 subjects, one subject with incomplete data was excluded from analysis of adipokines levels. Of 115 subjects, we observed no statistically difference of adiponectin (13.42 [10.73–18.71] vs 14.67 [9.74–22.58], $p = 0.300$), leptin (14.22 [6.04–24.37] vs 10.61 [4.25–19.99], $p = 0.104$), L/A ratio (0.88 [0.46–1.70] vs 0.66 [0.29–1.21], $p = 0.083$), and A-FABP levels (5.58 ± 2.35 vs 4.91 [3.35–6.43], $p = 0.460$) between both groups (Table 2).

Subgroup analysis using BMI cut point of 25, we observed significantly higher levels of leptin (13.70 [5.73–22.95] vs 9.28 [4.15–15.94], $p = 0.050$) and L/A ratio (0.83 [0.45–1.64] vs 0.49 [0.21–0.95], $p = 0.020$), trend of higher A-FABP level (4.99 ± 1.72 vs 4.42 [3.09–5.55], $p = 0.089$), but no significant difference of adiponectin in non-obese (BMI <25) FDR compared to non-obese non-FDR (Table 3).

In multivariate analysis, association between FDR status as independent variable and all adipokines as dependent variables were adjusted for age, sex, waist circumference, BMI, HbA1C, HOMA-IR, LDL-C, HDL-C, and triglyceride levels. Adiponectin became significantly inversely associated with FDR status with $p = 0.047$, whereas other adipokines remained not significantly associated with FDR status (Table 4). While in non-obese subgroup, after adjusting for age, sex, waist circumference, body mass index, HbA1C, HOMA-IR, LDL-C, HDL-C, and triglyceride levels, leptin became not significant, while L/A ratio remained significant (Table 5).

In addition, body fat percentage in both groups strongly and similarly correlated with leptin and L/A ratio but not with adiponectin (Table 6). Stratified by BMI, the correlations remained similar between FDR and non-FDR groups (Table 6).

4. Discussion

In this study, we tried to compare insulin resistance profile and circulating adipokines level in FDR to that in non-FDR as early as possible, when neither glucose dysregulation nor hypertension had occurred. Though there are already some studies reporting trends in adipokine changes in older FDR, some of which were without strict glucose or blood pressure criteria, there is still no report to date investigating adipokines in FDR in Indonesian population. Due to the fact that Asian population, including Indonesia, tend to eat high carbohydrate meal (i.e. rice, noodles) and have central obesity within the same BMI groups compared to Caucasian, it might contribute to the phenomenon of diabetes development in younger individuals [10–12]. Therefore, we believe that our study is needed to see early metabolic disturbance in Indonesian high-risk population, which is FDR, so that this could be the basis of early intervention strategy to prevent diabetes and to stimulate other studies about FDR.

This study found significantly higher insulin levels and HOMA-IR value in FDR group compared to non-FDR. This showed that even in similar metabolic and anthropometric characteristics where neither glycemic tolerance disturbance nor hypertension existed, FDR had tendency to be more hyperinsulinemic and insulin resistant, hence the greater risk of insulin resistance-related metabolic disorders including diabetes, dyslipidemia, and coagulation disorders [2]. This is in accordance with studies by Liu et al. and Lihn et al. which also found increased fasting insulin levels, increased HOMA-IR value, and reduced insulin sensitivity in FDR group compared to age and BMI-matched controls [3,13]. Shahid et al. also observed that young Southeast Asian male FDR subjects had significantly higher HOMA-IR and BMI compared to control group [14]. Being FDR is vulnerable to become insulin resistant because of impaired non-oxidative glucose metabolism, inherited defects in mitochondrial oxidative phosphorylation activity, defects in insulin activation of glucose transport activity, and reduced insulin stimulation of IRS-1 tyrosine phosphorylation [15–17].

