Serum Ectodysplasin A and Non-alcoholic Fatty Liver Disease in Patients with Type 2 Diabetes Mellitus: A Postprint

Authors: Qian Fangfang, Cai Zhensheng, Gu Tian, Li Haoxiang, Zhao Li, Yang Ling, Deng Xia, Yuan Guoyue

Date: 2022-09-30T18:40:31+00:00

Abstract

Background EDA (Ectodysplasin A, EDA) is a newly discovered hepatic factor that has been considered closely associated with diabetes, obesity, and insulin resistance in recent years. Objective To investigate the changes in serum Ectodysplasin A (EDA) concentration and its influencing factors in patients with type 2 diabetes mellitus (T2DM) complicated with non-alcoholic fatty liver disease (NAFLD). Methods A total of 130 patients with T2DM were recruited, and general clinical data were collected. Oral glucose tolerance test, insulin and C-peptide stimulation tests were performed. Abdominal ultrasound examination was conducted for all patients using a whole-body color Doppler diagnostic instrument. Based on the ultrasound results, patients were divided into a T2DM with NAFLD group (n=80) and a T2DM without NAFLD group (n=50). Differences in clinical indicators between the groups were compared. The correlation between EDA and clinical indicators was analyzed. Logistic regression analysis was used to explore the relationship between serum EDA levels and the risk of NAFLD in patients with T2DM. Results Compared with the non-NAFLD group, the NAFLD group had younger age and shorter duration of T2DM, while body mass index (BMI), fasting insulin (FIns), 2-hour postprandial insulin (2hIns), fasting C-peptide (FCP), 2-hour postprandial C-peptide (2hCP), HOMA-IR, triglyceride (TG), and EDA were significantly increased ($P<0.05$). EDA showed a significant positive correlation with age, FIns, 2hIns, HOMA-IR, β-cell function index (HOMA-β), AST, and serum creatinine (SCr) ($P<0.05$). Multiple linear regression analysis showed that 2hIns, age, AST, and waist-to-hip ratio (WHR) were independent correlates of EDA. Logistic regression analysis showed that after adjusting for multiple confounding factors, circulating EDA levels remained significantly associated with NAFLD in patients with T2DM ($P<0.05$). Conclusion Serum EDA levels were significantly increased in patients with T2DM complicated with NAFLD, suggesting that changes in EDA

levels in patients with T2DM may play a role in the occurrence and development of NAFLD.

Full Text

Association between Serum Ectodysplasin A and Non-alcoholic Fatty Liver Disease in Patients with Type 2 Diabetes Mellitus

QIAN Fangfang, CAI Zhensheng, GU Tian, LI Haoxiang, ZHAO Li, YANG Ling, DENG Xia*, YUAN Guoyue*

Department of Endocrinology and Metabolism, Affiliated Hospital of Jiangsu University, Zhenjiang, Jiangsu 212001, China

Funding: National Natural Science Foundation of China (81870548); Key Research and Development Program of Jiangsu Provincial Social Development (BE2018692); Natural Science Foundation of Jiangsu Province (BK20191222); Zhenjiang Fifth Phase "169 Project" Research Project; Beigu Elite Cultivation Program (BGYCB202206)

Corresponding authors:
YUAN Guoyue, Professor, Chief Physician; Email: yuanguoyue@ujs.edu.cn
DENG Xia, MD, Attending Physician; Email: dengxia11@outlook.com

Abstract

Background: Ectodysplasin A (EDA) is a newly discovered hepatokine that has recently been implicated in diabetes, obesity, and insulin resistance. **Objective:** This study aimed to investigate changes in serum EDA concentration and its influencing factors in patients with type 2 diabetes mellitus (T2DM) complicated by non-alcoholic fatty liver disease (NAFLD). **Methods:** A total of 130 patients with T2DM were recruited. General clinical data were collected, and oral glucose tolerance tests, insulin release tests, and C-peptide stimulation tests were performed. All patients underwent abdominal ultrasound examination using a whole-body color Doppler diagnostic instrument. Based on the ultrasound findings, patients were divided into a T2DM with NAFLD group (n=80) and a T2DM without NAFLD group (n=50). Inter-group differences in clinical parameters were compared, correlations between EDA and clinical indices were analyzed, and logistic regression was used to explore the relationship between serum EDA levels and NAFLD risk in T2DM patients. **Results:** Compared with the non-NAFLD group, the NAFLD group had lower age, shorter T2DM duration, and significantly higher body mass index (BMI), fasting insulin (FIns), 2-hour postprandial insulin (2hIns), fasting C-peptide (FCP), 2-hour postprandial C-peptide (2hCP), HOMA-IR, triglycerides (TG), and EDA ($P<0.05$). EDA was significantly positively correlated with age, FIns, 2hIns, HOMA-IR, HOMA-β, AST, and serum creatinine (SCr) ($P<0.05$). Multiple linear regression analysis

