Serum cathepsin S is associated with decreased insulin sensitivity and the development of diabetes type 2 in a community based cohort of elderly men

Short title: Jobs -Cathepsin S and insulin sensitivity

Elisabeth Jobs, MSc, Ulf Risérus, MMed, PhD, Erik Ingelsson, MD, PhD, Johan Sundström, MD, PhD, Magnus Jobs, MSc, PhD, Elisabet Nerpin, MMed, David Iggman, MD, Samar Basu, MSc PhD, Anders Larsson, MD, PhD, Lars Lind, MD, PhD, and Johan Ärnlöv, MD, PhD

Department of Public Health and Caring Sciences/ Section of Geriatrics (E.J, E.N, J.Ä.), Section of Clinical Nutrition and Metabolism (U.R, D.I.), Section of Oxidative Stress and Inflammation (S.B.); and Laboratoire de Biochimie, Biologie Moléculaire et Nutrition, Faculté de Pharmacie, Université d'Auvergne, Clermont-Ferrand, France (SB); Department of Medical Sciences (J.S.,A.L., L.L.), Uppsala University, Uppsala Sweden; Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden (E.I.) and School of Health and Social Studies, Dalarna University, Falun, Sweden (E.J., E.N., M.J., J.Ä.)

Correspondence to: Johan Ärnlöv, Department of Public Health and Caring Sciences/Geriatrics, Uppsala Science Park, SE-75185 Uppsala, Sweden

Fax: +46-18-6117976; phone: +46-70-6077703

E-mail: johan.arnlov@pubcare.uu.se

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Abstract

Objective

To investigate associations between serum cathepsin S, impaired insulin sensitivity, defective insulin secretion, and diabetes risk in a community-based sample of elderly men without diabetes.

Research design and Methods

Serum cathepsin S, insulin sensitivity (euglycaemic hyperinsulinaemic clamp) and insulin secretion (early insulin response during an oral glucose tolerance test) were measured in Uppsala Longitudinal Study of Adult Men (mean age 71 years, n=905). Thirty participants developed diabetes during 6 years follow-up.

Results

After adjustment for age, anthropometric variables and inflammatory markers, higher cathepsin S was associated with decreased insulin sensitivity (regression coefficient per SD increase -0.09, 95% CI, -0.14- -0.04 p=0.001) while no association with early insulin response was found. Moreover, higher cathepsin S was associated with higher risk for developing diabetes (odds ratio per SD increase 1.48, 95 % CI 1.08-2.01, p=0.01).

Conclusions

Cathepsin S activity appears to be involved in the early dysregulation of glucose and insulin metabolism.

Key words: Cathepsin S, insulin sensitivity, insulin secretion, cohort, epidemiology

Introduction

Adipokines, inflammatory cytokines secreted from adiopose tissue, have been suggested to play a key role in the development of insulin resistance and diabetes.(1) Cathepsin S, is a potent cysteine protease, that is highly expressed and secreted in adipose tissue of obese individuals (2) and has been suggested to be an important regulator of inflammatory activity.(3) Thus, we hypothesized that cathepsin S levels would be involved in the early dysregulation of glucose and insulin metabolism prior to development of diabetes. Accordingly, we investigated the association between serum cathepsin S and the two major underlying causes of diabetes, impaired insulin sensitivity and impaired insulin secretion in a community-based sample of elderly men without diabetes. In secondary analyses, we also investigated the longitudinal association between serum cathepsin S and the incidence of diabetes.

Research Design and Methods

The design and selection criteria of the Uppsala Longitudinal Study of Adult Men (ULSAM) have been described previously (4) and further details can be found on the Internet (http://www.pubcare.uu.se/ULSAM/). The present analyses are based on the third examination cycle (baseline1991-1995), n=1221, mean age 71 years) where 1161 men were free from diabetes. Of these, 905 men had valid measurements of cathepsin S and co-variates Follow-up data on diabetes status at the fourth examination cycle (1998-2002), was available in 597 participants.

Venous blood samples were drawn at baseline and stored at –70°C until analysis. Serum levels of cathepin S was measured by ELISA (human cathepsin S (Total), DY1183, R&D Systems) in frozen samples (mean freezer time 14.6 years [range 12.9-16.7 years].(5) Serum levels of high sensitivity C-reactive protein (CRP), interleukin (IL)-6, adiponectin, cystatin C, and triglycerides were performed as previously described. (5)Diabetes mellitus was diagnosed as fasting plasma glucose ≥ 7.0 mmol/l (≥ 126 mg/dl), or use of oral hypoglycaemic agents or insulin.

The euglycaemic- hyperinsulinaemic clamp (Clamp) technique according to De Fronzo (6) was used, with a slight modification to suppress hepatic glucose production (7), for estimation of in vivo sensitivity to insulin. The glucose infusion rate during the last hour (M-value) was used as the measure of insulin sensitivity. An oral glucose tolerance test (OGTT) was performed and beta cell function was estimated by the early insulin response: $([\text{insulin}_{30\text{min}}]-[\text{insulin}_{0\text{min}}]) / ([\text{glucose}_{30\text{min}}-\text{glucose}_{0\text{min}}])$.

