國立臺灣大學醫學院臨床醫學研究所

碩士論文

Graduate Institute of Clinical Medicine

College of Medicine

National Taiwan University

Master Thesis

ANGPTL8與肥胖和糖尿病病人腎功能惡化相關性與致病機轉之探討

Exploration of the role and related pathogenesis of ANGPTL8 in obesity and diabetes with chronic renal disease

黃則穎

Tse-Ying Huang

指導教授：張恬君 副教授

Advisor: Tien-Jyun Chang, Associate Professor

中華民國 112 年 9 月

September 2023

doi:10.6342/NTU202302402

國立臺灣大學碩士學位論文
口試委員會審定書
MASTER'S THESIS ACCEPTANCE CERTIFICATE
NATIONAL TAIWAN UNIVERSITY

ANGPTL8 與肥胖和糖尿病病人腎功能惡化相關性與致病機轉之探討

Exploration of the role of ANGPTL8 in obesity and diabetes related chronic renal disease and related pathogenesis

本論文係 黃則穎 (姓名) P10421323 (學號) 在國立臺灣大學 臨床醫學研究所 完成之碩士學位論文，於民國 112 年 7 月 28 日承下列考試委員審查通過及口試及格，特此證明。

The undersigned, appointed by the Institute of Clinical Medicine on 28 July, 2023 have examined a Master's thesis entitled above presented by HUANG, TSE-YING (P10421323) candidate and hereby certify that it is worthy of acceptance.

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doi:10.6342/NTU202302402

誌謝

兩年的臨床醫學研究所時光，短暫而寶貴，因有家人和師長的陪伴，這段旅程更加有意義。

謝謝我的指導老師張恬君老師，在研究所期間給予的指導和鼓勵，讓我克服種種挑戰，不斷進步。同時也感謝王治元主任和周祖述所長，在我分享研究過程中提供寶貴的意見和指導。

我也要感謝友人王國權，在資料分析上給予我相當多的幫助，讓我能對統計分析有更深入的認識。

最後，感謝我的太太陳嘉芳醫師，她在我成長的過程中給予充分的支持和鼓勵，讓我在忙碌的學業和臨床業務中感受到溫暖。也要感謝我的父母和家人，是你們給予我最大的後盾和動力。

研究所的學習經歷是我珍貴的回憶，儘管研究所只有兩年，但醫學研究和自我精進是終身學習的課題。期盼未來能繼續對醫學研究貢獻心力，再次感謝這些人們在我成長路上的扶持！

doi:10.6342/NTU202302402

摘要

背景與研究目的

肥胖與第 2 型糖尿病、心血管疾病和較低預期壽命相關。研究指出內臟脂肪堆積與胰島素阻抗、動脈粥樣硬化的脂質與糖尿病的發生之間相關聯。探究不同脂肪組織特定基因的表現與肥胖之間的關聯可以提供更多治療方向。而肥胖和脂毒性在糖尿病患者的慢性腎臟疾病中也有著重要作用，部分研究指出慢性腎臟疾病與血清 ANGPTL8 有關，關於 ANGPTL8 在合併糖尿病和慢性腎臟疾病患者上的調節機制仍需進一步研究。

Angiopoietin-like protein 8（ANGPTL8）是脂質代謝的重要調節因子。我們的研究目的其一是探討肥胖和非肥胖個體之間不同脂肪組織中 ANGPTL8 表現量的差異，以及其在合併糖尿病和慢性腎臟疾病族群中的調節機制。

方法

我們收集了 330 位接受肥胖手術或腹內手術患者的皮下脂肪組織和內臟脂肪組織。使用即時定量聚合酶連鎖反應測定 ANGPTL8 的表現量。分析 ANGPTL8 表現量與各種代謝指標間的相關性。另外我們從臺大醫院收集了 846 位第 2 型糖尿病的患者，收集其血液樣本，使用酵素連結免疫吸附分析測定空腹血漿中的 ANGPTL8 數值，從 2010 年至 2022 年進行長期追蹤。其中慢性腎臟疾病定義為 UACR $\geq$ 30mg/g 或 eGFR < 60 ml/min/1.73m^2 。

結果

肥胖者內臟脂肪中 ANGPTL8 的表現量較高，且與身體質量指數呈正相關。術前血漿 ANGPTL8 濃度最高的患者在減肥手術後 6 個月體重減少的百分比最低。而在血清中的 ANGPTL8 與腎功能惡化追蹤研究中，發現三酸甘油脂、身體質量指數、年齡、罹病時間長短、糖化血色素、收縮壓是糖尿病患者發生慢性腎臟疾病的危險因子，但與血漿中 ANGPTL8 濃度並無顯著相關。而且在 12 年的追蹤期內，

doi:10.6342/NTU202302402

血漿中 ANGPTL8 濃度高低並不影響慢性腎病變的發生率。

結論

肥胖者內臟脂肪組織中 ANGPTL8 的表現量較高，並且與身體質量指數呈正相關。而手術前血漿中 ANGPTL8 值越高者，其肥胖手術後的體重降幅越小，表示 ANGPTL8 在肥胖的病理生理過程中可能扮演重要角色。在第 2 型糖尿病人的世代研究中，雖然血漿 ANGPTL8 濃度在有或無慢性腎病變患者中並無顯著差異。但分析指出三酸甘油脂和身體質量指數是慢性腎臟疾病的危險因子，可能與脂毒性造成的腎功能異常相關。

關鍵詞

肥胖、內臟脂肪、慢性腎臟疾病、ANGPTL8、第二型糖尿病、脂毒性

doi:10.6342/NTU202302402

Abstract

Background and objective

Obesity is associated with type 2 diabetes, cardiovascular disease, and lower life expectancy. Obesity contributes to the development of insulin resistance related to type 2 diabetes, study had reported that visceral adipose tissue (VAT) was associated with insulin resistance, more atherogenic lipid profiles, prevalent diabetes, investigating the association between expression levels of specific genes in different depots of adipose tissue with obesity can yield more functional information.

Obesity and lipotoxicity also play crucial role on chronic kidney disease (CKD) on diabetic patients. The relationship between serum levels of angiopoietin-like protein 8 (ANGPTL8) and lipid metabolism had been studied extensively, and few studies had indicated that CKD is associated with serum ANGPTL8, in which may be due to lipotoxicity, but specific mechanisms for the regulation of ANGPTL8 on diabetes with CKD still need more investigation.

