Circulating levels of DDIT4 and mTOR, and contributions of BMI, inflammation and insulin sensitivity in hyperlipidemia

XINYAN PAN[1-3], ZHIMEI ZHANG[1,2], CHUNQIAO LIU[1,2], MING ZHAO[4], XING WANG[2], JUNQING ZHAI[5], CHAO WANG[2] and GUANGYAO SONG[1,2]

[1]Department of Internal Medicine, Hebei Medical University, Shijiazhuang, Hebei 050000; [2]Hebei Key Laboratory of Metabolic Diseases; [3]Department of Endocrinology; [4]Clinical Laboratory; [5]Hebei Provincial Physical Examination Center, Hebei General Hospital, Shijiazhuang, Hebei 050051, P.R. China

Received July 12, 2022; Accepted August 23, 2022

DOI: 10.3892/etm.2022.11602

Abstract. Evidence shows a high incidence of insulin resistance, inflammation and excess body mass index (BMI) in adults with hyperlipidemia. The present study aimed to determine the circulating levels of DNA damage inducible transcript 4 (DDIT4) and mTOR and assess the contributions of lipids, inflammatory markers, insulin sensitivity and BMI in hyperlipidemia. The study subjects were divided into a hyperlipidemia group and a normal control group (n=55 per group). Sex, age, blood pressure, waist circumference (WC), height, weight and BMI were recorded. Fasting venous blood samples were collected and an automatic biochemical analyzer was used to detect fasting blood glucose (FBG), fasting insulin (FINS), total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C). Quantitative ELISA kits were used to determine the levels of DDIT4, mTOR and inflammatory markers and calculate the homeostatic model assessment of insulin resistance (HOMA-IR). Compared with the normal control group, the hyperlipidemia group had significantly increased blood pressure, WC, weight, BMI, FBG, FINS, HOMA-IR, mTOR and inflammatory markers, but significantly reduced DDIT4. A concurrent correlation analysis showed that insulin resistance was positively correlated with blood pressure, BMI, lipid profiles (TG, TC, LDL-C), mTOR and inflammatory markers, but negatively correlated with HDL-C and DDIT4. Lipid profiles were positively correlated with BMI, mTOR and inflammatory markers, but negatively correlated with DDIT4. A factor analysis identified four domains in hyperlipidemia (inflammation-lipid 1 domain, 44.429%; overweight domain, 21.695%; insulin sensitivity domain, 11.782%; lipid 2 domain, 6.723%). In conclusion, people with hyperlipidemia have elevated mTOR and reduced DDIT4 and are accompanied by abnormal indicators such as insulin sensitivity, BMI and inflammatory factors. The identified domains may be applied to predict the outcomes of cardiovascular diseases and metabolic diseases in the future.

Correspondence to: Professor Guangyao Song, Hebei Key Laboratory of Metabolic Diseases, Hebei General Hospital, 348 Heping West Road, Shijiazhuang, Hebei 050051, P.R. China
E-mail sguangyao2@163.com

Abbreviations: DDIT4, DNA damage-inducible transcript 4; MCP-1, monocyte chemoattractant protein-1; TG, triglyceride; TC, total cholesterol; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; BMI, body mass index; FBG, fasting blood glucose; FINS, fasting insulin; HOMA-IR, homeostasis model assessment for insulin resistance index; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure

Key words: hyperlipidemia, insulin resistance, DNA damage-inducible transcript 4, mTOR, body mass index, inflammation

Introduction

Hyperlipidemia, a recognized risk factor for atherosclerosis, is closely related to insulin resistance and these two factors promote one another for the occurrence and development of diabetes (1).

In recent years, the incidence rate of hyperlipidemia has been increasing. In a survey on Chinese people aged >18 years, the total prevalence of hyperlipidemia was 40.4% (2). The study also found a considerable proportion of people with abnormal glucose metabolism who had not been diagnosed and treated in a timely manner among people with hyperlipidemia who had not yet experienced cardiovascular events, indicating that lipotoxicity is an important factor related to insulin resistance (3).

The mechanism for insulin resistance and glucose metabolism disorders in people with hyperlipidemia is not fully understood. mTOR is a key component of the mTOR signaling pathway that regulates cell growth and nutrient metabolism and is also involved in insulin resistance, adipose tissue physiological functions and body energy balance (4). Meanwhile, DNA damage inducible transcript 4 (DDIT4), an mTOR inhibitor (5), has roles in insulin signal transduction, hypoxic stress response, regulation of development and DNA damage (6-8), as well as a role in body metabolism. However, the relationships between DDIT4 and mTOR expression levels and hyperlipidemia and insulin resistance remain to be elucidated.

Several inflammatory markers are known to be related to blood lipid levels, atherosclerosis and insulin resistance, including IL-6, IL-1β, CRP, TNF-α, monocyte chemoattractant protein (MCP)-1, serum amyloid A, sCD40, adhesion molecules, chemokine-16, insulin-like growth factor, lipoprotein-associated phospholipase A2 and galectin-3, but their roles in hyperlipidemia and atherosclerosis are not well established (9,10). Inflammatory markers can act as independent risk factors for the development of diabetes and have a causal relationship with insulin resistance (11). Recent study has shown that Stem cell growth factor-beta (SCGF-β) levels were linked to insulin resistance and severity hepatic steatosis with the mediation role of CRP (12). Prediction of homeostatic model assessment (HOMA) values by SCGF-β levels, likely mediated by markers of inflammation, sheds some light on mechanisms inducing/worsening insulin resistance of male patients with obesity-related nonalcoholic fatty liver disease (NAFLD) (12). However, no studies have clarified the relationships between the expression levels of DDIT4 and mTOR and hyperlipidemia and the expression levels of inflammatory factors and insulin resistance.