Adipokines have recently studied as biomarkers predicting type 2 diabetes besides insulin. Despite significant difference in insulin resistance parameters, we found no significant differences in the levels of adiponectin, leptin, L/A ratio, and A-FABP in FDR of type 2 diabetes subjects compared to non-FDR subjects. It is most likely due to similarities in clinical characteristics such as age, sex, and anthropometric profile between both groups, as a result of strict inclusion criteria of research participants. This age, sex, and BMI-matched characteristics also explains the similarities found in lipid profile between both groups. In line with our previous study [9], overall, there was no difference in lipid profile between FDR and non-FDR group unless in male subgroup with age of 30–40 years. No difference was also found by Shahid et al. [14] in lipid profile between FDR and non-FDR group. After adjusting for age, sex, BMI, waist circumference, HOMA-IR, HbA1C, LDL-C, HDL-C, and triglyceride levels, adiponectin became significantly inversely associated with FDR status. This means that regardless of age, sex, anthropometric, and metabolic profiles, having parental history of type 2

Table 2
Comparison of adiponectin, leptin, A-FABP, and L/A ratio between FDR and non-FDR groups.

Variable	FDR (n = 56)	Non-FDR (n = 59)	*P* value
Adiponectin (ug/ml)	13.42 (10.73–18.71)	14.67 (9.74–22.58)	0.300
Leptin (ng/ml)	14.22 (6.04–24.37)	10.61 (4.25–19.99)	0.104
L/A ratio	0.88 (0.46–1.70)	0.66 (0.29–1.21)	0.083
A-FABP (ng/ml)	5.58 ± 2.35	4.91 (3.35–6.43)	0.460

Values are expressed as mean ± standard deviation or median (interquartile range).
FDR, first degree relative of type 2 diabetes; A-FABP, adipocyte fatty acid binding protein; L/A, leptin to adiponectin.

Table 3
Comparison of adiponectin, leptin, A-FABP, L/A ratio between FDR and non-FDR groups stratified by BMI.

		FDR	Non-FDR	*P* value
BMI <25 kg/m^2		**n = 42**	**n = 44**	
	Adiponectin (ug/ml)	13.75 (10.78–19.06)	15.84 (10.78–26.50)	0.288
	Leptin (ng/ml)	13.70 (5.73–22.95)	9.28 (4.15–15.94)	0.050[a]
	L/A ratio	0.83 (0.45–1.64)	0.49 (0.21–0.95)	0.020[a]
	A-FABP (ng/ml)	4.99 ± 1.72	4.42 (3.09–5.55)	0.089
BMI ≥ 25 kg/m^2		**n = 14**	**n = 15**	
	Adiponectin (ug/ml)	14.05 ± 6.02	10.01 (8.13–13.39)	0.217
	Leptin (ng/ml)	17.35 (7.51–34.36)	18.71 ± 11.82	0.847
	L/A ratio	1.07 (0.71–2.28)	1.71 ± 1.10	0.621
	A-FABP (ng/ml)	6.37 (4.98–8.80)	8.48 ± 3.29	0.217

Values are expressed as mean ± standard deviation or median (interquartile range).
FDR, first degree relatives of type 2 diabetes; BMI, body mass index; A-FABP, adipocyte fatty acid binding protein; L/A, leptin to adiponectin.
[a]Values denote statistical significance at $p < 0.05$ with Mann-Whitney test.

Table 4
Multivariate analysis for associations between FDR status and adipokines levels.

Dependent variables	Model	β (95% CI)	*P* value
Adiponectin	Model 1	−2.05 (−5.98; 1.88)	0.300
	Model 2	−2.37 (−6.21; 1.48)	0.225
	Model 3	−2.22 (−5.93; 1.49)	0.238
	Model 4	−3.77 (−7.49;-0.05)	0.047[a]
Leptin	Model 1	2.68 (−0.91; 6.27)	0.104
	Model 2	1.82 (−1.08; 4.71)	0.216
	Model 3	1.69 (−0.57; 3.94)	0.140
	Model 4	0.97 (−1.35; 3.28)	0.409
L/A ratio	Model 1	0.25 (−0.09; 0.59)	0.083
	Model 2	0.19 (−0.13; 0.51)	0.236
	Model 3	0.18 (−0.08; 0.43)	0.172
	Model 4	0.17 (−0.10; 0.44)	0.213
A-FABP	Model 1	0.19 (−0.79; 1.18)	0.460
	Model 2	0.14 (−0.83; 1.11)	0.775
	Model 3	0.09 (−0.75; 0.93)	0.831
	Model 4	−0.06 (−0.91; 0.78)	0.882