showed that 2hIns, age, AST, and waist-to-hip ratio (WHR) were independent correlates of EDA. Logistic regression analysis demonstrated that circulating EDA levels remained significantly associated with NAFLD in T2DM patients after adjusting for multiple confounders ($P<0.05$). **Conclusion:** Serum EDA levels are significantly elevated in T2DM patients with NAFLD, suggesting that altered EDA levels may play a role in the pathogenesis and progression of NAFLD in T2DM patients.

Keywords: type 2 diabetes mellitus; Ectodysplasin A; non-alcoholic fatty liver disease; hepatokine; insulin resistance

Introduction

With the continuous improvement of living standards, the prevalence of non-alcoholic fatty liver disease (NAFLD) has been increasing annually. Studies have shown that approximately 25% of adults are affected by NAFLD, with a total of up to 1 billion individuals worldwide [1]. NAFLD often begins as simple steatosis and progressively develops into steatohepatitis, which can further advance to cirrhosis and/or hepatocellular carcinoma, making it a leading cause of liver-related morbidity and mortality [2]. NAFLD frequently coexists with metabolic disorders including dyslipidemia, insulin resistance, obesity, hypertension, and diabetes mellitus, and is considered a major component of metabolic syndrome [3]. Research indicates that type 2 diabetes mellitus (T2DM) is a recognized risk factor for the development and progression of NAFLD, potentially exacerbating lipid metabolism disturbances and increasing cardiovascular disease incidence [4].

In recent years, multiple hepatokines have been found to participate in the pathogenesis of diabetes, obesity, metabolic syndrome, and NAFLD, and may serve as novel diagnostic biomarkers to elucidate the mechanisms underlying metabolic dysregulation [5]. The Ectodysplasin A (EDA) gene is located on chromosome Xq12-13 and was initially identified as a member of the tumor necrosis factor-related cytokine family. It belongs to the type II transmembrane protein family and can be secreted into the extracellular domain after furin protease cleavage [6]. Previous studies have demonstrated that EDA plays a crucial role in the development and maintenance of skin-derived structures such as teeth, hair, and sweat glands, with mutations in the EDA gene causing X-linked hypohidrotic ectodermal dysplasia [7] and selective non-syndromic tooth agenesis [8]. With further research, Awazawa et al. in 2017 reported a novel function of this gene—regulating systemic glucose metabolism and impairing skeletal muscle insulin sensitivity—identifying it as a hepatokine. Their study showed that hepatic and serum EDA levels were significantly elevated in high-fat diet-fed mice and db/db mice. Furthermore, EDA overexpression aggravated glucose intolerance, while EDA knockout markedly improved insulin sensitivity in db/db mice [9]. Currently, few studies have investigated the relationship between EDA and T2DM, and no research has examined serum EDA concentrations in T2DM patients with NAFLD. This study aims to explore the correlation between serum EDA

levels and NAFLD in T2DM patients and identify potential influencing factors, providing new theoretical basis for early screening or treatment of NAFLD.

Methods

Subjects

A total of 130 patients with T2DM who visited the Department of Endocrinology and Metabolism of our hospital between November 2017 and November 2020 were recruited. The diagnosis of T2DM was based on the 1999 World Health Organization (WHO) diagnostic criteria for diabetes [10]. The cohort included 74 males (56.92%) and 56 females (43.08%). According to ultrasound findings, patients were divided into an NAFLD group (n=80) and a non-NAFLD group (n=50). Exclusion criteria were: (1) special types of diabetes, type 1 diabetes, or acute diabetic complications; (2) various viral hepatitis, autoimmune diseases, hereditary hepatitis, or drug-induced liver disease; (3) excessive alcohol consumption; (4) pregnancy or lactation; (5) use of lipid-lowering medications; and (6) history of coronary heart disease, cerebral infarction, acute infection, or malignant tumors. This study was approved by the Biomedical Research Ethics Committee of the Affiliated Hospital of Jiangsu University, Jiangsu Province, China.