Angiopoietin-like protein 8 (*ANGPTL8*) is an important regulator of lipid metabolism. We aimed to investigate the difference of *ANGPTL8* expression in different depots of adipose tissues between individuals with and without obesity, and its specific mechanisms for the regulation of ANGPTL8 on diabetes with CKD.

doi:10.6342/NTU202302402

Methods

Subcutaneous (SAT) and visceral adipose tissue (VAT) samples were collected from patients who underwent bariatric or intra-abdominal surgery. Expression levels of *ANGPTL8*, monoglyceride lipase (*MGL*), N-alpha-acetyltransferase 10 (*NAA10*), monocyte chemoattractant protein-1 (*MCP-1*), leptin and adiponectin (*APM1*) were determined using real-time qPCR. The correlation of *ANGPTL8* expression with various metabolic parameters and other gene expression levels was analyzed using Pearson's correlation analysis. Logistic regression was used to establish a prediction model of obesity and CKD.

We also enrolled 846 patients form National Taiwan University Hospital (NTUH) with type 2 diabetes since 2010, baseline serum sample were collected, fasting serum ANGPTL8 levels were assessed using ELISA kits. Longitudinal follow up during 2010-2022. CKD is defined as UACR $\geq$ 30mg/g or eGFR < 60 ml/min/1.73m^2, and progression to CKD is defined as sustained eGFR < 60 ml/min/1.73m^2 in the following years.

Results

ANGPTL8 expression levels in VAT were higher in subjects with obesity, and positively correlated with BMI (O.R: 1.246, P = 0.038). On the other hand, the patients with the highest tertiles of pre-operative plasma ANGPTL8 level had the lowest

doi:10.6342/NTU202302402

percentage of BW reduction 6 months after bariatric surgery.

The analysis on diabetes and renal function revealed baseline serum ANGPTL8 showed no significant difference on cross sectional and longitudinal follow up.

In the longitudinal follow-up study of type 2 diabetes patients at NUTH, it was found that plasma triglyceride levels, body mass index, age, diabetes duration, HbA1c, and systolic blood pressure are risk factors for chronic kidney disease in diabetic patients. Plasma ANGPTL8 concentrations were not significantly correlated with CKD in diabetic patients. Moreover, during the 12-year follow-up period, the level of plasma ANGPTL8 did not affect the incidence of chronic kidney disease.

Conclusion

The expression of *ANGPTL8* in visceral adipose tissue of obese people is higher, and it is positively correlated with body mass index. The higher the pre-operative plasma ANGPTL8 level, the lesser the weight loss after obesity surgery, indicating that ANGPTL8 may play an important role in the pathophysiological process of obesity. In a cohort study of people with type 2 diabetes, although serum ANGPTL8 concentrations were not significantly different in patients with or without CKD, triglycerides and body mass index are positive risk factors for chronic kidney disease, which may be associated

doi:10.6342/NTU202302402

with abnormal renal function caused by lipotoxicity.

Key words

Obesity, Visceral fat, Chronic kidney disease, ANGPTL8, Type 2 diabetes, Lipotoxicity

doi:10.6342/NTU202302402

目　錄

doi:10.6342/NTU202302402

Introduction

Worldwide, the prevalence of obesity has increased dramatically during the decades. Obesity is a major public health issue in the world. It has been estimated that there are approximately 1.9 billion adults who are either overweight or obese (body mass index, BMI ≥25 kg/m^2) [1]. In Taiwan, obesity is recognized as a significant health concern that requires attention and intervention.

Obesity has been linked to type 2 diabetes, cardiovascular disease, malignancy and a decreased life expectancy [2]. Obesity plays a significant role in the development of insulin resistance, which is strongly associated with type 2 diabetes. The relationship between obesity and disease risk is mediated through the dysregulation of several processes, including chronic inflammation, hormonal imbalances, and insulin resistance. These factors are closely tied to the abnormal accumulation of adipose tissue [3]. Body mass index (BMI) is commonly used to define obesity; however, it is acknowledged that BMI is an imperfect measure of excessive or abnormal accumulation of body fat or adipose tissue [3].

Visceral adipose tissue (VAT) and abdominal subcutaneous adipose tissues (SAT) contribute to obesity but may have different metabolic and atherosclerosis risk profiles

doi:10.6342/NTU202302402

[4]. Studies have reported a connection between visceral adipose tissue (VAT) and insulin resistance, as well as higher levels of atherogenic lipid profiles and a higher prevalence of diabetes [4]. VAT plays an importance role on insulin resistance, more atherogenic lipid profiles, prevalent diabetes, metabolic syndrome, hepatic steatosis, and aortic plaques. SAT demonstrated modest associations with inflammatory biomarkers and leptin, but no independent association with dyslipidemia, insulin resistance, or atherosclerosis [4]. By examining the expression levels of specific genes in various adipose tissue depots in relation to obesity, researchers can gain more valuable insights into its functional implications.

Furthermore, the abnormal VAT accumulation may lead to lipotoxicity and organ damage, and obesity and lipotoxicity also play a crucial role on chronic renal disease (CKD) in diabetic patients. Individuals with both diabetes and chronic kidney disease are at a higher risk for kidney failure, atherosclerotic cardiovascular disease, heart failure, and premature mortality. In recent times, there has been an emergence of new treatment approaches specifically targeting diabetes and CKD [5].

Lipotoxicity defined as harmful effect of high concentrations of lipids or lipid derivatives in the cells of non-fatty tissues, causing disturbances in their metabolism and loss of function or apoptosis [6]. It predominantly affects cells in the pancreas, liver,

doi:10.6342/NTU202302402

skeletal muscles, heart muscle, and kidney [7].

Angiopoietin-like protein 8 (*ANGPTL8*) is an important regulator of lipid metabolism [8]. The relationship between serum levels of ANGPTL8 and lipid metabolism had been studied extensively, and few studies had indicated that CKD is associated with serum ANGPTL8 [9], in which may be due to lipotoxicity, but specific mechanisms for the regulation of ANGPTL8 on diabetes with CKD still need more investigation.

Our study has two main objectives. Firstly, we aimed to investigate the difference of *ANGPTL8* expression in different depots of adipose tissues between individuals with and without obesity, and studied whether pre-operative plasma ANGPTL8 levels predict the extent of BW reduction and changes of other metabolic parameters.

Secondly, we aimed to compare the plasma ANGPTL8 levels between diabetes with and without CKD at baseline and investigated whether plasma ANGPTL8 levels at baseline can predict the development of incident CKD during 11yr follow-up. We hope to enhance our understanding of the role of ANGPTL8 in the development and progression of diabetes patients with CKD.

doi:10.6342/NTU202302402

Research designs and methods

Participants

In our first study, we used residual specimens to obtain SAT and VAT from 330 subjects who underwent either bariatric surgery or elective intra-abdominal surgery at Min-Sheng General Hospital and National Taiwan University Hospital Yun-Lin Branch. A batch of specimens was collected from 1997 to 2001. All participants signed informed consent before enrolment, and the institutional review board of National Taiwan University Hospital reviewed and approved the study protocol (NTUH-REC No.: 202006141RIND).