Multivariate linear regression with FDR status as independent variable where β value denotes adipokines level difference of the FDR group has in comparison to non-FDR group.
Model 1: Unadjusted.
Model 2: Adjusted for age and sex.
Model 3: Adjusted for age, sex, waist circumference, and body mass index.
Model 4: Adjusted for age, sex, waist circumference, body mass index, HbA1C, HOMA-IR, LDL-C, HDL-C, and triglyceride levels.
FDR, first degree relatives of type 2 diabetes; A-FABP, adipocyte fatty acid binding protein; L/A, leptin to adiponectin.
[a]Values denote statistical significance at $p < 0.05$.

diabetes alone already affects serum adiponectin level.

Adiponectin is hormone released by adipose tissue with insulin sensitizing properties. It increases glucose uptake in adipose tissue, improves insulin secretion in regards to glycemic environment, decreases hepatic gluconeogenesis, stimulates GLUT4-mediated glucose uptake in skeletal muscle, and enhances systemic insulin sensitivity [18,19]. Adiponectin also has anti-inflammatory role, downregulating expression and release of proinflammatory immune mediators and acting directly on NF-κB and inflammatory cells [20]. These anti-inflammatory and insulin sensitizing nature of adiponectin make its antidiabetic effect, as has been described by Bidulescu et al. in observing incident type 2 diabetes among 3363 African Americans. They showed inverse association between adiponectin and incident type 2 diabetes [19].

Contradicting to adiponectin, leptin comprises proinflammatory properties through stimulation of T-helper cell proliferation and upregulation of TNF-α and IL-6, inducing hepatic CRP production [20]. Leptin also suppresses insulin-stimulated glucose uptake, reduces responsiveness of β-cell receptors and inhibiting insulin gene expression in pancreatic cells [18]. In obese individuals, leptin level is increased that leads to reduced responsiveness of β-cell receptors. This results in hyperinsulinemia, which in turn would worsen obesity and further increase leptin levels creating a diabetogenic circle [20]. In line with this, Bidulescu et al. also found significant association between leptin and incident type 2 diabetes that is mediated by insulin resistance [19].

Nonetheless, the association between FDR status and adipokines level has been inconsistent. Our study observed lower adiponectin level in FDR group compared to that of non-FDR group but failed to reach statistical significance. However, in multivariate analysis we found that there was a significant association between FDR status and adiponectin level after further adjustment for age, sex, anthropometric and metabolic profiles, implicating that adiponectin level is independently associated with parental history of type 2

Table 5
Multivariate analysis for associations between FDR status and adipokines level in subjects with BMI<25 kg/m^2.

Dependent variables	Model	β (95% CI)	*P* value
Adiponectin	Model 1	−3.66 (−8.57; 1.25)	0.288
	Model 2	−3.90 (−8.81; 1.00)	0.117
	Model 3	−3.50 (−8.39; 1.40)	0.159
	Model 4	−4.91 (−10.06; 0.24)	0.061
Leptin	Model 1	3.57 (0.01; 7.12)	0.050*
	Model 2	2.73 (−0.16; 5.62)	0.064
	Model 3	2.36 (−0.09; 4.80)	0.058
	Model 4	2.15 (−0.51; 4.80)	0.111
L/A ratio	Model 1	0.41 (0.09; 0.73)	0.020*
	Model 2	0.33 (0.04; 0.63)	0.028*
	Model 3	0.30 (0.03; 0.57)	0.029*
	Model 4	0.35 (0.06; 0.64)	0.019*
A-FABP	Model 1	0.66 (−0.12; 1.44)	0.089
	Model 2	0.60 (−0.19; 1.40)	0.135
	Model 3	0.54 (−0.23; 1.30)	0.167
	Model 4	0.56 (−0.23; 1.35)	0.162