The present study aimed to determine the changes in DDIT4, mTOR, inflammatory markers, circulating lipid profiles and insulin sensitivity in subjects with hyperlipidemia and heathy control subjects. It further sought to elucidate the relationships among DDIT4, mTOR, blood lipids, inflammation and insulin sensitivity and to clarify their contributions to metabolic risk using a factor analysis.

Materials and methods

Selection of subjects. Clinical samples were obtained from the Hebei Provincial Physical Examination Center and 55 subjects with elevated blood lipids in the physical examination population between May 2021 and October 2021 were randomly selected as the hyperlipidemia group (HL group; 29 men, 26 women; age: 46.25±11.75 years). A further 55 healthy subjects matched for age and sex with the subjects in the HL group were randomly selected as the normal control group (CON group; 28 men, 27 women; age: 45.47±12.45 years).

Selection basis of sample size was according to the method of sample size estimation in the experimental design of the comparison of two population means (the outcome indicator is quantitative) in the simple random sampling study:

$$n_1 = n_2 = 2\left[\frac{u_{\alpha/2} + u_{\beta}}{\delta/\sigma}\right]^2 + \frac{1}{4}u_{\alpha/2}{}^2$$

Set double test level α=0.05, β=0.15, 1-β= 0.85, δ/σ=0.05, 55 cases in each group.

The inclusion criteria for the HL group were: Hyperlipidemia diagnosed in accordance with the 2016 Guidelines for the Prevention and Treatment of Dyslipidemia in Chinese Adults (2) by elevated blood lipids meeting at least one of total cholesterol (TC) ≥6.2 mmol/l, triglyceride (TG) ≥2.3 mmol/l, low-density lipoprotein cholesterol (LDL-C) ≥4.1 mmol/l or high-density lipoprotein cholesterol (HDL-C) <1.0 mmol/l (13).

The inclusion criteria for the CON group were: No history of chronic diseases such as hypertension and diabetes; body mass index (BMI) 18.5-24 kg/m^2; blood glucose 3.9-6.1 mmol/l; blood lipids comprising TC <5.2 mmol/l, TG <1.7 mmol/l, LDL-C <3.4 mmol/l.

The exclusion criteria were: Dysfunction or abnormality of the heart, liver, kidney, or thyroid, combined with hypertension, diabetes, blood system diseases, mental diseases, acute and chronic infectious diseases, autoimmune diseases and tumor history; receipt of drugs, hormones and immunosuppressants that affect insulin sensitivity, or hypoglycemic, lipid-lowering and antihypertensive drugs within previous six months; pregnancy, lactation, or long-term oral contraceptive use; and recent surgical history.

Physical examination, specimen collection and ethics statement. All subjects filled out a questionnaire to collect data on their age, sex, history of present illness, medication history, allergy history, tobacco and alcohol history and family history. The subjects underwent a physical examination that included measurements of height, weight, waist circumference (WC), systolic blood pressure (SBP) and diastolic blood pressure (DBP) and calculation of BMI as weight (kg)/height squared (m^2). Overnight-fasting peripheral blood samples were obtained from all subjects. All subjects signed informed consent and the personal information was withheld. The study was approved by Hebei General Hospital Ethics Committee (approval no. 2021057; Shijiazhuang, China).

Biochemical index detection. An automatic biochemical analyzer [PML-AU5821; Beckman Coulter Commercial Enterprise (China) Co., Ltd.] was used to detect fasting blood glucose (FBG), fasting insulin (FINS), TC, TG, HDL-C and LDL-C. FBG (cat. no. AUZ8807), TC (cat. no. AUZ8916), TG (cat. no. AUZ8708), HDL-C (cat. no. AUZ8826) and LDL-C (cat. no. AUZ8744) were measured using kits from the Beckman Coulter Laboratory Systems (Suzhou) Co., Ltd. FINS (cat. no. 47675203) was measured using kits from Roche Diagnostics (Shanghai) Co., Ltd. The islet function evaluation index was calculated as follows: Homeostatic model assessment of insulin resistance (HOMA-IR)=FBG (mmol/l) x FINS (mIU/l)/22.5 (14). HOMA-IR ≥2.69 was set as the cut-off point for insulin resistance (15).

The serum concentrations of DDIT4 (cat. no. CSB-EL006590HU) and mTOR (cat. no. CSB-E09038h) were measured using ELISA kits from Cusabio according to manufacturer's protocol. The serum concentrations of CRP (cat. no. CHE0104), IL-6 (cat. no. CHE0009), TNF-α (cat. no. CHE0019) and MCP-1 (cat. no. CHE0103) were measured using ELISA kits from 4A Biotech Co., Ltd. in accordance with the manufacturer's instructions.

Statistical methods. All statistical analyses were performed using SPSS 22.0 software (IBM Corp.). Continuous variables were expressed as the mean ± standard deviation. For comparisons between the groups, unpaired Student's t-test was used for normally distributed data and the Mann-Whitney U test was used for non-normally distributed data. Correlations between parameters were evaluated by Pearson product moment correlation analysis. P<0.05 was considered to indicate a statistically significant difference.

A factor analysis was conducted using the principal component method with a Varimax rotation to examine

Table I. Comparisons of clinical characteristics and metabolic parameters between the two groups.