Methods

General clinical data including height, weight, waist circumference (WC), hip circumference (HC), and blood pressure were collected. BMI and WHR were calculated. All subjects underwent oral glucose tolerance tests. Insulin and C-peptide levels were measured by chemiluminescence assay. Serum triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), total cholesterol (TC), and high-density lipoprotein cholesterol (HDL-C) were determined by enzymatic methods. Blood glucose levels were measured using the glucose oxidase method. Glycated hemoglobin (HbA1c) was assayed by high-performance liquid chromatography. Serum uric acid (UA), serum creatinine (Scr), and blood urea nitrogen (BUN) levels were detected using an automatic biochemical analyzer.

The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as FPG $\times$ FIns / 22.5, and the homeostasis model assessment of β-cell function (HOMA-β) was calculated as FIns $\times$ 20 / (FPG - 3.5).

Serum EDA levels were measured using a commercial human enzyme-linked immunosorbent assay (ELISA) kit (Wuhan EIAab Science Co., Ltd., Catalog No. E1976H). The assay sensitivity was less than 20 pg/mL, with intra-assay coefficient of variation (CV) $\leq$ 7.8% and inter-assay CV $\leq$ 8.9%. Procedures were performed according to the manufacturer's instructions. Absorbance at 450 nm was measured using a microplate reader (ThermoFisher, Multiskan GO), and standard curves were generated. The ELISA detection range was 78–5000 pg/mL. Abdominal ultrasound examinations were performed by professional

sonographers using a whole-body color Doppler diagnostic system (LOGIQ-9). The diagnosis of NAFLD was based on the Guidelines for the Diagnosis and Treatment of Non-alcoholic Fatty Liver Disease established by the Chinese Society of Hepatology [11].

Statistical Analysis

All statistical analyses were performed using SPSS version 25.0, with $P<0.05$ considered statistically significant. Normally distributed continuous variables were expressed as mean ± standard deviation ($x \pm SD$) and compared between groups using t-tests. Non-normally distributed continuous variables were expressed as median and interquartile range [M (P25, P75)] and compared between groups using Mann-Whitney U tests. Categorical variables were expressed as number (percentage) and compared using ^{2} tests. Spearman/Pearson correlation analysis was used to evaluate associations between serum EDA and other variables. Linear regression analysis was employed to identify independent influencing factors of serum EDA. Logistic regression analysis was used to explore the relationship between EDA levels and NAFLD development in T2DM patients.

Results

Comparison of Clinical and Biochemical Data Between Groups

Table 1 summarizes the baseline clinical characteristics of the study subgroups. There were no statistically significant differences between the two groups in terms of gender, history of hypertension, WHR, FPG, 2hPG, HbA1c, HOMA-β, TC, HDL-C, LDL-C, SCr, or smoking history (Table 1). Compared with the non-NAFLD group, the NAFLD group had younger age and shorter duration of T2DM. The NAFLD group also showed significantly higher BMI, 2hIns, FIns, 2hCP, FCP, HOMA-IR, TG, UA, AST, and ALT ($P<0.05$ or $P<0.01$), and significantly lower BUN ($P<0.05$). Most importantly, serum EDA concentration was significantly higher in the NAFLD group compared with the non-NAFLD group ($P<0.01$).

Table 1 Comparison of Clinical and Biochemical Data Between Two Groups

Note: Normally distributed data are expressed as mean ± standard deviation ($x \pm s$), while non-normally distributed data are expressed as median and interquartile range [M(P25, P75)]. "a" represents Z-value, "b" represents ^{2} value, and others represent t-values. BMI=body mass index, WHR=waist-to-hip ratio, SBP=systolic blood pressure, DBP=diastolic blood pressure, FPG=fasting plasma glucose, 2hPG=2-hour postprandial glucose, FIns=fasting insulin, 2hIns=2-hour postprandial insulin, FCP=fasting C-peptide, 2hCP=2-hour postprandial C-peptide, HbA1c=glycated hemoglobin, HOMA-IR=homeostasis model assessment of insulin resistance, HOMA-β=homeostasis model assessment of β-cell function, TG=triglycerides, TC=total cholesterol, HDL-C=high-density lipoprotein cholesterol, LDL-C=low-density lipoprotein cholesterol,

ALT=alanine aminotransferase, AST=aspartate aminotransferase, BUN=blood urea nitrogen, SCr=serum creatinine, UA=uric acid; EDA=Ectodysplasin A.