Multivariate linear regression with FDR status as independent variable where β value denotes adipokines level difference of the FDR group has in comparison to non-FDR group.
Model 1: Unadjusted.
Model 2: Adjusted for age and sex.
Model 3: Adjusted for age, sex, waist circumference, and body mass index.
Model 4: Adjusted for age, sex, waist circumference, body mass index, HbA1C, HOMA-IR, LDL-C, HDL-C, and triglyceride levels.
FDR, first degree relatives of type 2 diabetes; BMI, body mass index; A-FABP, adipocyte fatty acid binding protein; L/A, leptin to adiponectin.
*Values denote statistical significance at $p < 0.05$.

Table 6
Correlation between body fat percentage and BMI, adiponectin, leptin, and L/A ratio.

	FDR (N = 56)		Non-FDR (N = 59)	
	r	*P*-value	r	*P*-value
BMI	0.62	<0.001	0.52	<0.001
Adiponectin	0.10	0.163	0.07	0.581
Leptin	0.80	<0.001	0.85	<0.001
L/A ratio	0.65	<0.001	0.76	<0.001
BMI < 25 kg/m^2	**FDR (N = 42)**		**Non-FDR (N = 44)**	
Adiponectin	0.03	0.859	0.16	0.288
Leptin	0.84	<0.001	0.79	<0.001
L/A ratio	0.70	<0.001	0.69	<0.001
BMI ≥ 25 kg/m^2	**FDR (N = 14)**		**Non-FDR (N = 15)**	
Adiponectin	0.19	0.527	0.38	0.164
Leptin	0.63	0.016	0.79	0.001
L/A ratio	0.67	0.009	0.55	0.033

FDR, first degree relatives of type 2 diabetes; BMI, body mass index; L/A, leptin to adiponectin.

diabetes. Studies in various populations such as those by Liu et al. [3] and Akbarzadeh et al. [21] have reported lower adiponectin serum levels in the FDR group compared to non-FDR group as well. Meanwhile Lihn et al. did not find any difference in serum adiponectin levels, but only a lower levels of mRNA expression in adipose tissue [13]. Lihn included relatively younger subjects compared to Liu and Akbarzadeh, but older than our subjects. The conflicting results by Lihn might be due to its relatively small sample size compared to our study and to that of studies by Liu and Akbarzadeh. In addition, Lihn did not conduct further adjustment to potentially confounding factors.

We observed a slightly higher leptin level in the FDR group, however, it did not reach statistical significance. A previous study by Shahid et al. among young adult FDR subjects, aged 15–25 years, showed that subjects with parents living with type 2 diabetes had significant higher leptin level than non-FDR subjects [14]. In contrast, Moran et al. did not find any significant difference in baseline leptin levels between FDR and non-FDR group [22]. The inconsistency observed between those studies might be explained by the fact that BMI among FDR group in the study by Shahid was higher in comparison to non-FDR group, while the fat mass in the study by Moran was similar between FDR and non-FDR group, which is similar to our finding. Further analysis in our study revealed that leptin level was significantly higher in non-obese FDR compared to non-obese non-FDR but became not significant after adjusting for age, sex, anthropometric and metabolic profiles. This might be caused by small size of sample after stratification by BMI. As has been explained before by Margetic et al. obesity itself strongly increases leptin levels and there was one study discovering euglycemic

hyperinsulinemia increased leptin secretion only in lean rats, not in obese rats which already had significantly higher baseline leptin level [23]. In line with Nyholm et al. percentage body fat and sex determined leptin levels, but not family history of type 2 DM [24]. Additionally, correlation analysis between body fat percentage and leptin and L/A ratio in our study showed that body fat affected adipokines similarly. These suggested that FDR status seems to affect leptin, but when obesity has occurred, the renowned effect of obesity to leptin level is more prominent than that of FDR status.