Clinical characteristics and metabolic parameters	CON group	HL group	t-value	P-value
n	55	55		
Male/female	28/27	29/26	-0.190	0.849
Age (years)	45.47±12.45	46.25±11.75	-0.339	0.735
SBP (mmHg)	114.42±11.83	129.49±15.40[a]	-5.756	<0.001
DBP (mmHg)	73.65±8.25	86.76±10.97[a]	-7.085	<0.001
WC (cm)	78.4±6.58	91.49±11.63[a]	-7.264	<0.001
Height (cm)	167.27±8.00	168.05±8.27	-0.504	0.615
Weight (kg)	61.78±7.40	76.24±14.91[a]	-6.439	<0.001
BMI (kg/m^2)	22.03±1.50	26.83±3.79[a]	-8.748	<0.001
TG (mmol/l)	0.96±0.30	3.98±2.15[a]	-10.291	<0.001
TC (mmol/l)	4.33±0.64	6.91±0.67[a]	-20.677	<0.001
HDL-C (mmol/l)	1.35±0.27	1.27±0.24	1.673	0.097
LDL-C (mmol/l)	2.63±0.45	4.43±0.72[a]	-15.763	<0.001
FBG (mmol/l)	5.26±0.41	5.94±1.28[a]	-3.770	<0.001
FINS (mIU/l)	7.04±3.51	13.89±8.73[a]	-5.407	<0.001
HOMA-IR	1.67±0.89	3.75±2.72[a]	-5.402	<0.001

[a]P<0.05 vs. CON group. CON group, normal control group; HL group, hyperlipidemia group; SBP, systolic blood pressure; DBP, diastolic blood pressure; WC, waist circumference; BMI, body mass index; TG, triglyceride; TC, total cholesterol; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; FBG, fasting blood glucose; FINS, fasting insulin; HOMA-IR, homeostasis model assessment for insulin resistance index.

whether insulin resistance was clustered with clinical characteristics, metabolic parameters and inflammatory variables. WC, weight, BMI, height, FBG, FINS, HOMA-IR, TG, TC, HDL-C, LDL-C, DDIT4, mTOR, IL-6, CRP, TNF-α and MCP-1 were included as variables in the factor analysis. Factor loading with an absolute value ≥±0.5 or was used as the cut-off value for data interpretation. The total variance explained by each factor was presented to indicate the individual effect of the factor in the analysis.

Results

Clinical characteristics and metabolic parameters of the study subjects. The study cohort comprised 55 subjects in the normal control group (CON group) and 55 subjects in the hyperlipidemia group (HL group). As shown in Table I, there were no significant differences between the two groups for male-to-female ratio, age and height (P>0.05). However, compared with the CON group, the HL group had significantly increased systolic blood pressure, diastolic blood pressure, waist circumference, weight and BMI (P<0.05).

The metabolic parameters are also shown in Table I. Compared with the CON group, the HL group had significantly increased plasma TC, TG and LDL-C levels (P<0.05). Meanwhile, the HL group had lower HDL-C than the CON group (P>0.05), but the difference was not significant.

The HL group had significantly increased FBG, FINS and HOMA-IR compared with the CON group. These data indicate a significant degree of insulin resistance in the HL group compared with the CON group. The clinical characteristics and metabolic parameters of the participants are summarized in Table I.

Levels of DDIT4, mTOR and inflammatory parameters. Compared with the CON group, the HL group had increased mTOR and decreased DDIT4 (P<0.05). The HL group also had significantly higher CRP, IL-6, TNF-α and MCP-1 compared with the CON group (Table II).

Correlations among clinical data, metabolic markers and inflammatory markers. A correlation analysis was performed for all lipid profiles, metabolic markers and inflammatory markers (Table III). The insulin resistance indexes (FINS, HOMA-IR) were found to be positively correlated with SBP, DBP, WC, weight, BMI, mTOR, lipid profiles (TG, TC, LDL-C) and inflammatory markers (CRP, IL-6, TNF-α, MCP-1), but negatively correlated with HDL-C and DDIT4 (P<0.05). Meanwhile, the lipid profiles (TG, TC, LDL-C) were found to be positively correlated with WC, weight, BMI, mTOR and inflammatory markers (CRP, IL-6, TNF-α, MCP-1), but negatively correlated with DDIT4 (P<0.05). HDL-C was negatively correlated with WC, weight, BMI and DDIT4, but positively correlated with mTOR. DDIT4 was negatively correlated with mTOR and inflammatory markers, while mTOR was positively correlated with inflammatory markers.

The present study conducted a factor analysis using the principal component method with a Varimax rotation to examine whether fasting insulin level was clustered with clinical data and metabolic risk factors among subjects with hyperlipidemia. The variables included in the factor analysis were WC, weight, BMI, height, FBG, FINS, HOMA-IR, TG, TC, HDL-C, LDL-C, DDIT4, mTOR, IL-6, CRP, TNF-α and MCP-1 (Table IV). In total, four domains were identified that could explain 84.629% of the total variance (domain 1: 44.429%; domain 2: 21.695%; domain 3: 11.782%; domain 4:

Table II. Comparisons of DDIT4, mTOR, and inflammatory factors between the two groups.

Factor	CON group	HL group	t-value	P-value
DDIT4 (ng/ml)	1.05±0.40	0.51±0.06[a]	9.734	<0.001
mTOR (ng/ml)	5.22±1.74	15.35±6.08[a]	11.879	<0.001
CRP (mg/l)	6.14±0.98	10.55±1.24[a]	20.693	<0.001
IL-6 (pg/ml)	8.21±0.93	16.93±1.82[a]	31.641	<0.001
TNF-α (pg/ml)	7.85±1.15	15.28±1.98[a]	24.065	<0.001
MCP-1 (pg/ml)	79.87±9.16	122.80±12.76[a]	20.269	<0.001

[a]P<0.05 vs. CON group. DDIT4, DNA damage-inducible transcript 4; CON group, normal control group; HL group, hyperlipidemia group; MCP-1, monocyte chemoattractant protein-1.

6.723%). The first domain was designated the inflammation-lipid 1 domain, in which IL-6, CRP, TNF-α, MCP-1, mTOR, TC and LDL-C were positively loaded and DDIT4 was negatively loaded. The second domain was denoted the overweight domain, in which weight, WC and BMI were positively loaded. The third domain was termed the insulin sensitivity domain, in which FBG, FINS and HOMA-IR were positively loaded. The fourth domain was called the lipid 2 domain, in which TG was positively loaded and HDL-C was negatively loaded.