In our second retrospective cohort study population, we recruited patients with diabetes from NTUH hospital cohort from 2010 to 2022, we excluded patients with type 1 diabetes and end-stage renal disease (ESRD) population. We also collected basic profiles. Venous blood sampling was performed after overnight fasting for the determination of plasma glucose, hemoglobin A1c, serum total cholesterol, HDL-cholesterol, LDL-cholesterol, triglyceride, and creatinine, spot urine microalbumin/cre (UACR) at baseline date. In the longitudinal follow up since 2010 to 2022, we followed up the renal function decline. All participants signed informed consent before enrolment, and the institutional review board of National Taiwan University Hospital reviewed and

doi:10.6342/NTU202302402

approved the study protocol (NTUH-REC No.: 202006141RIND).

Measurement of various biochemical parameters

Blood samples were also obtained from residual blood samples of the 330 subjects. Briefly, blood samples were taken after at least 8 h of fasting before the operation. Fasting plasma glucose, serum triglyceride, total cholesterol, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol levels were measured using an automatic analyzer (Toshiba TBA 120FR, Toshiba Medical Systems Co., Ltd. Tokyo, Japan). Insulin levels were measured using a microparticle enzyme immunoassay with an automatic analyzer (Abbott AxSYM system, Abbott Laboratories, Abbott Park, IL, USA). We used updated computer models for homeostasis model assessment to calculate the insulin sensitivity indices (HOMA2%S), insulin resistance (HOMA2-IR), and beta cell function (HOMA2%B) [10].

Measurement of anthropometric data

Body weight, body height, waist circumference, and hip circumference of each subject were measured by trained nurses in the morning after fasting for at least 8 h. Blood pressure was recorded to the nearest 2 mmHg using a mercury sphygmomanometer with the arm supported at the heart level after sitting quietly for 10 min. Well-trained nurses recorded three separate readings at 1-min intervals. The average of the last two readings

doi:10.6342/NTU202302402

was used for analysis.

Adipose tissue RNA extraction and reverse transcription

Total RNA of SAT and VAT from subjects with obesity (BMI ≥ 27 kg/m^2) and without obesity (BMI < 27 kg/m^2) was isolated using TRIzol Reagent (Thermo Fisher Scientific Inc., Waltham, MA, USA) following the manufacturer's instructions. Reverse transcription was performed with 1 μg of total RNA and 0.5 μg of random hexamers in a final volume of 25 μl containing 200 U of Maloney murine leukemia virus reverse transcriptase, 20 nmol/l deoxynucleotide triphosphate, and 25 U of rRNasin for 1 h at 37 °C using Superscript III First-Strand Synthesis System (Invitrogen, Waltham, MA, USA) according to the manufacturer's instructions. The complementary DNA (cDNA) products were diluted to 100 μl with distilled deionized water before polymerase chain reaction (PCR) for amplification.

Quantitation of mRNA by real-time PCR

PCR amplification was performed using Power SYBR™ Green PCR Master Mix (Thermo Fisher Scientific Inc.). Expression of several candidate genes was performed using the following primers:

ANGPTL8: forward: 5'- AAT CTG CCT GGA TGG AAC TG

doi:10.6342/NTU202302402

reverse: 5'- CTG CGT CTG TCT CTG CTC TG

LEPTIN: forward: 5'- CCC ATC CAA AAA GTC CAA G

reverse:5'-CCCAGGAATGAAGTCCAAAC

Monocyte chemoattractant protein-1 (*MCP1*)

forward: 5'-TCA GCC AGA TGC AAT CAA TG

reverse: 5'-ATG GTC TTG AAG ATC ACA GC

Monoglyceride lipase (*MGL*)

forward: 5'-CAT GTG GAT TCC ATG CAG AAA G

reverse: 5'-AGG ATT GGC AAG AAC CAG AGG

Adiponectin (*APM1*)

forward: 5'-TGTTGCTGGGAGCTGTTCTACTG

reverse: 5'-ATGTCTCCCTTAGGACCAATAAG

N-alpha-acetyltransferase 10 (*NAA10*)

forward: 5'-GATCAGTGAAGTGGAGCC

reverse: 5'- AGCTCGGAGGTGAATTG

Succinate dehydrogenase complex flavoprotein subunit A (*SDHA*)

forward: 5'- TGGGAACAAGAGGGCATCTG

reverse: 5'- CCACCACTGCATCAAATTCATG

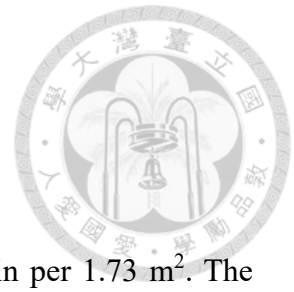
doi:10.6342/NTU202302402

Real-time quantitative PCR (qPCR) was performed according to the users' manual of Applied Biosystems 7900HT FAST (Thermo Fisher Scientific Inc.). Samples with a high starting copy number of cDNA showed an increase in fluorescence earlier in the PCR process, thus resulting in a low Ct number. Relative gene expression was calculated by normalization to succinate dehydrogenase complex flavoprotein subunit A (*SDHA*) as a housekeeping gene in human adipose tissue [11]. The comparative Ct method was used to display target gene expression (*ANGPTL8* in this study) relative to that of the endogenous control gene (*SDHA* in this study), that is, ΔCt = Ct number of *SDHA* − Ct number of *ANGPTL8*. ΔCt indicates a $\log_2$ transformation of *ANGPTL8* mRNA expression relative to that of *SDHA*. In other words, the higher the ΔCt level, the higher the *ANGPTL8* gene expression level relative to *SDHA* expression.

Measurement of ANGPTL8 levels

ANGPTL8 levels in the fasting serum were quantified using commercially available ELISA kits (Novus Biologicals, USA; NBP2-68217). The procedures were performed according to the manufacturer's instructions, with intra-assay of coefficient of variation ≤ 6.46%, and an inter-assay coefficient of variation ≤ 6.72%. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm.

doi:10.6342/NTU202302402



Outcome assessment

CKD was defined as UACR ≥ 30 mg/g or eGFR < 60 mL/min per 1.73 m^2. The eGFR was calculated using the CKD-EPI Creatinine Equation (2021) [12]. Progression of kidney disease defined as sustained eGFR < 60 mL/min per 1.73 m^2 in the following years.