While adiponectin and leptin have been shown to have an opposite effect, L/A ratio has been suggested as a better marker to see the balance of these two antagonistic adipokines, as L/A ratio was associated stronger with risk of type 2 diabetes, correlated independently stronger with carotid intima media thickness, and was better independent predictor to first cardiovascular event than adiponectin or leptin alone [20,25,26]. However, there was only one study investigating L/A ratio in FDR of type 2 diabetes subjects. A study by Abdullah K et al. found significantly lower L/A ratio in FDR subjects compared to type 2 diabetes subjects. However, compared to healthy control subjects, the L/A ratio of FDR subjects was not significantly higher [27]. In line with Abdullah et al. L/A ratio in our study had shown higher trend compared to non-FDR but failed to reach statistical significance. However, in subgroup analysis, FDR with BMI <25 kg/m^2 showed significantly higher value of L/A ratio compared to that of non-FDR even after adjusting for age, sex, anthropometric and metabolic profiles. This result showed that L/A ratio might potentially be a better marker of increased risk in FDR than adiponectin and leptin alone before obesity occurs, as leptin and adiponectin alone had not shown significant difference.

Another adipokine, A-FABP, was also associated with obesity, HOMA-IR, and was significantly higher in patients with type 2 diabetes compared to normal subjects [28]. As intracellular lipid chaperones mainly expressed in adipose tissue and macrophages, A-FABP also interacts with hormone-sensitive lipase to modulate its catalytic activity and is associated with some inflammatory responses through JNK/inhibitor of kappa kinase (IKK) [29]. In obesity-induced oxidative stress condition, A-FABP affinity shifts to preferably bind palmitic acid, a saturated fatty acid, instead of polyunsaturated fatty acids. This bond increases inflammatory responses such as TNFα–NF–κB signalling pathways which is related to insulin resistance leading to type 2 diabetes [30]. In a study including 408 Chinese subjects without diabetes, it was found that high baseline A-FABP was predictive of type 2 diabetes, independent of obesity, insulin resistance, or glycemic indexes with relative risk of 2.25 [31]. While in FDR population, Hu et al. reported a significantly higher A-FABP level compared to non-FDR group [32]. Our study, also observed a higher level of A-FABP in the FDR group, however it did not reach statistical significance. This might be due to a relatively low power of our study in comparison to the study by Hu et al. In addition, our study also limited the age and BMI of participants, excluding severely obese participants. We did subgroup analysis in subjects with BMI <23 kg/m^2 (data not shown) and it showed significantly higher A-FABP in FDR subjects compared to non-FDR. This might suggest that FDR status already causes metabolic alteration, reflected in higher A-FABP level, before obesity occurs. But when obesity already exists, the stronger influence of obesity subverts the effect of FDR status. This is supported by Cabre et al. who found that A-FABP was positively correlated with BMI and was strikingly higher in diabetes subjects with metabolic syndrome, whose BMI was significantly higher, compared to diabetes subject without metabolic syndrome and to healthy control subjects [33].

Present study suggested that significant independent association between FDR status and circulating level of adiponectin had been observed in young normoglycemic-normotensive population alongside the increase in HOMA-IR and insulin levels in FDR population compared to those of non-FDR with similar metabolic and anthropometric measure. Development of hyperinsulinemia or insulin resistance may potentially dysregulate adiponectin gene expression, increase leptin gene expression and secretion especially in lean subjects, promote A-FABP gene transcription and prevent downregulation of A-FABP secretion from fat cells [23,32,34].

There are limitations to our study. Firstly, this is a cross-sectional study. Therefore, this study did not show a cause-effect relationship. Secondly, we restricted BMI of subjects and this might affect adipokines levels so that we did not find significant difference in other adipokines levels between FDR and non-FDR in general. Thirdly, we did not evaluate pro-inflammatory cytokines which also have potential as early predictor of type 2 diabetes development.

In summary, by having a proper control group, we observed that parental history of type 2 diabetes was independently associated with adiponectin level. Furthermore, higher L/A ratio was more pronounced in non-obese FDR than those of non-FDR subjects, suggesting that despite similar effect of body fat to adipokines, FDR status may already play a role in the development of adipokines dysregulation before obesity occurs. Thus, well-powered larger prospective studies are needed to further confirm our findings and to establish causal relationship between adipokines dysregulation, insulin resistance, and type 2 diabetes in FDR population.