Discussion

Insulin resistance is prevalent in people with hyperlipidemia (16) and is a common risk factor for diabetes and cardiovascular disease. Insulin resistance increases the risk of hyperlipidemia, leading to increased incidence of atherosclerosis, coronary heart disease and stroke (17,18) and the interaction of the two factors aggravates the occurrence of metabolic syndrome.

mTOR is a highly conserved serine/threonine kinase that can integrate growth-, stress- and nutrition-related signals and coordinate cell growth, proliferation and metabolism (19,20). Previous studies show that the mTOR signaling pathway functions by controlling downstream p70 ribosomal protein S6 kinase (p70S6K) and eukaryotic initiation factor phospho-eI F4E-binding protein 1 (p-4EBP1) (21) and participates in a variety of human diseases, including cancer, diabetes, obesity and neurodegenerative diseases (20). Other studies confirmed that mTOR is a key signaling component for regulation of insulin metabolism (22,23), in which mTOR and its downstream regulator p70S6K phosphorylate insulin receptor substrate (IRS)-1 on serine residues to block the activation of phosphatidylinositol 3-kinase (PI3K) (22,24), while inhibition of the mTOR pathway can improve glucose and lipid metabolism disorders. The present study confirmed that serum mTOR was significantly higher in the HL group compared with the CON group and that high mTOR expression was positively correlated with blood glucose, blood lipids, insulin resistance and inflammatory markers, indicating that mTOR may have roles in glucose and lipid metabolism disorders, insulin resistance and inflammation, consistent with the related reports. In animal experiments, mTOR phosphorylation is found to be increased in obese mice fed a high-fat and high-sugar diet (25). Furthermore, when the mTOR pathway is negatively regulated, it promotes hepatocyte autophagy and improves lipid metabolism disorders in obese mice (25). Meanwhile, mTOR expression is increased in a rat model of NAFLD and a corn silk aqueous extract alleviates high-fat diet-induced NAFLD and its mechanism of action may be related to inhibition of PI3K/Akt/mTOR pathway activation (26).

DDIT4, also known as RTP801/Dig2/REDD1, negatively regulates mTOR activity and serves important roles in oxidative metabolism and insulin-stimulated signaling (6-8,27). Dungan and Williamson (28,29) and other researchers (30) demonstrate that DDIT4 knockout or deletion decreased insulin-stimulated tyrosine phosphorylation of IRS-1, leading to the development of insulin resistance. Regazzetti *et al* (30) report that reduced DDIT4 levels following gene knockdown limits insulin-stimulated activation of IRS-1 and Akt. Decreased DDIT4 protein expression overactivates basal mTORC1 signaling, which produces negative feedback and inhibits IRS-1, thereby suppressing signaling responses to insulin (31). In DDIT4 KO mice, inhibition of mTORC1 signaling following rapamycin treatment limits negative feedback on IRS-1 and mitogen-activated protein kinase kinases (MEK) 1/2 activation, thereby enhancing signaling responses to insulin (28,31). In the present study, serum DDIT4 was significantly lower than in the HL group compared with the CON group and DDIT4 was negatively correlated with blood glucose, blood lipids, insulin resistance and inflammatory markers, indicating that DDIT4 overexpression may be beneficial for alleviation of glucose and lipid metabolism, insulin resistance and inflammation. The present results are basically consistent with the findings of Wang *et al* (32) and Yang *et al* (33). These researchers demonstrated that DDIT4 expression is decreased in cells cultured in the presence of high glucose, suggesting that the occurrence and development of diabetic nephropathy may be related to DDIT4 and DNA damage. It is also reported that DDIT4 expression is reduced in rat renal tubular epithelial cells cultured under high glucose conditions, while overexpressed DDIT4 can alleviate DNA damage caused by hyperglycemia (34).

Some studies found that certain drugs interfere with the DDIT4/mTOR pathway to improve adverse metabolic responses to high sugar, high fat, or stress (35-38), which inspired the present study. A study confirmed that salidroside is able to protect human umbilical vein endothelial

Table III. Correlations among clinical data and metabolic and inflammatory parameters.