Statistical analysis

Means ± standard deviations [13] are shown for normally distributed data. Medians (interquartile ranges) are shown for variables not normally distributed. We analyzed the correlation between the expression levels of *ANGPTL8* in different adipose tissue depots and metabolic parameters [systolic blood pressure (SBP), diastolic blood pressure (DBP), BMI, lipid profiles, fasting glucose, HOMA2-IR, HAMA2%B, and HOMA2%S, etc.] using Pearson's correlation. We compared *ANGPTL8* expression levels in SAT and VAT and the above metabolic parameters between subjects with and without obesity using Student's *t*-test. Logistic regression was used to establish a prediction model of obesity and to identify risk factors for CKD, respectively. The Cox regression analysis was performed to assess the association between the ordinal levels of plasma ANGPTL8 and the development of incident CKD. All statistical analyses were conducted using R Software (R-4.3.1 for Windows). Significance was set at $P < 0.05$.

doi:10.6342/NTU202302402

Results

Clinical characteristics of subjects with or without obesity

This study recruited 330 subjects, including 281 subjects with obesity and 49 subjects without obesity. Table 1 summarizes the clinical profiles of all subjects. The average age of subjects with and without obesity was 31 years old (range: 26–42 y/o) and 50 years (range: 36.5–64 y/o), respectively. The obesity group was predominantly female (73.31%). The obesity group had higher BMI, waist circumference, SBP, DBP, fasting plasma insulin levels, HOMA2%B, and HOMA2-IR (Table 1). In contrast, the obesity group had lower fasting plasma glucose levels, HOMA2%S, plasma triglyceride, and adiponectin levels (Table 1). We also analysed *ANGPTL8* gene expression in SAT and VAT by qPCR, and the expression levels of *ANGPTL8* in VAT were significantly higher in the obesity group than in the non-obesity group (Table 1).

Correlation between *ANGPTL8* expression and clinical variables

We used the Pearson's correlation to analyse the association of *ANGPTL8* expression in different depots of adipose tissue with various metabolic parameters (Table 2). The expression of *ANGPTL8* in VAT was positively correlated with BMI (R = 0.1168, P = 0.0416).

doi:10.6342/NTU202302402

ANGPTL8 in VAT positively associated with obesity

In univariate analysis, SBP and *ANGPTL8* gene expression levels in VAT were positively associated with obesity. Age, male sex, and plasma adiponectin levels were negatively associated with obesity. In the logistic regression model, after adjustment for age, sex, SBP, and plasma adiponectin levels, *ANGPTL8* gene expression levels in VAT remained positively associated with obesity (Table 3).

Correlation of *ANGPTL8* expression with expression of various candidate genes involved in lipolysis, adipocyte-mediated thermogenesis, inflammatory responses, obesity and insulin resistance in respective VAT and SAT

To further explore the possible mechanisms of *ANGPTL8* expression positively associated with BMI and obesity, we measured the mRNA expression of several candidate genes involved in lipolysis (monoglyceride lipase, *MGL*), adipocyte-mediated thermogenesis (N-alpha-acetyltransferase 10, *NAA10*), inflammatory responses (monocyte chemoattractant protein-1, *MCP-1*), obesity and insulin resistance (leptin; adiponectin, *APM1*) in VAT and SAT, respectively. The gene expression of *ANGPTL8* in SAT and VAT was negatively correlated with the gene expression of *APM1* in respective SAT and VAT (SAT: r = -0.308, $P = 0.0001$, VAT: r=-0.294, $P = 0.0001$, Fig. 1). The gene

doi:10.6342/NTU202302402

expression of *ANGPTL8* in SAT and VAT was not significantly associated with the gene expression of *MGL*, *MCP-1*, and leptin in respective SAT and VAT (data not shown).

Higher pre-operative plasma ANGPTL8 levels is associated with less post-operative weight loss.

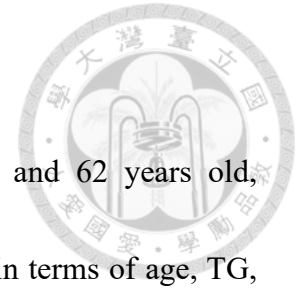
We measured plasma ANGPTL8 levels in the obesity group, and then categorized them into tertiles. We followed up with the patients for 6 months and 12 months after surgery to assess the extent of weight loss. The results revealed that patients with the highest tertiles of pre-operative plasma ANGPTL8 levels had less post-operative weight reduction. (Fig. 2)

Clinical characteristics in subjects with or without chronic kidney disease

In our second study, a total of 1060 patients were included for analysis. We excluded patients with incomplete eGFR data and those with undetectable plasma ANGPTL8 levels (below 0). Additionally, patients with type 1 diabetes mellitus (T1DM) and end-stage renal disease (ESRD) were also excluded. Ultimately, a total of 846 patients were included in the analysis.

Table. 4 provides a summary of the clinical characteristics of all the subjects. The

doi:10.6342/NTU202302402



average age of patients with CKD and without CKD was 68 and 62 years old, respectively. The CKD group exhibited significantly higher values in terms of age, TG, BMI, and DM duration compared with those of the non-CKD group ($P < 0.05$). We also conducted an analysis considering medication, which revealed a higher proportion of patients receiving metformin in the non-CKD group, and higher proportion of patients receiving insulin and antiplatelet drugs in the CKD group may suggest a higher prevalence of comorbidities in this population.

Relationship of plasma ANGPTL8 expression with various metabolic parameters

Using Pearson's correlation analysis, we examined the association between plasma ANGPTL8 levels and various metabolic parameters (Table 5). The results indicated a significant positive correlation between HbA1c and ANGPTL8 expression ($P < 0.05$), as well as a significant negative correlation between SBP and ANGPTL8 expression. However, the correlations between ANGPTL8 and other metabolic parameters were not statistically significant.

Risk factors associated with chronic kidney disease

Additionally, we performed logistic regression analysis to identify factors associated with CKD (Table 6), which revealed age, TG, BMI, SBP, HbA1c and DM duration were positively associated with CKD, but plasma ANGPTL 8 showed no significant

doi:10.6342/NTU202302402

association.

Event-free survival analysis between plasma ANGPTL8 levels and incident CKD

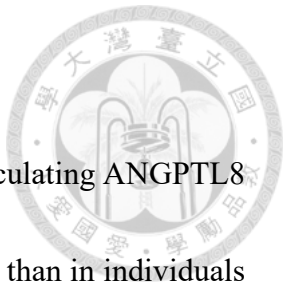
We categorized the plasma ANGPTL 8 values into quartiles and performed Cox regression analysis. A total of 530 non-CKD patients were included in the analysis at baseline since November 2010, and among them, 109 patients (20.57%) progressed to CKD by July 2022. There was no significant different progression rate to incident CKD among different quartiles of baseline plasma ANGPTL8 levels (Fig 3).