Author contribution statement

Dyah Purnamasari: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Cindya Klarisa Simanjuntak: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Christian Tricaesario: Analyzed and interpreted the data; Wrote the paper.

Dicky Levenus Tahapary, Dante Saksono Harbuwono, Em Yunir: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

Funding statement

Dr. Dyah Purnamasari was supported by Kementerian Riset Teknologi Dan Pendidikan Tinggi Republik Indonesia {NKB-2776/UN2.RST/HKP.05.00/2020}.

Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors thank Yesinta Diandra and Cut Neubi Getha for their assistance in the subject recruitment process and Tika Pradnjaparamita for performing the ELISA.

This work was supported by PUPTN 2020 Grant by Ministry of Research, Technology, and Higher Education of Republic of Indonesia [NKB-2776/UN2.RST/HKP.05.00/2020].

References

[1] Y. Zhang, A.O.Y. Luk, E. Chow, G.T.C. Ko, M.H.M. Chan, M. Ng, et al., High risk of conversion to diabetes in first-degree relatives of individuals with young-onset type 2 diabetes: a 12-year follow-up analysis, Diabet. Med. 34 (2017) 1701–1709, https://doi.org/10.1111/dme.13516.
[2] R.A. DeFronzo, E. Ferrannini, L. Groop, R.R. Henry, W.H. Herman, J.J. Holst, et al., Type 2 diabetes mellitus, Nat. Rev. Dis. Prim. 1 (2015), 15019, https://doi.org/10.1038/nrdp.2015.19.
[3] J. Liu, F. Wang, Y. Cha, Z. Chen, H. Ding, Adiponectin levels in non-obese first-degree relatives of type 2 diabetes patients and non-diabetic subjects: a 5-year follow-up study, J. Int. Med. Res. 38 (2010) 792–802, https://doi.org/10.1177/147323001003800306.
[4] G.R. Soodini, Adiponectin and leptin in relation to insulin sensitivity, Metab. Syndr. Relat. Disord. 2 (2004) 114–123, https://doi.org/10.1089/met.2004.2.114.
[5] Q. Zhuo, Z. Wang, P. Fu, J. Piao, Y. Tian, J. Xu, et al., Comparison of adiponectin, leptin and leptin to adiponectin ratio as diagnostic marker for metabolic syndrome in older adults of Chinese major cities, Diabetes Res. Clin. Pract. 84 (2009) 27–33, https://doi.org/10.1016/j.diabres.2008.12.019.
[6] J.-H. Yoon, J.K. Park, S.S. Oh, K.-H. Lee, S.-K. Kim, I.-J. Cho, et al., The ratio of serum leptin to adiponectin provides adjunctive information to the risk of metabolic syndrome beyond the homeostasis model assessment insulin resistance: the Korean Genomic Rural Cohort Study, Clin. Chim. Acta 412 (2011) 2199–2205, https://doi.org/10.1016/j.cca.2011.08.003.
[7] I. Simón, X. Escoté, N. Vilarrasa, J. Gómez, J.M. Fernández-Real, A. Megía, et al., Adipocyte fatty acid-binding protein as a determinant of insulin sensitivity in morbid-obese women, Obesity 17 (2009) 1124–1128, https://doi.org/10.1038/oby.2008.665.
[8] Y. Xiao, X. Xiao, A. Xu, X. Chen, W. Tang, Z. Zhou, Circulating adipocyte fatty acid-binding protein levels predict the development of subclinical atherosclerosis in type 2 diabetes, J. Diabet. Complicat. 32 (2018) 1100–1104, https://doi.org/10.1016/j.jdiacomp.2018.09.001.
[9] D. Purnamasari, R. Aulia, M.S. Abdaly, A. Hazim, Hypercholesterolemia as the first manifestation of metabolic abnormalities in normoglycemic young adult male with family history of type 2 diabetes mellitus, Diabetes Metabol. Syndr. 13 (2019) 969–974, https://doi.org/10.1016/j.dsx.2018.12.019.
[10] V. Mohan, V. Ruchi, R. Gayathri, M. Bai, V. Sudha, R. Anjana, et al., Slowing the diabetes epidemic in the world health organization south-east asia region: the role of diet and physical activity. WHO south-east asia, J. Public Health 5 (2016) 5–16, https://doi.org/10.4103/2224-3151.206554.
[11] A. Misra, U. Shrivastava, Obesity and dyslipidemia in south asians, Nutrients 5 (2013) 2708–2733, https://doi.org/10.3390/nu5072708.
[12] U. Lim, T. Ernst, S.D. Buchthal, M. Latch, C.L. Albright, L.R. Wilkens, et al., Asian women have greater abdominal and visceral adiposity than Caucasian women with similar body mass index, Nutr. Diabetes 1 (2011) e6, https://doi.org/10.1038/nutd.2011.2.
[13] A.S. Lihn, T. Østergård, B. Nyholm, S.B. Pedersen, B. Richelsen, O. Schmitz, Adiponectin expression in adipose tissue is reduced in first-degree relatives of type 2 diabetic patients, Am J Physiol Metab 284 (2003) E443–E448, https://doi.org/10.1152/ajpendo.00358.2002.