P-value/r	Age	SBP	DBP	WC	Height	Weight	BMI	TG	TC	HDL-C	LDL-C	FBG	FINS	HOMA-IR	DDIT4	mTOR	CRP	IL-6	TNF-α	MCP-1
Age	-	0.088[a]	-0.095[a]	-0.236[a]	-0.534[b]	-0.377[b]	-0.149[a]	0.057[a]	0.144[a]	0.262[b]	0.079[a]	0.168[a]	-0.277[a]	-0.215[a]	-0.235[b]	0.133[b]	0.125[a]	0.091[a]	0.107[a]	0.135[a]
SBP	0.360[a]	-	0.820[b]	0.537[b]	0.202[a]	0.464[b]	0.461[b]	0.373[b]	0.441[b]	-0.203[a]	0.413[a]	0.115[a]	0.287[b]	0.277[b]	-0.332[a]	0.360[b]	0.417[b]	0.447[b]	0.433[b]	0.414[a]
DBP	0.323[a]	0.000[b]	-	0.615[b]	0.202[a]	0.572[b]	0.589[b]	0.410[b]	0.504[b]	-0.234[a]	0.497[a]	0.050[b]	0.424[a]	0.370[b]	-0.417[a]	0.386[b]	0.483[b]	0.514[b]	0.494[b]	0.483[a]
WC	0.013[a]	0.000[b]	0.000[b]	-	0.460[b]	0.924[b]	0.888[b]	0.355[b]	0.474[b]	-0.263[b]	0.491[a]	0.154[b]	0.585[b]	0.54[a]	-0.396[b]	0.344[b]	0.461[b]	0.504[b]	0.480[b]	0.456[a]
Height	0.000[b]	0.034[a]	0.034[a]	0.000[b]	-	0.625[b]	0.178[a]	0.018[a]	-0.039[a]	-0.218[a]	-0.004[b]	-0.154[a]	0.164[a]	0.111[b]	0.129[a]	-0.033[b]	-0.038[a]	-0.003[a]	-0.016[a]	-0.039[b]
Weight	0.000[b]	0.000[b]	0.000[b]	0.000[b]	0.000[b]	-	0.875[b]	0.323[b]	0.403[b]	-0.287[b]	0.427[b]	0.099[b]	0.628[b]	0.568[b]	-0.320[b]	0.276[a]	0.403[b]	0.447[b]	0.424[b]	0.393[b]
BMI	0.120[a]	0.000[b]	0.000[b]	0.000[b]	0.062[a]	0.000[b]	-	0.404[b]	0.536[b]	-0.247[b]	0.549[a]	0.229[b]	0.694[b]	0.653[b]	-0.479[a]	0.372[b]	0.536[a]	0.573[b]	0.551[b]	0.525[a]
TG	0.553[a]	0.000[b]	0.000[b]	0.000[b]	0.852[a]	0.001[b]	0.000[b]	-	0.606[b]	-0.356[b]	0.461[a]	0.302[b]	0.437[b]	0.444[b]	-0.475[a]	0.485[b]	0.598[b]	0.645[a]	0.620[b]	0.593[a]
TC	0.133[a]	0.000[b]	0.000[b]	0.000[b]	0.685[a]	0.000[b]	0.000[b]	0.000[b]	-	0.075[a]	0.943[a]	0.310[b]	0.410[b]	0.405[b]	-0.861[a]	0.923[b]	0.979[b]	0.967[b]	0.974[a]	0.972[a]
HDL-C	0.006[b]	0.034[a]	0.014[a]	0.005[b]	0.022[a]	0.002[b]	0.009[b]	0.000[b]	0.435[a]	-	-0.026[b]	-0.145[a]	-0.326[a]	-0.325[b]	-0.211[a]	0.131[b]	0.066[b]	-0.020[b]	0.024[a]	0.071[b]
LDL-C	0.412[a]	0.000[a]	0.000[a]	0.000[a]	0.968[b]	0.000[b]	0.000[a]	0.000[a]	0.000[a]	0.788[b]	-	0.269[b]	0.410[b]	0.393[b]	-0.794[a]	0.882[b]	0.925[b]	0.913[b]	0.921[b]	0.916[a]
FBG	0.080[a]	0.230[a]	0.605[b]	0.108[b]	0.107[a]	0.306[b]	0.016[b]	0.001[b]	0.001[b]	0.130[a]	0.004[b]	-	0.318[a]	0.543[a]	-0.289[a]	0.237[a]	0.303[a]	0.309[b]	0.303[b]	0.286[a]
FINS	0.003[a]	0.002[b]	0.000[a]	0.000[b]	0.087[a]	0.000[b]	0.000[b]	0.000[b]	0.000[b]	0.001[a]	0.000[b]	0.001[a]	-	0.956[b]	-0.358[a]	0.307[b]	0.408[b]	0.423[b]	0.412[b]	0.389[b]
HOMA-IR	0.024[a]	0.003[b]	0.000[b]	0.000[a]	0.248[b]	0.000[b]	0.000[b]	0.000[b]	0.000[b]	0.001[b]	0.000[b]	0.000[a]	0.000[b]	-	-0.35[a]	0.305[b]	0.402[b]	0.418[b]	0.407[b]	0.383[a]
DDIT4	0.014[b]	0.000[a]	0.000[a]	0.000[b]	0.178[a]	0.001[b]	0.000[a]	0.000[a]	0.000[a]	0.027[a]	0.000[a]	0.002[a]	0.000[a]	0.000[a]	-	-0.708[b]	-0.833[b]	-0.774[b]	-0.799[b]	-0.825[a]
mTOR	0.165[b]	0.000[b]	0.000[b]	0.000[b]	0.728[b]	0.004[a]	0.000[b]	0.000[b]	0.000[b]	0.174[b]	0.000[b]	0.013[a]	0.001[b]	0.001[b]	0.000[b]	-	0.917[b]	0.899[b]	0.916[b]	0.919[a]
CRP	0.194[a]	0.000[b]	0.000[b]	0.000[b]	0.693[a]	0.000[b]	0.000[a]	0.000[b]	0.000[b]	0.495[b]	0.000[b]	0.001[a]	0.000[b]	0.000[b]	0.000[b]	0.000 [b]	-	0.984[b]	0.994[b]	0.995[a]
IL-6	0.346[a]	0.000[b]	0.000[b]	0.000[b]	0.978[a]	0.000[b]	0.000[b]	0.000[a]	0.000[b]	0.838[b]	0.000[b]	0.001[b]	0.000[b]	0.000[b]	0.000[b]	0.000[b]	0.000[b]	-	0.994[b]	0.984[a]
TNF-α	0.264[a]	0.000[b]	0.000[b]	0.000[b]	0.870[a]	0.000[b]	0.000[b]	0.000[b]	0.000[a]	0.804[a]	0.000[b]	0.001[b]	0.000[b]	0.000[b]	0.000[b]	0.000[b]	0.000[b]	0.000[b]	-	0.994[a]
MCP-1	0.161[a]	0.000[a]	0.000[a]	0.000[a]	0.687[b]	0.000[b]	0.000[a]	0.000[a]	0.000[a]	0.464[b]	0.000[a]	0.002[a]	0.000[b]	0.000[a]	0.000[a]	0.000[a]	0.000[a]	0.000[a]	0.000[a]	-

The lower left portion of the table shows the P-values; the upper right area shows the positive or negative correlation coefficients. [a]Significantly correlated at the 0.05 level (two-sided); [b]significantly correlated at the 0.01 level (two-sided). SBP, systolic blood pressure; DBP, diastolic blood pressure; WC, waist circumference; BMI, body mass index; TG, triglyceride; TC, total cholesterol; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; FBG, fasting blood glucose; FINS, fasting insulin; HOMA-IR, homeostasis model assessment for insulin resistance index; DDIT4, DNA damage-inducible transcript 4; MCP-1, monocyte chemoattractant protein-1.