Conclusion and discussion

In our first study, we showed higher *ANGPTL8* expression in the VAT of subjects with obesity than in those without obesity, and that *ANGPTL8* expression levels in VAT were positively correlated with BMI. On the other hand, the gene expression of *ANGPTL8* was also negatively correlated with the expression of *APM1* in VAT and SAT. To the best of our knowledge, this is the first study to demonstrate the positive correlation of *ANGPTL8* expression in specific depots of adipose tissue with BMI, and that the expression levels of *ANGPTL8* in VAT can serve as a positive indicator of obesity. The above findings indicated that ANGPTL8 may be a potential biomarker of obesity.

ANGPTL8 is a member of the ANGPTL family, and is associated with lipid

doi:10.6342/NTU202302402



regulation and glucose metabolism [14]. One study reported that circulating ANGPTL8 levels were lower in patients with morbid obesity and type 2 diabetes than in individuals with obesity and without type 2 diabetes. However, expression levels of ANGPTL8 in white adipose tissue was negatively correlated with plasma ANGPTL8 levels but positively correlated with type 2 diabetes [15]. Another study reported a biphasic change in plasma ANGPTL8 levels following bariatric surgery, with a short-term increase but a long-term reduction in plasma ANGPTL8 levels [16]. Al-Shawaf et al. also found that ANGPTL8 levels were higher in type 2 diabetes patients, regardless of weight, compared with non-obese individuals without type 2 diabetes [16]. These contradictory results indicate that plasma levels of ANGPTL8 may vary depending on the status of type 2 diabetes and obesity [17], and currently there is no reliable causal relationship between them. Therefore, more systematic studies are warranted to investigate the mechanistic relationships between circulating ANGPTL8 levels and type 2 diabetes and obesity.

Angptl8 knockout (*Angptl8*$^{-/-}$) mice have been found to gain weight more slowly than wild-type littermates due to selective reduction in adipose tissue expansion, which indicates that *ANGPTL8* is required to direct fatty acids to adipose tissue for storage in the fed state. However, although the absence of ANGPTL8 remarkably disrupts triglyceride metabolism, it has no influence on glucose homeostasis [18]. Vatner *et al.*

doi:10.6342/NTU202302402

ever reported that omental *ANGPTL8* expression is higher in morbid obese subjects with fatty liver and insulin resistance (BMI: 48 kg/m^2, HOMA-IR: 13.5) compared with those in BMI-matched controls (BMI: 50 kg/m^2, HOMA-IR: 2.1) [19]. Our study showed that the expression levels of *ANGPTL8* in VAT were not correlated with HOMA-IR or HOMA-beta, but was positively correlated with BMI (Table 2).

To further explore the possible mechanisms of *ANGPTL8* expression positively associated with BMI and obesity, we analyzed the correlation of *ANGPTL8* in VAT and SAT with gene expression involved in lipolysis (*MGL*), adipocyte-mediated thermogenesis (*NAA10*), inflammatory responses (*MCP-1*), obesity and insulin resistance (*LEPTIN*, *APM1*) in VAT and SAT, respectively. Though a previous study showed ANGPTL8 inhibits lipolysis in 3T3-L1 adipocytes [20], no significant correlation between gene expression of *ANGPTL8* and *MGL* was found in our study. There is also no significant correlation between gene expression of *ANGPTL8* and *MCP1* & *LEPTIN* in our study. Interestingly, we found the gene expression of ANGPTL8 in SAT and VAT was negatively correlated with the gene expression of *APM1* in respective SAT and VAT. Adiponectin (*APM1*) is an adipokine [21],which has been reported that serum levels of adiponectin decrease with obesity and are positively associated with insulin sensitivity [22, 23]. Therefore, we inferred that ANGPTL8 is positively associated with BMI and

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obesity maybe through decreased APM1 leading to increase adiposity.

We also observed that pre-operation plasma ANGPTL8 levels may predict the extent of post-operation weight reduction. Elevated ANGPTL8 could be related to lipid metabolism and obesity.

Previous research has shown a positive association between plasma ANGPTL8 levels and the decline of renal function in cross-sectional and longitudinal follow-up [24]. However, in our current analysis, the correlation analysis revealed only a positive relationship with HbA1c and plasma ANPTL8, while no significant correlation was observed with other metabolic variables.

We observed higher levels of triglycerides (TG) and BMI in patients with diabetes with CKD, supporting our hypothesis that renal function impairment may be linked to lipotoxicity. Elevated TG levels could potentially contribute to the deterioration of renal function in these patients.

The cross-sectional data showed that patients with CKD had significantly higher TG, BMI, DM duration and SBP. In our hypothesis, lipotoxicity may play crucial role on renal function decline, study had pointed out that lipid may be related to CKD [25]. Consistently, according to cross-sectional logistic regression analysis in our study, TG is

doi:10.6342/NTU202302402

positively correlated with CKD. In a previous study, it indicated that patients with high plasma ANPTL8 levels were at an increased risk of renal function decline in cross-sectional and long-term follow-up [9]. However, the study only included 23.5% of diabetes patients. By contrast, there was no significant different progression rate to incident CKD among different quartiles of baseline plasma ANGPTL8 levels in our study. Considering the potential influence of comorbidity, drug history, and diabetes duration in our study, these factors may partially contribute to the different finding between our study and the previous reports.

Our first study has some strengths. First, this is a large cohort of 281 subjects with obesity undergoing bariatric surgery and 49 subjects without obesity undergoing intra-abdominal surgery. Second, this is the first human study to compare ANGPTL8 expression in different depots of adipose tissue and to analyze the correlation with various metabolic parameters. However, there are some limitations to this study. First, this was a cross-sectional study, so no causal relationship between ANGPTL8 expressions in different depots of adipose tissue with various post-operative metabolic parameters could be established. Second, a relatively small number of individuals without obesity were recruited in this study. Third, there were significant differences in age and gender between participants with and without obesity, which may be potential confounding factors in the

doi:10.6342/NTU202302402

statistical analyses.

In conclusion, patients with obesity had higher *ANGPTL8* expression levels in VAT than subjects without obesity, and the expression levels of *ANGPTL8* in VAT were positively correlated with BMI in human subjects. We further inferred that ANGPTL8 is positively associated with BMI and obesity maybe through decreased *APM1* leading to increase adiposity. We also found that higher pre-operative plasma ANGPTL8 levels is associated with less post-operative weight loss. This confirmed the role of ANGPTL8 in the pathophysiology of obesity and may pave the way for novel treatment of obesity. Although our secondary study showed no significant difference in plasma ANGPTL8 with renal function decline in cross-sectional and longitudinal follow up, we still figured out TG, BMI, HbA1c and SBP as the classical risk factors associated with progression and development of incident CKD in diabetes patients. It was compatible with our current guideline for better glycemic, lipid, blood pressure and BW control to decrease diabetes-related complications.

doi:10.6342/NTU202302402

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doi:10.6342/NTU202302402

Figure legends

Figure 1. Correlation of gene expression of *ANGPTL8* with genes involved in obesity and insulin resistance in subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT), respectively. *ANGPTL8* gene expression was negatively correlated with *APM1* in SAT (A) and VAT (B), respectively.