[14] A. Shahid, K. Lone, S. Saeed, M. Arslan, Male offspring of both diabetic parents have higher insulin resistance and serum leptin levels compared to those with one diabetic parent, Hormones (Basel) 7 (2008) 313–319, https://doi.org/10.14310/horm.2002.1212.
[15] K.F. Petersen, S. Dufour, D. Befroy, R. Garcia, G.I. Shulman, Impaired mitochondrial activity in the insulin-resistant offspring of patients with type 2 diabetes, N. Engl. J. Med. 350 (2004) 664–671, https://doi.org/10.1056/NEJMoa031314.
[16] K. Morino, K.F. Petersen, G.I. Shulman, Molecular mechanisms of insulin resistance in humans and their potential links with mitochondrial dysfunction, Diabetes 55 (2006), https://doi.org/10.2337/db06-S002. S9–15.
[17] A. Vaag, M. Lehtovirta, P. Thye-Rönn, L. Groop, Metabolic impact of a family history of Type 2 diabetes. Results from a European multicentre study (EGIR), Diabet. Med. 18 (2001) 533–540, https://doi.org/10.1046/j.1464-5491.2001.00496.x.
[18] J.H. Stern, J.M. Rutkowski, P.E. Scherer, Adiponectin, leptin, and fatty acids in the maintenance of metabolic homeostasis through adipose tissue crosstalk, Cell Metabol. 23 (2016) 770–784, https://doi.org/10.1016/j.cmet.2016.04.011.
[19] A. Bidulescu, P.C. Dinh, S. Sarwary, E. Forsyth, M.C. Luetke, D.B. King, et al., Associations of leptin and adiponectin with incident type 2 diabetes and interactions among African Americans: the Jackson heart study, BMC Endocr. Disord. 20 (2020) 31, https://doi.org/10.1186/s12902-020-0511-z.
[20] P. López-Jaramillo, D. Gómez-Arbeláez, J. López-López, C. López-López, J. Martínez-Ortega, A. Gómez-Rodríguez, et al., The role of leptin/adiponectin ratio in metabolic syndrome and diabetes, Horm. Mol. Biol. Clin. Invest. 18 (2014) 37–45, https://doi.org/10.1515/hmbci-2013-0053.
[21] S. Akbarzadeh, I. Nabipour, M. Assadi, A. Movahed, S.M. Jafari, N. Motamed, et al., The normoglycemic first-degree relatives of patients with type 2 diabetes mellitus have low circulating omentin-1 and adiponectin levels, Cytokine 58 (2012) 295–299, https://doi.org/10.1016/j.cyto.2012.02.005.
[22] C.N. Moran, N.D. Barwell, D. Malkova, S.J. Cleland, I. McPhee, C.J. Packard, et al., Effects of diabetes family history and exercise training on the expression of adiponectin and leptin and their receptors, Metabolism 60 (2011) 206–214, https://doi.org/10.1016/j.metabol.2009.12.026.
[23] S. Margetic, C. Gazzola, G. Pegg, R. Hill, Leptin: a review of its peripheral actions and interactions, Int. J. Obes. 26 (2002) 1407–1433, https://doi.org/10.1038/sj.ijo.0802142.
[24] B. Nyholm, S. Fisker, S. Lund, N. Møller, O. Schmitz, Increased circulating leptin concentrations in insulin-resistant first-degree relatives of patients with non-insulin-dependent diabetes mellitus: relationship to body composition and insulin sensitivity but not to family history of non-insulin-dependent d, Eur. J. Endocrinol. 136 (1997) 173–179, https://doi.org/10.1530/eje.0.1360173.
[25] G.D. Norata, S. Raselli, L. Grigore, K. Garlaschelli, E. Dozio, P. Magni, et al., Leptin:Adiponectin ratio is an independent predictor of intima media thickness of the common carotid artery, Stroke 38 (2007) 2844–2846, https://doi.org/10.1161/STROKEAHA.107.485540.
[26] P.J.W.H. Kappelle, R.P.F. Dullaart, A.P. van Beek, H.L. Hillege, B.H.R. Wolffenbuttel, The plasma leptin/adiponectin ratio predicts first cardiovascular event in men: a prospective nested case–control study, Eur. J. Intern. Med. 23 (2012) 755–759, https://doi.org/10.1016/j.ejim.2012.06.013.
[27] K. Abdullah, M. AL-Habori, E. Al-Eryani, Ramadan intermittent fasting affects adipokines and leptin/adiponectin ratio in type 2 diabetes mellitus and their first-degree relatives, BioMed Res. Int. 2020 (2020), https://doi.org/10.1155/2020/1281792.
[28] G. Niu, J. Li, H. Wang, Y. Ren, J. Bai, Associations of A-FABP with anthropometric and metabolic indices and inflammatory cytokines in obese patients with newly diagnosed type 2 diabetes, BioMed Res. Int. 2016 (2016), 9382092, https://doi.org/10.1155/2016/9382092.