Table IV. Results of the principal component factor analysis with a Varimax rotation among subjects with hyperlipidemia.

Variable	Factor 1	Factor 2	Factor 3	Factor 4
IL-6	0.970[a]	-0.099	-0.069	-0.048
CRP	0.969[a]	-0.078	-0.045	-0.040
TNF-α	0.965[a]	-0.084	-0.052	-0.039
DDIT4	-0.960[a]	0.036	0.015	0.036
MCP-1	0.949[a]	-0.098	-0.087	-0.047
mTOR	0.947[a]	-0.119	-0.036	-0.063
TC	0.925[a]	-0.090	-0.039	-0.072
LDL-C	0.716[a]	0.060	0.065	-0.341
Weight	-0.122	0.978[a]	0.023	-0.020
WC	-0.119	0.924[a]	0.055	-0.083
BMI	-0.177	0.843[a]	0.297	-0.124
Height	0.014	0.711[a]	-0.389	0.140
HOMA-IR	0.009	0.468	0.805[a]	0.237
FBG	-0.089	-0.221	0.768[a]	-0.015
FINS	0.028	0.593	0.638[a]	0.267
TG	-0.081	-0.107	0.048	0.936[a]
HDL-C	0.371	-0.133	-0.355	-0.566[a]
% Total variance (%)	44.429	21.695	11.782	6.723
% Cumulative variance	44.429	66.124	77.906	84.629

[a]Loadings ≥0.5. WC, waist circumference; BMI, body mass index; TG, triglyceride; TC, total cholesterol; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; FBG, fasting blood glucose; FINS, fasting insulin; HOMA-IR, homeostasis model assessment for insulin resistance index; DDIT4, DNA damage-inducible transcript 4; MCP-1, monocyte chemoattractant protein-1.

cells against H_2O_2-induced apoptosis by activating the PI3K/Akt/mTOR-dependent pathway and inhibiting ROS through activation of DDIT4 (35). Other studies found that increased expression of activator of transcription 4 (ATF4) is sufficient to promote DDIT4 transcription, thereby inhibiting expression of mTORC1 and alleviating endoplasmic reticulum stress (36,37). These antioxidant and anti-stress effects of DDIT4 may serve synergistic roles in insulin resistance in people with hyperlipidemia. Meanwhile, 1,25-dihydroxyvitamin D3 inhibits high glucose-induced rat mesangial cell proliferation through the DDIT4/mTOR signaling pathway, thereby exerting a renoprotective effect in diabetes (38). Therefore, the role of the DDIT4/mTOR pathway in insulin resistance caused by high glucose and high fat is worthy of further investigation.

Hyperlipidemia is a known risk factor for atherosclerosis (39). Studies have demonstrated that inflammatory responses can be triggered by the formation, development and even complications of hyperlipidemia and that the activity and expression of various inflammatory factors, such as CRP, MCP-1, TNF-α and IL-6, are simultaneously enhanced (9,40). Another study showed that increased blood CRP levels can lead to glucose and lipid metabolism disorders and promote the occurrence of atherosclerosis (41). A change in the CRP index level is more meaningful than a change in LDL-C in cardiovascular diseases. The present study found that the inflammatory state and insulin resistance were significantly increased in the HL group compared with the CON group. The correlation analysis further revealed that insulin resistance (fasting insulin level and HOMA-IR) was positively correlated with blood pressure, BMI, blood lipids (TG, TC and LDL-C), inflammatory markers (CRP, IL-6, MCP-1 and TNF-α) and mTOR, but negatively correlated with HDL-C and DDIT4. In addition, inflammatory markers were positively correlated with blood lipids (TG, TC and LDL-C), BMI and mTOR, but negatively correlated with DDIT4. These findings reflected a higher incidence of metabolic syndrome, inflammation and excess body weight in the subjects in the HL group.

We have attempted to build a model that can be applied to clinical events from experimental data, but we have not found significant linear or non-linear correlations between the data. We consulted the references and a factor analysis was conducted to help with understanding of the metabolic, inflammatory and lipid variables in metabolic syndrome, which are influenced by disease, participant characteristics and other specific variables (42). A previous study assessed the risk domains in metabolic syndrome and cardiovascular events by factor analysis (43). Other studies confirmed that elevated blood lipids were associated with coronary heart disease morbidity and mortality (44), while elevated serum IL-6 and CRP levels were associated with development of type 2 diabetes (45). Among the variables, the present study attempted to elucidate the contributions of insulin sensitivity, blood lipids, body mass index and inflammatory factors in hyperlipidemia and conducted a factor analysis to determine the factor structures of these variables in hyperlipidemia. The results identified four domains that could explain 84.629% of the total variance among the subjects with hyperlipidemia:

inflammation-lipid 1 domain, overweight domain, insulin sensitivity domain and lipid 2 domain. TC and TG belonged to different domains, indicating that the effects of these indicators were different and not fully synergistic in hyperlipidemia. The major strength of the present study is its comprehensive analysis of the contributions of various physiological indexes in hyperlipidemia by the factor analysis. Further dimensionality reduction analysis of these variables could be applied to future predictions and analyses of diseases, such as diabetes, coronary heart disease and atherosclerosis, in people with hyperlipidemia. However, the smaller sample size is a limitation of the present study. Future research will continue to increase the number of enrolled cases and improve the data to support the results demonstrated.

In conclusion, blood lipid levels in adults warrant special early attention because insulin resistance, inflammation, blood lipids and abnormal body weight can increase the risk of cardiovascular disease and diabetes in adults. Serum DDIT4 and mTOR levels are closely related to blood lipid levels, insulin resistance and inflammatory status and may be potential predictors of hyperlipidemia. The present study also found that inflammatory-lipid 1, insulin sensitivity, overweight and lipid 2 domains predominantly exist in the data of people with hyperlipidemia. These domains may be useful to predict the outcomes of diabetes and cardiovascular diseases in the future.

