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Microdialysis and proteomics of subcutaneous interstitial fluid reveals increased galectin-1 in type 2 diabetes patients

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ARTICLE INFO

Article history:
Received 1 February 2016
Accepted 7 April 2016

Keywords:
Microdialysis
Proteomics
Galectin-1
Obesity
Adipose tissue

ABSTRACT

Objective. To identify a potential therapeutic target for type 2 diabetes by comparing the subcutaneous interstitial fluid from type 2 diabetes patients and healthy men.

Methods. Proteomics was performed on the interstitial fluid of subcutaneous adipose tissue obtained by microdialysis from 7 type 2 diabetes patients and 8 healthy participants. 851 proteins were detected, of which 36 (including galectin-1) showed significantly altered expression in type 2 diabetes. We also measured galectin-1 expression in: (1) adipocytes isolated from adipose tissue biopsies from these participants; (2) subcutaneous adipose tissue of 24 obese participants before, during and after 16 weeks on a very low calorie diet (VLCD); and (3) adipocytes isolated from 6 healthy young participants after 4 weeks on a diet and lifestyle intervention to promote weight gain. We also determined the effect of galectin-1 on glucose uptake in human adipose tissue.

Results. Galectin-1 protein levels were elevated in subcutaneous dialysates from type 2 diabetes compared with healthy controls ($p < 0.05$). In agreement, galectin-1 mRNA expression was increased in adipocytes from the type 2 diabetes patients ($p < 0.05$). Furthermore, galectin-1 mRNA expression was decreased in adipose tissue after VLCD ($p < 0.05$) and increased by overfeeding ($p < 0.05$). Co-incubation of isolated human adipocytes with galectin-1 reduced glucose uptake ($p < 0.05$) but this was independent of the insulin signal.

Abbreviations: VLCD, Very low calorie diet; PPAR-γ, Peroxisome proliferator-activated receptor-γ.

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http://dx.doi.org/10.1016/j.metabol.2016.04.003

Conclusion. Proteomics of the interstitial fluid in subcutaneous adipose tissue *in vivo* identified a novel adipokine, galectin-1, with a potential role in the pathophysiology of type 2 diabetes.

1. Introduction

The global prevalence of diabetes has more than doubled since 1980 and at least 350 million people worldwide currently suffer from the disease [1], 90% of whom have type 2 diabetes [2]. Adipose tissue dysfunction is a major contributor to the development of insulin resistance, orchestrated through several different proteins including the adipokines leptin, adiponectin and TNF-α [3,4]. Exploiting the adipose tissue to identify novel therapies for type 2 diabetes has been successful, and several drugs that target the peroxisome proliferator-activated receptor-γ (PPAR-γ) pathway are now available [5]. However, there is still a need for additional treatment strategies.

Proteomic analysis of biopsied adipose tissue, cultured adipocytes and plasma has identified candidate proteins [6–8], but none have so far been exploited as therapeutic targets of type 2 diabetes. To date, no study has investigated human subcutaneous adipose tissue *in vivo* to find a future therapeutic target for this disease. We have previously shown that microdialysis is an attractive method for acquisition of proteins from the subcutaneous interstitial fluid *in situ* [9]. Indeed, the *in vivo* metabolic milieu is more accurately reflected in samples obtained by microdialysis than in tissue biopsies or blood samples, and the catheterization procedure for microdialysis is minimally invasive [10].

Here we combined microdialysis of human subcutaneous adipose tissue with tandem mass spectrometry, and showed increased levels of galectin-1 in the subcutaneous adipose tissue of type 2 diabetes patients. We chose to investigate this protein further as it was a secreted protein, upregulated in the diabetes group.

2. Methods

2.1. Participants

Seven men with type 2 diabetes and 8 age-matched healthy men were recruited from primary health care centers and through advertisement in local newspapers. Inclusion criteria for participants with type 2 diabetes: Caucasian males, 40–65 years, diabetes duration <5 years, weight stable (±3 kg last three months) and heredity for type 2 diabetes. Inclusion criteria for healthy controls: Caucasian males, 40–65 years, HbA1c <6.5% (48 mmol/mol), waist <94 cm and no diabetes heredity. Exclusion criteria for all participants: medications with possible effects on metabolism such as cortisol, natural products or other supplements; nicotine use; ongoing infection; and uncontrolled blood pressure. The healthy controls were drug naive. In the diabetes group, one patient used candesartan, two patients used metformin and one patient

Table 1 – Clinical Characteristics of Study Participants [a].

Clinical characteristics	Control	Type 2 diabetes	p Value
Gender, male	8	7	
Diabetes duration, years	–	1.6 ± 1.8	
Age, years	54 ± 10	55 ± 8	0.83
Body mass index, kg/m^2	23.4 ± 1.6	25.9 ± 1.8	**0.02**
Waist, cm	88 ± 6	97 ± 5	**0.01**
Waist/hip ratio	0.90 ± 0.06	0.96 ± 0.04	**0.04**
Body fat, %	19 ± 6	26 ± 8	0.11
Fat cell diameter, μm	89 ± 5	98 ± 4	0.17
HbA1c, % (mmol/mol) [b]	5.2 ± 0.2 (33.4 ± 2.1)	6.4 ± 0.7 (46.7 ± 8.2)	**<0.01**
Fasting P-Glucose, mmol/l	5.1 ± 0.4	8.0 ± 1.4	**<0.01**
Fasting S-Insulin, mU/l	3.7 ± 2.0	6.6 ± 2.2	**0.02**
HOMA index [c]	0.8 ± 0.5	2.3 ± 0.8	**<0.01**
S-HDL, mmol/l	1.44 ± 0.36	1.36 ± 0.29	0.68
S-LDL, mmol/l	3.5 ± 0.3	3.1 ± 0.2	0.37
S-Triglycerides, mmol/l	1.0 ± 0.7	1.2 ± 0.6	0.49
Systolic blood pressure, mm Hg	137 ± 14	137 ± 18	0.97
Diastolic blood pressure, mm Hg	85 ± 6	90 ± 11	0.25

[a] Data presented as mean ± SD.
[b] HbA1c normal interval: 5.0–6.4% and 31–46 mmol/mol, respectively.
[c] HOMA = Homeostatic model assessment.