[29] M. Furuhashi, G.S. Hotamisligil, Fatty acid-binding proteins: role in metabolic diseases and potential as drug targets, Nat. Rev. Drug Discov. 7 (2008) 489–503, https://doi.org/10.1038/nrd2589.
[30] M. Furuhashi, Fatty acid-binding protein 4 in cardiovascular and metabolic diseases, J. Atherosclerosis Thromb. 26 (2019) 216–232, https://doi.org/10.5551/jat.48710.
[31] A.W.K. Tso, A. Xu, P.C. Sham, N.M.S. Wat, Y. Wang, C.H.Y. Fong, et al., Serum adipocyte fatty acid–binding protein as a new biomarker predicting the development of type 2 diabetes, Diabetes Care 30 (2007) 2667–2672, https://doi.org/10.2337/dc07-0413.
[32] X. Hu, X. Pan, X. Ma, Y. Luo, Y. Xu, Q. Xiong, et al., Contribution of a first-degree family history of diabetes to increased serum adipocyte fatty acid binding protein levels independent of body fat content and distribution, Int. J. Obes. 40 (2016) 1649–1654, https://doi.org/10.1038/ijo.2016.147.
[33] A. Cabré, I. Lázaro, J. Girona, J.M. Manzanares, F. Marimón, N. Plana, et al., Fatty acid binding protein 4 is increased in metabolic syndrome and with thiazolidinedione treatment in diabetic patients, Atherosclerosis 195 (2007), https://doi.org/10.1016/j.atherosclerosis.2007.04.045 e150–8.
[34] A.S. Lihn, S.B. Pedersen, B. Richelsen, Adiponectin: action, regulation and association to insulin sensitivity, Obes. Rev. 6 (2005) 13–21, https://doi.org/10.1111/j.1467-789X.2005.00159.x.