Acknowledgements

Not applicable.

Funding

No funding was received.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

XP and GS conceived and designed the study. XP, ZZ, CL, MZ, XW, JZ and CW acquired and analyzed the data. XP, CW and GS confirm the authenticity of the raw data. XP prepared the draft of the manuscript. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

All subjects provided signed informed consent. The study was approved by Hebei General Hospital Ethics Committee (approval no. 2021057).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Tomlinson B, Chan P and Lam CW: Postprandial hyperlipidemia as a risk factor in patients with type 2 diabetes. Expert Rev Endocrinol Metab 15: 147-157, 2020.
2. Zhu JR, Gao RL and Zhao SP: Guidelines for the prevention and treatment of dyslipidemia in Chinese adults (Revised edition 2016). Chinese Circulation Journal 31: 937-953, 2016 (in Chinese).
3. Wang L, Lu MY, Ren JY and Chen H: Correlation study on different dyslipidemia classification and glucose metabolism in patients with hyperlipidemia. Beijing Da Xue Xue Bao Yi Xue Ban 43: 427-431, 2011 (In Chinese).
4. Koundouros N and Blenis J: Targeting mTOR in the context of diet and whole-body metabolism. Endocrinology 163: bqac041, 2022.
5. Dennis MD, Coleman CS, Berg A and Jefferson LS and Kimball SR: REDD1 enhances protein phosphatase 2A-mediated dephosphorylation of Akt to repress mTORC1 signaling. Sci Signal 7: ra68, 2014.
6. Ellisen LW, Ramsayer KD, Johannessen CM, Yang A, Beppu H, Minda K, Oliner JD, McKeon F and Haber DA: REDD1, a developmentally regulated transcriptional target of p63 and p53, links p63 to regulation of reactive oxygen species. Mol Cell 10: 995-1005, 2002.
7. Kimball SR, Do AN, Kutzler L, Cavener DR and Jefferson LS: Jefferson LS rapid turnover of the mTOR complex 1 (mTORC1) repressor REDD1 and activation of mTORC1 signaling following inhibition of protein synthesis. Biol Chem 283: 3465-3475, 2008.
8. Katiyar S, Liu E, Knutzen CA, Lang ES, Lombardo CR, Sankar S, Toth JI, Petroski MD, Ronai Z and Chiang GG: REDD1, an inhibitor of mTOR signalling, is regulated by the CUL4A-DDB1 ubiquitin ligase. EMBO Rep 10: 866-872, 2009.
9. Papapanagiotou A, Siasos G, Kassi E, Gargalionis AN and Papavassiliou AG: Novel inflammatory markers in hyperlipidemia: Clinical implications. Curr Med Chem 22: 2727-2743, 2015.
10. Siasos G, Tousoulis D, Oikonomou E, Zaromitidou M, Stefanadis C and Papavassiliou AG: Inflammatory markers in hyperlipidemia: From experimental models to clinical practice. Curr Pharm Des 17: 4132-4146, 2011.
11. Butani L, Dharmar M, Devaraj S and Jialal I: Preliminary report of inflammatory markers, oxidative stress, and insulin resistance in adolescents of different ethnicities. Metab Syndr Relat Disord 14: 182-186, 2016.
12. Tarantino G, Citro V, Balsano C and Capone D: Could SCGF-beta levels be associated with inflammation markers and insulin resistance in male patients suffering from obesity-related NAFLD?. Diagnostics (Basel) 10: 395, 2020.
13. Chinese Adult Dyslipidemia Prevention and Treatment Guidelines Revision Joint Committee: Guidelines for the prevention and treatment of dyslipidemia in Chinese adults (Revised edition 2016). Chinese Journal of General Practitioners 16: 15-35, 2017 (in Chinese).
14. Sun J, Du Q and Wang GP: Research on β cell function and insulin resistance in patients with type 2 diabetes mellitus. Chin J Diabetes 23: 592-594, 2015.
15. Xiao-Yan X, Wen-Ying Y and Zhao-Jun Y: The diagnostic significance of homeostasis model assessment of insulin resistance in metabolic syndrome among subjects with different glucose tolerance. Chinese J Diabetes Mellitus 2004: 31-35, 2004.
16. Yuan H and Yuan F: Study on the relationship between pure hyperlipidemia and insulin resistance index. Chinese Journal of Practical Internal Medicine 27: 246-247, 2007 (in Chinese).
17. Qiu LQ: Comparison of clinical effects of two statins in the treatment of primary hyperlipidemia. Guide of China Medicine 9: 85-86, 2011 (in Chinese).
18. Wang XG and Zhao X: Research progress of pathogenesis and treatment of hyperlipidemia. Journal of Liaoning University of Traditional Chinese Medicine 22: 196-200, 2020 (in Chinese).
19. Schmelzle T and Hall MN: TOR, a central controller of cell growth. Cell 103: 253-262, 2000.
20. Sudarsanam S and Johnson DE: Functional consequences of mTOR inhibition. Curr Opin Drug Discov Devel 13: 31-40, 2010.
21. Laplante M and Sabatini DM: mTOR signaling in growth control and disease. Cell 149: 274-293, 2012.
22. Carlson CJ, White MF and Rondinone CM: Mammalian target of rapamycin regulates IRS-1 serine 307 phosphorylation. Biochem Biophys Res Commun 316: 533-539, 2004.
23. Sarbassov DD, Ali SM and Sabatini DM: Growing roles for the mTOR pathway. Curr Opin Cell Biol 17: 596-603, 2005.