Correlation Analysis Between Serum EDA Concentration and Various Indices

Correlation analysis revealed that serum EDA was significantly positively correlated with age, FIns, 2hIns, HOMA-IR, HOMA-β, AST, and SCr ($P<0.05$ or $P<0.01$) (Table 2). Further multiple linear regression analysis with EDA as the dependent variable showed that 2hIns, age, AST, and WHR entered the equation, indicating that these four parameters were independent correlates of EDA (Table 3).

Table 2 Correlation Analysis of EDA with Clinical and Biochemical Indexes in T2DM Patients

Table 3 Multiple Linear Regression Analysis of EDA and Related Indexes

Binary Logistic Regression Analysis of Factors Affecting NAFLD Development in T2DM Patients

Using the presence or absence of NAFLD as the dependent variable, binary logistic regression analysis was performed. The results showed that the risk of NAFLD increased with serum EDA concentration in every model ($P<0.05$ or $P<0.01$) (Table 4). In the basic unadjusted model, serum EDA was independently associated with NAFLD (OR 95% CI: 1.001–1.008, $P<0.05$). After adjusting for age and sex, the OR for NAFLD was 1.005 (95% CI: 1.001–1.009, $P<0.01$) (Table 4, Model 1). Further adjustment for BMI and WHR on top of Model 1 yielded an OR for NAFLD of 1.006 (95% CI: 1.001–1.010, $P<0.01$) (Table 4, Model 2). Additional adjustment for smoking and alcohol consumption history, hypertension history, insulin use, and oral hypoglycemic and antihypertensive medication history showed that the OR for NAFLD was 1.006 (95% CI: 1.002–1.010, $P<0.01$) (Table 4, Model 3).

Table 4 Binary Logistic Regression Analysis of Factors Affecting NAFLD in T2DM Patients

Note: Model 1 adjusted for age and disease duration; Model 2 adjusted for sex, BMI, and WHR in addition to Model 1; Model 3 adjusted for smoking history, alcohol consumption history, and hypertension history in addition to Model 2.

Discussion

The results of this study demonstrate that serum EDA concentration is significantly higher in the T2DM with NAFLD group compared with the non-NAFLD group, and that 2hIns, AST, and WHR are independent correlates of EDA. More importantly, the risk of NAFLD increases with rising serum EDA concentration,

suggesting that EDA may be an important risk factor for the development and progression of NAFLD in T2DM patients.

Obesity is recognized as a major risk factor for NAFLD development. NAFLD is associated with other metabolic abnormalities including hyperlipidemia, impaired glucose tolerance, insulin resistance, and type 2 diabetes mellitus [12,13]. Among these, lipid metabolism disturbance plays a crucial role, as hepatic lipid content balance is influenced by multiple factors including hepatic lipid transport, uptake, synthesis, and secretion. Disruption of this balance further exacerbates hepatic steatosis [14,15]. Our study results show that TG, ALT, AST, uric acid, and BMI were significantly higher in the NAFLD group compared with the non-NAFLD group. Additionally, FIns, 2hIns, FCP, 2hCP, and HOMA-IR were also significantly elevated in the NAFLD group, indicating that these patients compensate for insulin resistance by secreting more insulin. Previous studies have also found that indicators reflecting glucose and lipid metabolism abnormalities are significantly higher in T2DM patients with NAFLD than in those with T2DM alone [16], collectively confirming that insulin resistance and obesity are important contributors to NAFLD development in T2DM patients.

Insulin resistance and increased hepatic TG-derived fatty acid (FA) release lead to elevated hepatic FA concentrations, while lipotoxicity from lipid deposition further drives hepatocyte injury and inflammatory progression, ultimately resulting in liver fibrosis [17]. As a newly discovered hepatokine, EDA has recently been implicated in obesity, insulin resistance, T2DM, and NAFLD [18]. Awazawa et al.'s clinical study demonstrated that in 33 obese male patients, hepatic EDA expression was positively correlated with liver fat content and with histologically determined inflammation and steatosis scores in non-alcoholic steatohepatitis [9]. Another case-control study showed that serum EDA concentration was higher in the NAFLD group than in the control group [19]; however, no studies have investigated abnormal serum EDA expression in T2DM patients with NAFLD. Our study is the first to find that EDA is significantly elevated in the T2DM with NAFLD group compared with the T2DM alone group, and that it is significantly positively correlated with FIns, 2hIns, HOMA-IR, HOMA-β, and AST, suggesting that EDA may contribute to systemic lipid metabolism disorders and hepatic dysfunction through insulin resistance. Previous research has shown that insulin resistance can alter systemic lipid metabolism, leading to dyslipidemia characterized by increased secretion of LDL-C and TG, decreased HDL-C levels, and accelerated NAFLD development [20]. Yang et al. similarly found that EDA was significantly positively correlated with HOMA-IR, but also with BMI, WHR, FPG, and HbA1c—associations not observed in our study. Additionally, Bayliss et al. found no significant correlation between plasma EDA and HOMA-IR, FPG, or HbA1c [21]. The discrepancies with our results may be due to differences in study populations: our study focused on T2DM patients with NAFLD, Yang et al. studied NAFLD patients without T2DM, and Bayliss et al. investigated NAFLD patients with T2DM. Pathological characteristics at different disease stages may influence outcomes. Moreover, our study and Yang et al. used abdominal ultrasound for NAFLD diagnosis, whereas Bayliss