Figure 2.

Percentage of weight loss after bariatric surgery (0, 6m, 12m) according to pre-operative plasma ANGPTL8 (n=104)

Figure 3.

Event-free survival analysis between plasma ANGPTL8 levels and incident CKD, according to quartile of plasma ANGPTL8 levels

doi:10.6342/NTU202302402

Table 1. Clinical characteristics in subjects with or without obesity

Variables	Obesity (BMI ≥ 27 kg/m^2)	Non-Obesity (BMI < 27 kg/m^2)	*P* value
N	281	49	
Age (y/o) *	31 (26–42)	50 (36.5–64)	**<0.001**
Male (%)	75 (26.69%)	30 (61.22%)	**<0.01**
Body mass index (kg/m^2)	40.31 (6.17)	22.34 (3.60)	**<0.001**
WC (cm)	118.06 (13.89)	84.33 (2.08)	**<0.001**
HC (cm)	164.70 (8.27)	162.57 (8.13)	0.0964
WHR	0.92 (0.08)	0.83 (0.02)	0.0538
SBP (mmHg)	132.45 (17.59)	125.70 (14.92)	**0.012**
DBP (mmHg)	82.55 (13.84)	77.04 (10.98)	**0.0086**
Fasting plasma glucose (mmol/l) *	5.16 (4.83–5.79)	5.5 (4.91–8.16)	**0.0029**
Fasting plasma insulin (μU/ml) *	16.20 (11.80–23.38)	9.90 (5.18–14.38)	**<0.001**
HOMA2%B (%)*	184.31 (117.29–276.27)	65.92 (37.81–176.06)	**<0.001**
HOMA2%S (%)*	0.25 (0.17–0.37)	0.37 (0.19–0.83)	**0.0140**
HOMA2-IR*	3.92 (2.71–6.03)	2.70 (1.21–5.45)	**0.0140**
Total cholesterol (mmol/l)	5.05 (0.98)	4.85 (1.59)	0.4420
HDL cholesterol (mmol/l)	1.11 (0.24)	1.17 (0FPG.45)	0.1962
LDL cholesterol (mmol/l)	3.48 (0.85)	3.16 (1.28)	0.2249
Triglyceride (mmol/l) *	3.57 (2.67–4.84)	5.36 (3.43–8.37)	**0.0004**
TG/HDL	4.64 (0.52)	6.5 (0.89)	0.1521
Adiponectin (μg/ml) *	6.19 (3.95–8.66)	8.22 (6.24–Tot12.23)	**<0.001**
ΔCtANGPTL8_SAT	−2.52 (3.48)	−3.41 (2.81)	0.1286
ΔCtANGPTL8_VAT	−3.54 (3.00)	−4.77 (2.30)	**0.0096**

doi:10.6342/NTU202302402

Means (standard deviations) are shown.

* Medians (interquartile ranges) are shown for variables not normally distributed. Statistical analyses were performed after log transformation.

DM: diabetes mellitus; WC: waist circumference; HC: hip circumference; WHR: waist hip ratio; SBP: systolic blood pressure; DBP: diastolic blood pressure; HOMA2%B: homeostasis model assessment-beta cell function; HOMA2%S: homeostasis model assessment-insulin sensitivity indices; HOMA2-IR: homeostasis model assessment-insulin resistance; HDL: high density lipoprotein; LDL: low density lipoprotein; ANGPTL8: angiopoietin-like protein 8; ΔCtANGPTL8_SAT: relative expression levels of *ANGPTL8* in subcutaneous adipose tissue; ΔCtANGPTL8_VAT: relative expression levels of *ANGPTL8* in visceral adipose tissue.

doi:10.6342/NTU202302402

Table 2. Relationship of ANGPTL8 expression in different depots of adipose tissue with various metabolic parameters

	SAT_ANGPTL8		VAT_ANGPTL8	
Variables	**R**	***P* value**	**R**	***P* value**
Body mass index	0.0445	0.4742	0.1168	**0.0416**
SBP	0.0020	0.9740	-0.0860	0.1345
DBP	-0.0318	0.6096	-0.0640	0.2660
Fasting plasma glucose	0.0199	0.7543	-0.0366	0.5339
Fasting plasma insulin	0.0067	0.9143	-0.0001	0.9980
HOMA2%B	-0.0108	0.8669	0.0024	0.9675
HOMA2%S	-0.0030	0.9627	-0.0096	0.8719
HOMA2-IR	0.0110	0.8634	-0.0322	0.5863
Total cholesterol	0.1435	**0.0289**	0.0410	0.5019
HDL-cholesterol	0.0560	0.3688	-0.0329	0.5683
LDL-cholesterol	0.0892	0.1816	0.0243	0.6955
Triglyceride	0.0117	0.8508	-0.0652	0.2570
TG / HDL	-0.0039	0.9499	-0.086	0.1345
Adiponectin	-0.0035	0.9601	-0.0532	0.4207

SAT: subcutaneous adipose tissue; VAT: visceral adipose tissue; ANGPTL8: angiopoietin-like protein 8; SBP: systolic blood pressure; DBP: diastolic blood pressure; HOMA2%B: homeostasis model assessment-beta cell function; HOMA2%S: homeostasis model assessment-insulin sensitivity indices; HOMA2-IR: homeostasis model assessment-insulin resistance; HDL: high density lipoprotein; LDL: low density lipoprotein;

doi:10.6342/NTU202302402

Table 3. Logistic regression to identify factors associated with obesity

	Univariable analysis			Logistic regression model		
Variable	O.R.	95% C.I.	*P* value	O.R.	95% C.I.	*P* value
Age	0.914	0.891 – 0.937	**<0.001**	0.930	0.902–0.958	**<0.001**
Sex	0.231	0.122 – 0.434	**<0.001**	0.125	0.047–0.335	**<0.001**
SBP	1.026	1.006 – 1.047	**0.012**	1.059	1.026–1.093	**<0.001**
Adiponectin	0.875	0.818 – 0.937	**<0.001**	0.866	0.786–0.954	**0.004**
ΔCt ANGPTL8_VAT	1.208	1.049 – 1.391	**0.009**	1.246	1.013–1.533	**0.038**

SBP: systolic blood pressure; △Ct ANGPTL8_VAT: relative expression level of ANGPTL8 in visceral adipose tissue.