Statistical analysis

Linear regression analyses were used to assess cross-sectional associations between cathepsin S (independent variable), insulin sensitivity (dependent variable) or insulin secretion (dependent variable), in separate multivariable models (see Table 1). Logistic regression was used to investigate the longitudinal association between cathepsin S and the development of diabetes.

Results

Baseline characteristics of the study population is presented in Supplementary Table 1. Higher serum cathepsin S was significantly associated with decreased insulin sensitivity (glucose disposal rate, M) in all multivariable (Models A-E, Table 1) while no association was found between cathepsin S and early insulin response. The results were similar in participants with BMI<30 kg/m^2 (Table 1).

At the follow-up after 6 years (median follow-up 6.5 years [range 4.5-9.2 years]), 41 participants had developed diabetes. One SD increase in cathepsin S at baseline was associated with 41-48% risk of developing diabetes in all multivariable models (Model D: Odds ratio per SD increase 1.48, 95 % CI 1.08-2.01, p=0.01, Supplementary Table 2).

Conclusions

Our study suggests that increased cathepsin S levels are involved in the early dysregulation of glucose and insulin metabolism, prior to the development of diabetes.

Comparison with the literature

Previous data on the association between circulating cathepsin S and the underlying causes of diabetes is scarce. In a previous small study in women, no associations between serum cathepsin S and insulin sensitivity, as evaluated by the Quantitative Insulin-sensitivity Check Index (QUICKI), was observed. (8) However, QUICKI have limitations as an indicator of insulin sensitivity (9) which may explain the discrepancy with the present study

One previous study reported increased cathepsin S levels in patients with type 2 diabetes. (10) However, the longitudinal association of cathepsin S and diabetes incidence has not been reported previously.

Potential mechanisms

Our understanding of the importance of adipose tissue-induced inflammation in the development of insulin resistance and diabetes is increasing (1) Cathepsin S may play a part in this process. Cathepsin S is released by macrophages.(11) and participate in the pathophysiologic remodeling of extracellular matrix (ECM). (12) which lead to adipogenesis and/or adipose cell hyperthrophy. (13) This adipose tissue expansion may trigger hypoxia which in turn results in local low grade inflammation that has been suggested to be a causal link to insulin resistance. (14) Also, cystatin C, the endogenous inhibitor of cathepsin S, has been found to be elevated in obese subjects, both in the circulation and in adipose tissue expression, independently of reduced eGFR, which could be a reflection of adipose tissue growth control through cathepsin inhibitions.(13) However, the fact that associations between cathepsin S and insulin sensitivity were independent of adiposity measures, inflammatory markers and cystatin C in the present study would argue against adipose tissue-derived inflammation as the sole mechanistic explanation of our results. Still we cannot rule out that there may be substantial residual confounding as both the adiposity measurements and circulating inflammatory markers used in the present study may be poor proxies for specific inflammation in adipose tissue. Cathepsin S has also been shown to be associated with triglyceridemia(8) and an increased cardiovascular risk(5), but these mechanisms did not appear to mediate the present associations (see Table, Model E)

Clinical implications

The development of selective inhibitors of cathepsin S is currently pursued by several pharmaceutical companies (15) but whether cathepsin S inhibitors improve insulin sensitivity or prevent diabetes remains to be established.

Limitations

Limitations of the study include the unknown generalizability to women and other age- and ethnic groups, the large number of participants lost to follow-up, and the modest number of incident diabetes events during follow-up. Also, it is not possible to establish causality with a cross-sectional study design, and there is a risk of reverse causation. Further studies are needed for validation, for exploration of the underlying pathophysiology, and for evaluation of the clinical utility of measuring cathepsin S.

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Author contributions

Author contributions: J.Ä. researched data, edited manuscript, contributed to discussion, provided funding. E.J. wrote manuscript, researched data, contributed to discussion. U.R. reviewed manuscript, contributed to discussion. E.I. reviewed manuscript, contributed to discussion. J.S reviewed manuscript, contributed to discussion. M.J reviewed manuscript. E.N reviewed manuscript. D.I reviewed manuscript. S.B reviewed manuscript. A.L reviewed manuscript, contributed with data, L.L reviewed manuscript.