et al. used liver biopsy and histological assessment, and different diagnostic criteria can affect results. Furthermore, our study found that older age, higher 2hIns, and higher AST were associated with higher serum EDA concentration. While age is a non-modifiable risk factor, 2hIns and AST are modifiable, suggesting that improvements in insulin resistance and hepatic function may reduce serum EDA levels and thereby alleviate lipid metabolism disorders. After adjusting for common clinical risk factors, circulating EDA levels remained closely associated with high NAFLD incidence. Our results also showed that age and disease duration were significantly lower in the T2DM with NAFLD group compared with the non-NAFLD group. Considering the characteristics of our study subjects, older patients have poorer fat storage capacity, higher proportions of hepatic fibrosis, longer duration of T2DM intervention and treatment, and higher mortality rates, all of which may have influenced the results to some extent [22].

This study has several limitations. First, it is a cross-sectional study with a small sample size, and causality cannot be established, which may affect the accuracy of the results. Second, the lack of normal subjects as controls may reduce the credibility of the findings. Third, NAFLD was diagnosed by ultrasound rather than liver biopsy, which may have impacted the results.

Conclusion

In summary, serum EDA levels are significantly elevated in T2DM patients with NAFLD and are closely associated with insulin resistance, revealing that EDA is an important risk factor for NAFLD development in T2DM patients and may participate in NAFLD pathogenesis and progression. However, the underlying mechanisms remain to be elucidated and require further in-depth investigation.

Conflict of Interest: All authors declare no conflict of interest.

Author Contributions: YUAN Guoyue conceived the study; DENG Xia and YUAN Guoyue conducted feasibility analysis and provided research guidance; GU Tian, CAI Zhensheng, and LI Haoxiang participated in data collection and organization; QIAN Fangfang collected data, performed statistical analysis, and wrote the manuscript; ZHAO Li and YANG Ling provided writing guidance and revised the manuscript.

References

Steinman J, Salomao M, Pajvani U. Zonation in NASH-a key paradigm for understanding pathophysiology and clinical outcomes. *Liver International*: Official Journal of the International Association for the Study of the Liver. 2021. doi: 10.1111/liv.15025.

Semmler G, Datz C, Reiberger T, Trauner M. Diet and Exercise in NAFLD/NASH: Beyond the Obvious. *Liver International*: Official Journal of the International Association for the Study of the Liver. 2021. doi:

10.1111/liv.15024.

Varghese D, Ali B. Pathological Crosstalk Between Oxidized LDL and ER Stress in Human Diseases: A Comprehensive Review. *Frontiers in Cell and Developmental Biology*. 2021;9:674103. doi: 10.3389/fcell.2021.674103.

刘君, 杨艳君, 韩伟. 尿微量白蛋白与尿肌酐比值对 2 型糖尿病合并非酒精性脂肪肝的影响. 安徽医药. 2021;25:1656-1659. doi: 10.3969/j.issn.1009-6469.2021.08.042.

de Oliveira Dos Santos A, de Oliveira Zanuso B, Miola V, et al. Adipokines, Myokines, and Hepatokines: Crosstalk and Metabolic Repercussions. *International Journal of Molecular Sciences*. 2021;22(5). doi: 10.3390/ijms22052639.

Ezer S, Bayés M, Elomaa O, Schlessinger D, Kere J. Ectodysplasin is a collagenous trimeric type II membrane protein with a tumor necrosis factor-like domain and co-localizes with cytoskeletal structures at lateral and apical surfaces of cells. *Human Molecular Genetics*. 1999;8(11):2079-2086. doi: 10.1093/hmg/8.11.2079.