doi:10.6342/NTU202302402

Table 4. Clinical characteristics in subjects with or without chronic kidney disease (N = 846)

Variable	Non-CKD (n=525)	CKD (n=321)	*P* value
Age (y/o)*	61.59 ± 9.79	68.10 ± 11.04	**<0.001**
Male**	288 (55%)	169 (53%)	0.570
Hemoglobin A1c (%)	7.1 (6.7 - 7.6)	7.40 (6.6 - 8)	**0.049**
eGFR*(ml/min/1.73m^2)	87.24 ± 13.66	65.59 ± 23.19	**<0.001**
UACR (mg/g)	8 (5 - 13)	71 (35 - 239)	**<0.001**
ANGPTL8 (pg/ml)	76.92 (25.68 - 170.03)	63.52 (24.92 - 1470.20)	0.129
LDL cholesterol (mg/dL)*	90.75 ± 25.47	89.19 ± 27.90	0.415
Triglyceride (mg/dL)	105 (76 - 145)	126 (87 - 183)	**<0.001**
Body mass index (kg/m^2)	24.88 (22.85 - 27.01)	26.18 (23.85 - 29.10)	**<0.001**
Diabetes duration (years)	10 (6 - 15)	12 (8 - 18)	**<0.001**
SBP (mmHg)	130 (120 - 140)	136 (128 - 148)	**<0.001**
DBP (mmHg)	80 (70 - 82)	80 (70 - 84)	0.580
TG/HDL	2.4(1.53 - 3.62)	2.88 (1.89 - 4.81)	**<0.001**
ARB or ACEI**	221 (42%)	204 (64%)	**<0.001**
Stain or ezetimibe	259 (49%)	151 (47%)	0.524
Fibrate or niacin	16 (3%)	13 (4%)	0.442
Sulfonylurea	283 (54%)	153 (48%)	0.089
Thiazolidinedione	149 (28%)	87 (27%)	0.752
Metformin	445 (85%)	235 (73%)	**<0.001**
Insulin	36 (7%)	36 (11%)	**0.031**
Antiplatelets	112 (21%)	94 (29%)	**0.010**

*: t-test ; **: fisher exact test

CKD: chronic kidney disease; eGFR: estimated Glomerular filtration rate; UACR: urine albumin/creatinine ratio; ANGPTL8: angiopoietin-like proteins 8

LDL: low density lipoprotein; SBP: systolic blood pressure; DBP: diastolic blood pressure; ARB: angiotensin receptor blocker; ACEI: angiotensin Converting Enzyme Inhibitors

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Table. 5 Relationship of plasma ANGPTL8 expression with various metabolic parameters

Variables	R	*P* value
Age	0.00134	0.9689
LDL cholesterol	0.00292	0.9324
Hemoglobin A1c	0.07811	**0.02308**
eGFR	0.02099	0.5419
UACR	0.00981	0.7758
Triglyceride	0.01142	0.7401
Body mass index	0.01522	0.6584
Diabetes duration	0.01233	0.7201
SBP	-0.0725	**0.0350**
DBP	-0.0354	0.3037
TG/HDL	0.019	0.582

ANGPTL8: angiopoietin-like proteins 8; LDL: low density lipoprotein; eGFR: estimated Glomerular filtration rate;
UACR: urine albumin/creatinine ratio; SBP: systolic blood pressure; DBP: diastolic blood pressure

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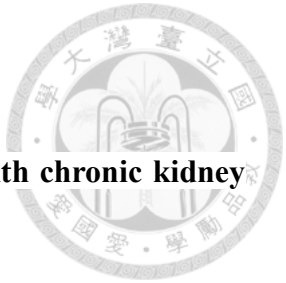

Table 6. Logistic regression to identify risk factors associated with chronic kidney disease

	Logistic regression model		
Variable	O.R.	95% C.I.	*P* value
Age	1.076	0.952 - 0.986	**<0.01**
Triglyceride	1.08	1.044 - 1.136	**<0.01**
Body mass index	1.016	0.983 - 1.050	**<0.01**
SBP	10009	0.999 - 1.004	**<0.01**
Hemoglobin A1c	1.107	1.045 - 1.191	**0.033**
Diabetes duration	1.012	1.045 - 1.191	**0.043**
ANGPTL8	0.998	0.998 - 1.006	0.3755

SBP: systolic blood pressure; ANGPTL8: angiopoietin-like proteins 8

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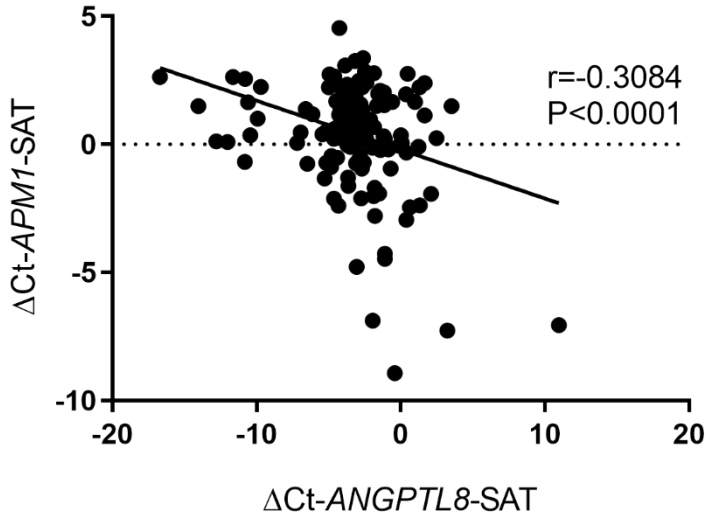
Figure 1.

A.



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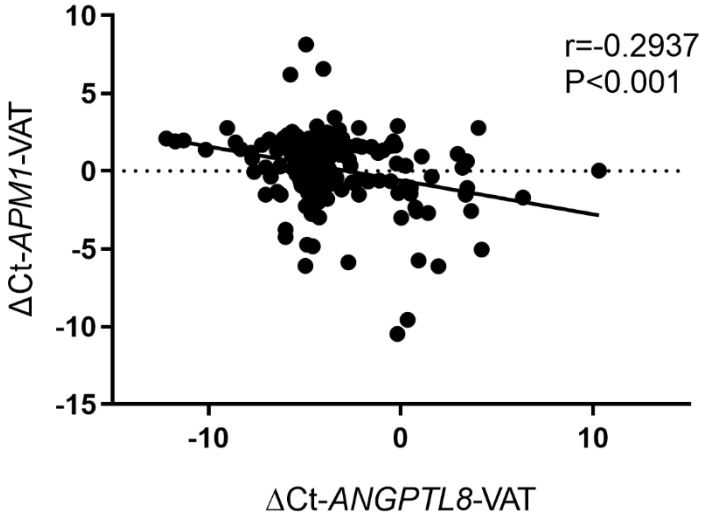

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Figure 2.