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References

1. Li ZY, Wang P, Miao CY: Adipokines in inflammation, insulin resistance and cardiovascular disease. *Clinical and experimental pharmacology & physiology* 38:888-896, 2011

2. Taleb S, Lacasa D, Bastard JP, Poitou C, Cancello R, Pelloux V, Viguerie N, Benis A, Zucker JD, Bouillot JL, Coussieu C, Basdevant A, Langin D, Clement K: Cathepsin S, a novel biomarker of adiposity: relevance to atherogenesis. *Faseb J* 19:1540-1542, 2005

3. Jobs E, Riserus U, Ingelsson E, Helmersson J, Nerpin E, Jobs M, Sundstrom J, Lind L, Larsson A, Basu S, Arnlov J: Serum Cathepsin S Is Associated with Serum C-Reactive Protein and Interleukin-6 Independently of Obesity in Elderly Men. *J Clin Endocrinol Metab* 95:4460-4464, 2010

4. Hedstrand H: A study of middle-aged men with particular reference to risk factors for cardiovascular disease. *Upsala journal of medical sciences. Supplement* 19:1-61, 1975

5. Jobs E, Ingelsson E, Riserus U, Nerpin E, Jobs M, Sundstrom J, Basu S, Larsson A, Lind L, Arnlov J: Association between serum cathepsin S and mortality in older adults. *JAMA : the journal of the American Medical Association* 306:1113-1121, 2011

6. DeFronzo RA, Tobin JD, Andres R: Glucose clamp technique: a method for quantifying insulin secretion and resistance. *The American journal of physiology* 237:E214-223, 1979

7. Pollare T, Vessby B, Lithell H: Lipoprotein lipase activity in skeletal muscle is related to insulin sensitivity. *Arteriosclerosis and thrombosis : a journal of vascular biology / American Heart Association* 11:1192-1203, 1991

8. Naour N, Rouault C, Fellahi S, Lavoie ME, Poitou C, Keophiphath M, Eberle D, Shoelson S, Rizkalla S, Bastard JP, Rabasa-Lhoret R, Clement K, Guerre-Millo M: Cathepsins in human obesity: changes in energy balance predominantly affect cathepsin s in adipose tissue and in circulation. *J Clin Endocrinol Metab* 95:1861-1868, 2010

9. Singh B, Saxena A: Surrogate markers of insulin resistance: A review. *World journal of diabetes* 1:36-47, 2010

10. Liu J, Ma L, Yang J, Ren A, Sun Z, Yan G, Sun J, Fu H, Xu W, Hu C, Shi GP: Increased serum cathepsin S in patients with atherosclerosis and diabetes. *Atherosclerosis* 186:411-419, 2006

11. Shi GP, Munger JS, Meara JP, Rich DH, Chapman HA: Molecular cloning and expression of human alveolar macrophage cathepsin S, an elastinolytic cysteine protease. *The Journal of biological chemistry* 267:7258-7262, 1992

12. Reddy VY, Zhang QY, Weiss SJ: Pericellular mobilization of the tissue-destructive cysteine proteinases, cathepsins B, L, and S, by human monocyte-derived macrophages. *Proceedings of the National Academy of Sciences of the United States of America* 92:3849-3853, 1995

13. Lafarge JC, Naour N, Clement K, Guerre-Millo M: Cathepsins and cystatin C in atherosclerosis and obesity. *Biochimie* 92:1580-1586, 2010

14. Trayhurn P, Wood IS: Adipokines: inflammation and the pleiotropic role of white adipose tissue. *Br J Nutr* 92:347-355, 2004

15. Gupta S, Singh RK, Dastidar S, Ray A: Cysteine cathepsin S as an immunomodulatory target: present and future trends. *Expert Opin Ther Targets* 12:291-299, 2008

Table. Cross-sectional associations between cathepsin S, insulin sensitivity and early insulin response, n=905.

	Insulin sensitivity (M-value)				Early insulin response*			
Model	**Total cohort**		**BMI<30**		**Total cohort**		**BMI<30***	
	β-coefficient(95% CI)	**P-value**	**β-coefficient(95% CI)**	**P-value**	**β-coefficient(95% CI)**	**P-value**	**β-coefficient(95% CI)**	**P-value**
Model A	-0.13(-0.19- -0.06)	<0.001	-0.12(-0.18- -0.05)	<0.001	-0.01(-0.07-0.06)	0.82	-0.01(-0.08-0.05)	0.68
Model B	-0.09(-0.14- -0.04)	<0.001	-0.10(-0.15- -0.04)	0.001	-0.02(-0.08-0.05)	0.58	-0.02(-0.08-0.05)	0.61
Model C	-0.10(-0.16- -0.03)	0.001	-0.10(-0.16- -0.03)	0.003	-0.01(-0.08-0.05)	0.71	-0.02(-0.09-0.05)	0.55
Model D	-0.09(-0.14- -0.04)	0.001	-0.09(-0.14- -0.03)	0.002	-0.02(-0.08-0.05)	0.59	-0.02(-0.09-0.05)	0.53
Model E	-0.06(-0.11—0.01	0.02	-0.06(-0.12- -0.00)	0.03	-0.02(0.09-0.04)	0.50	-0.03(-0.10-0.04)	0.45

Data are regression coefficients per 1 SD increase ;Primary models)Model A: age; Model B: age, BMI and waist; Model C: age, C-reactive protein, Interleukin-6 and adiponectin; Model D; combined (model A-C), Secondary models:, Model E: Model D+triglycerids, previous cardiovascular disease and cystatin C. *n=805