Chen Y, Molloy SS, Thomas L, Gambee J, Bächinger HP, Ferguson B, et al. Mutations within a furin consensus sequence block proteolytic release of ectodysplasin-A and cause X-linked hypohidrotic ectodermal dysplasia. *Proceedings of the National Academy of Sciences of the United States of America*. 2001;98(13):7218-7223. doi: 10.1073/pnas.131076098.

Nikopensius T, Annilo T, Jagomägi T, Gilissen C, Kals M, Krjutškov K, et al. Non-syndromic tooth agenesis associated with a nonsense mutation in ectodysplasin-A (EDA). *Journal of Dental Research*. 2013;92(6):507-511. doi: 10.1177/0022034513487210.

Awazawa M, Gabel P, Tsaousidou E, Nolte H, Krüger M, Schmitz J, et al. A microRNA screen reveals that elevated hepatic ectodysplasin A expression contributes to obesity-induced insulin resistance in skeletal muscle. *Nature Medicine*. 2017;23(12):1466-1473. doi: 10.1038/nm.4420.

Diagnosis and Classification of Diabetes Mellitus and its Complications. In: Report of a WHO Consultation, Part 1: Diagnosis and Classification of Diabetes Mellitus. World Health Organization; 1999.

中华医学会肝病学分会脂肪肝和酒精性肝病学组. 非酒精性脂肪性肝病诊疗指南 (2010 年修订版). 中华肝脏病杂志. 2010;18(3):167-170.

Maurice J, Manousou P. Non-alcoholic fatty liver disease. *Clinical Medicine* (London, England). 2018;18(3):245-250. doi: 10.7861/clinmedicine.18-3-245.

Nagashimada M, Honda M. Effect of Microbiome on Non-Alcoholic Fatty Liver Disease and the Role of Probiotics, Prebiotics, and Biogenics. *International Journal of Molecular Sciences*. 2021;22(15). doi: 10.3390/ijms22158008.

Watt MJ, Miotto PM, De Nardo W, Montgomery MK. The Liver as an Endocrine Organ-Linking NAFLD and Insulin Resistance. *Endocrine Reviews*. 2019;40(5):1367-1393. doi: 10.1210/er.2019-00034.

Li Y, Su X, Rohatgi N, Zhang Y, Brestoff JR, Shoghi KI, et al. Hepatic lipids promote liver metastasis. *JCI Insight*. 2020;5(17). doi: 10.1172/jci.insight.136215.

卢亚男, 刘丽俊, 李伟. GA/HbA1c 比值与 2 型糖尿病合并非酒精性脂肪肝相关. 基础医学与临床. 2020;40(12). doi: 10.16352/j.issn.1001-6325.2020.12.006.

Fuchs C, Radun R, Dixon E, et al. Hepatocyte-specific deletion of adipose triglyceride lipase (ATGL/PNPLA2) ameliorates dietary induced steatohepatitis in mice. *Hepatology* (Baltimore, Md). 2021. doi: 10.1002/hep.32112.

Han H, Jiang Y, Wang M, et al. Intestinal dysbiosis in nonalcoholic fatty liver disease (NAFLD): focusing on the gut-liver axis. *Critical Reviews in Food Science and Nutrition*. 2021:1-18. doi: 10.1080/10408398.2021.1966738.

Yang J, Zhou W, Zhu J, Wu Y, Xu L, Wang Y, et al. Circulating ectodysplasin A is a potential biomarker for nonalcoholic fatty liver disease. *Clinica Chimica Acta*; International Journal of Clinical Chemistry. 2019;499:134-141. doi: 10.1016/j.cca.2019.09.009.

Ormazabal V, Nair S, Elfeky O, Aguayo C, Salomon C, Zuñiga F. Association between insulin resistance and the development of cardiovascular disease. *Cardiovascular Diabetology*. 2018;17(1):122. doi: 10.1186/s12933-018-0762-4.

Bayliss J, Ooi G, De Nardo W, Shah Y, Montgomery M, McLean C, et al. Ectodysplasin A Is Increased in Non-Alcoholic Fatty Liver Disease, But Is Not Associated With Type 2 Diabetes. *Frontiers in Endocrinology*. 2021;12:642432. doi: 10.3389/fendo.2021.642432.

Cardoso AL, Fernandes A, Aguilar-Pimentel JA, de Angelis MH, Guedes JR, Brito MA, et al. Towards frailty biomarkers: Candidates from genes and pathways regulated in aging and age-related diseases. *Ageing Research Reviews*. 2018;47:214-277. doi: 10.1016/j.arr.2018.07.004.

Note: Figure translations are in progress. See original paper for figures.