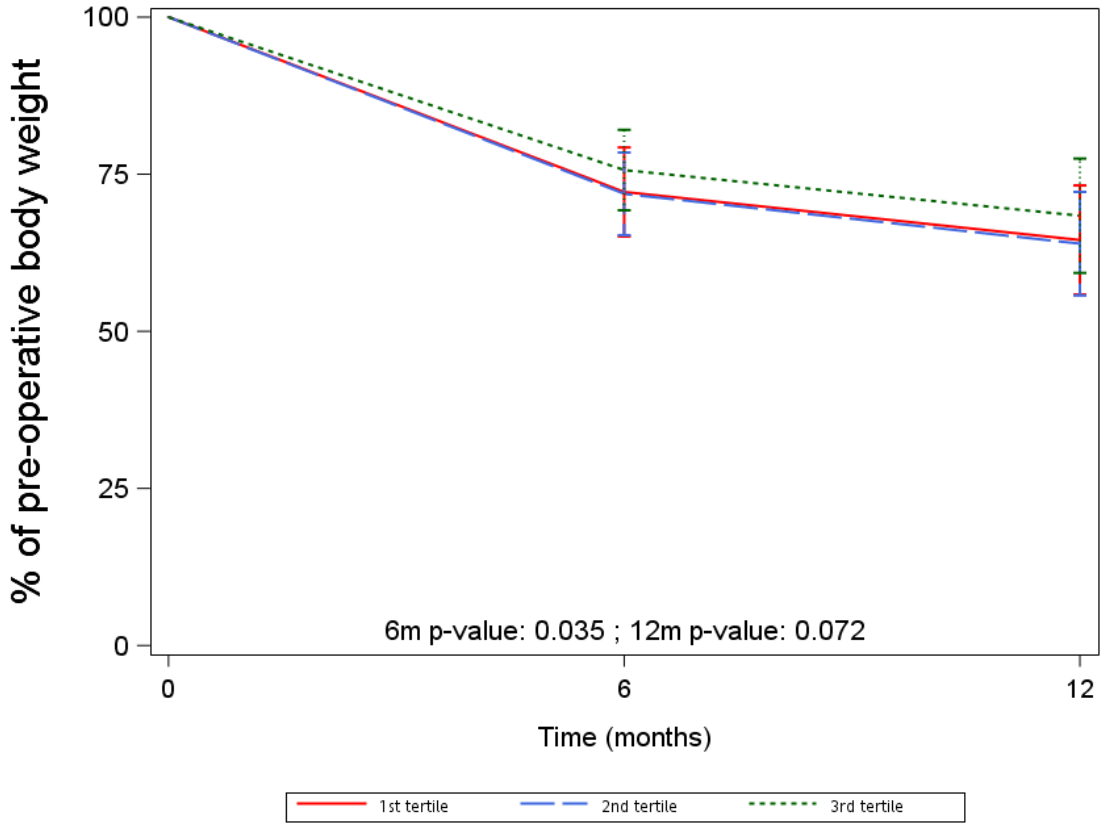

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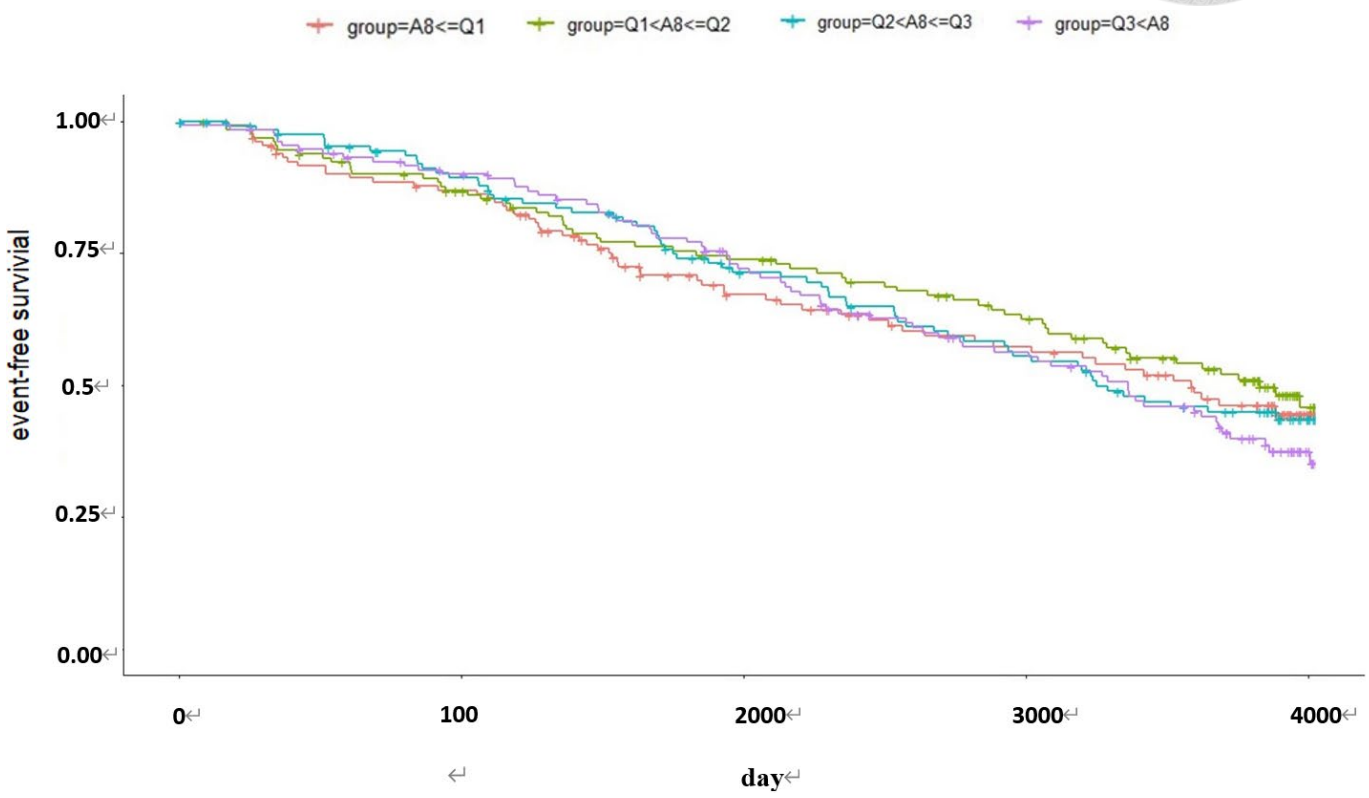
Figure 3.



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