24. Ozes ON, Akca H, Mayo LD, Gustin JA, Maehama T, Dixon JE and Donner DB: A phosphatidylinositol 3-kinase/Akt/mTOR pathway mediates and PTEN antagonizes tumor necrosis factor inhibition of insulin signaling through insulin receptor substrate-1. Proc Natl Acad Sci USA 98: 4640-4645, 2001.
25. Ting H, Zhen-Zhen L, Yang B and Hui-Shuang L: Probiotics improve lipid metabolism of obese mice induced by high-fat and high-sucrose diet. Basic Clin Med 41: 1260-1265, 2021.
26. Ding L and Liu YC: Study on improving nonalcoholic fatty liver disease of high fat diet induced rats by corn silk aqueous extract. China Food Additives 32: 51-57, 2021 (in Chinese).
27. Gordon BS, Williamson DL, Lang CH, Jefferson LS and Kimball SR: Nutrient-induced stimulation of protein synthesis in mouse skeletal muscle is limited by the mTORC1 repressor REDD1. J Nutr 145: 708-713, 2015.
28. Dungan CM and Williamson DL: Regulation of skeletal muscle insulin-stimulated signaling through the MEK-REDD1-mTOR axis. Biochem Biophys Res Commun 482: 1067-1072, 2017.
29. Dungan CM, Wright DC and Williamson DL: Lack of REDD1 reduces whole body glucose and insulin tolerance, and impairs skeletal muscle insulin signaling. Biochem Biophys Res Commun 453: 778-783, 2014.
30. Regazzetti C, Dumas K, Marchand-Brustel YL, Peraldi P, Tanti JF and Giorgetti-Peraldi S: Regulated in development and DNA damage responses -1 (REDD1) protein contributes to insulin signaling pathway in adipocytes. PLoS One 7: e52154, 2012.
31. Sarbassov DD, Ali SM, Sengupta S, Sheen JH, Hsu PP, Bagley AF, Markhard AL and Sabatini DM: Prolonged rapamycin treatment inhibits mTORC2 assembly and Akt/PKB. Mol Cell 22: 159-168, 2006.
32. Wang H, Wang JM, Qu H, Wei H, Ji B, Yang Z, Wu J, He Q, Luo Y, Liu D, *et al*: In vitro and in vivo inhibition of mTOR by 1,25-dihydroxyvitamin D3 to improve early diabetic nephropathy via the DDIT4/TSC2/mTOR pathway. Endocrine 54: 348-359, 2016.
33. Yang Z, Liu F, Qu H, Wang H, Xiao X and Deng H: $1,25(OH)_2D3$ protects cell against high glucose-induced apoptosis through mTOR suppressing. Mol Cell Endocrinol 414: 111-119, 2015.
34. Liang D, Zhao SQ, Li ZY, Xiao YW, Ding J, Xiao Y and Guo B: miR-22 promotes DNA damage of renal tubular epithelial cells with diabetic kidney disease by inhibiting DDIT4. Chin J Pathophysiol 37: 1933-1941, 2021 (In Chinese).
35. Xu MC, Shi HM, Wang H and Gao XF: Salidroside protects against hydrogen peroxide-induced injury in HUVECs via the regulation of REDD1 and mTOR activation. Mol Med Rep 8: 147-153, 2013.
36. Jin HO, Seo SK, Woo SH, Kim ES, Lee HC, Yoo DH, An S, Choe TB, Lee SJ, Hong SI, *et al*: Activating transcription factor 4 and CCAAT/enhancer-binding protein-beta negatively regulate the mammalian target of rapamycin via Redd1 expression in response to oxidative and endoplasmic reticulum stress. Free Radic Biol Med 46: 1158-1167, 2009.
37. Whitney ML, Jefferson LS and Kimball SR: ATF4 is necessary and sufficient for ER stress-induced upregulation of REDD1 expression. Biochem Biophys Res Commun 379: 451-455, 2009.
38. Chen DP, Ma YP, Zhuo L, Zhang Z, Zou GM, Yang Y, Gao HM and Li WG: 1,25-Dihydroxyvitamin D3 inhibits the proliferation of rat mesangial cells induced by high glucose via DDIT4. Oncotarget 9: 418-427, 2018.
39. Chen H, Chen Y, Wu W, Chen Z, Cai Z, Ch Z, Yan X and Wu S: Prolonged hyperlipidemia exposure increases the risk of arterial stiffness in young adults: A cross-sectional study in a cohort of Chinese. BMC Public Health 20: 1091-1099, 2020.
40. Miao J, Zang X, Cui X and Zhang J: Autophagy, hyperlipidemia, and atherosclerosis. Adv Exp Med Biol 1207: 237-264, 2020.
41. López-Mejías R, Genre F, Remuzgo-Martínez S, González-Juanatey C, Robustillo-Villarino M, Llorca J, Corrales A, Vicente E, Miranda-Filloy JA, Magro C, *et al*: Influence of elevated-CRP level-related polymorphisms in non-rheumatic Caucasians on the risk of subclinical atherosclerosis and cardiovascular disease in rheumatoid arthritis. Sci Rep 6: 31979, 2016.
42. Chang CJ, Jian DY, Lin MW, Zhao JZ, Ho LT and Juan CC: Evidence in obese children: contribution of hyperlipidemia, obesity-inflammation, and insulin sensitivity. PLoS One 10: e0125935, 2015.
43. Lin MW, Hwu CM, Huang YH, Sheu WH, Shih KC, Chiang FT, Olshen R, Chen YD, Curb JD, Rodriguez B, *et al*: Directly measured insulin resistance and the assessment of clustered cardiovascular risks in hypertension. Am J Hypertens 19: 1118-1124, 2006.
44. Ding D, Li X, Qiu J, Li R, Zhang Y, Su D, Li Z, Wang M, Lv X and Wang D, *et al*: Serum lipids, apolipoproteins, and mortality among coronary artery disease patients. Biomed Res Int 2014: 709756, 2014.
45. Wang X, Bao W, Liu J, Ouyang YY, Wang D, Rong S, Xiao X, Shan ZL, Zhang Y, Yao P and Liu LG: Inflammatory markers and risk of type 2 diabetes: A systematic review and meta-analysis. Diabetes Care 36: 166-175, 2013.