

Urocortin 3 levels are impaired in overweight humans with and without type 2 diabetes and modulated by exercise

Sina Kavalakatt^{1#}, Abdelkrim Khadir^{1#}, Dhanya Madhu¹, Maha Hammad¹, Sriraman Devarajan², Jehad Abubaker¹, Fahd Al-Mulla², Jaakko Tuomilehto^{2,3} and Ali Tiss^{1*}

¹ Biochemistry and Molecular Biology department, Research Division, Dasman Diabetes Institute, Kuwait

² Research Division, Dasman Diabetes Institute, Kuwait

³ Department of Public Health, University of Helsinki, Helsinki, Finland; Department of Public Health Solutions, National Institute for Health and Welfare, Helsinki, Finland

Supplementary Information

Table S1: List of primers used for RT-PCR analysis

Gene	Catalogue # or sequence
Human Ucn3	Hs00846499 (<i>Applied Biosystem, USA</i>)
Human Gapdh	Hs02786624 (<i>Applied Biosystem, USA</i>)
Mouse Ucn3	Mm00453206 (<i>Applied Biosystem, USA</i>)
Mouse Gapdh	Mm99999915 (<i>Applied Biosystem, USA</i>)
<i>PPARG</i>	Mm00440940 (<i>Applied Biosystem, USA</i>)
<i>CRHR2</i>	Mm00438308 (<i>Applied Biosystem, USA</i>)
<i>FABP4</i>	Mm00445878 (<i>Applied Biosystem, USA</i>)
<i>FASN</i>	Mm00662319 (<i>Applied Biosystem, USA</i>)
<i>LPL</i>	Mm00434764 (<i>Applied Biosystem, USA</i>)
<i>LIPE</i>	Mm00495359 (<i>Applied Biosystem, USA</i>)
<i>TNF</i>	Mm00443258 (<i>Applied Biosystem, USA</i>)
<i>IL6</i>	Mm00446190 (<i>Applied Biosystem, USA</i>)
<i>C/EBP</i>	5'-GAACAGCAACGAGTACCGGGTA-3' 5'-GCCATGGCCTTGACCAAGGAG-3'
<i>ACC</i>	5'-GAACAGCAACGAGTACCGGGTA-3' 5'-GCCATGGCCTTGACCAAGGAG-3'

Table S2: Multivariate Linear Regression analysis with UCN3 as dependent variable

Independent variables	Normal-weight		All Non-Diabetic	
	β coefficient	p value	β coefficient	p value
Age	-0.374	0.929	-0.022	0.840
Gender	0.389	0.429	-0.033	0.883
TGL	-0.057	0.511	-0.090	0.828
FPG	0.423	0.922	-0.080	0.498
HbA1c	-0.184	0.512	0.365	0.588
Insulin	-0.062	0.766	-0.045	0.007*
GLP-1	-0.128	0.904	0.140	0.731
Glucagon	0.117	0.889	-0.088	0.403
Leptin	0.075	0.854	-0.055	0.545
Visfatin	0.355	0.903	0.221	0.717
RANTES	-0.143	0.561	-0.217	0.099

Table S3: Odds Ratio analysis by binary logistic regression models

Covariates	Overweight Non-Diabetic	Overweight - Diabetic	
	Reference	OR (95% CI)	p value
UCN3	1.00	2.24 (1.01 – 4.96)	0.019*
TGL	1.00	1.77 (1.13 – 2.79)	0.014*
RANTES	1.00	1.12 (1.01 – 1.27)	0.031*
C-Peptide	1.00	1.03 (0.99 – 1.08)	0.072
GLP1	1.00	1.01 (1.00 – 1.05)	0.039*
Visfatin	1.00	1.04 (1.00 – 1.11)	0.030*

Model adjusted for age and gender, * $p < 0.05$ significant

Table S4: Effect of exercise in overweight subjects

		Non-Diabetic (n=39)	Diabetic (n=39)
Gender (M/F)		17/22	25/14
BMI (kg/m ²)	Before	31.04±4.12	32.17±3.41
	After	30.28±4.41**	31.86±3.26
PBF (%)	Before	35.84±5.78	35.45±5.34
	After	34.81±6.48**	34.80±5.35[§]
Waist (cm)	Before	98.25±8.35	107.74±8.76
	After	94.61±8.29**	105.34±9.12[§]
VO ₂ max (ml/kg/min)	Before	17.95±3.68	17.69±3.59
	After	21.22±4.72***	18.52±4.42
Cholesterol (mmol/l)	Before	5.21±0.97	4.78±1.27
	After	5.19±1.03	4.29±0.91[§]
GLU (mmol/l)	Before	5.21±0.62	8.18±2.98
	After	5.51±1.12*	7.89±2.74
HbA1c (%)	Before	5.93±1.21	7.71±1.95
	After	5.70±0.48	7.10±1.38^{§§}
Insulin (ng/ml)	Before	4.18±1.57	2.96±1.22
	After	3.41±1.28*	2.80±1.53
Ghrelin (ng/ml)	Before	647.63±205.29	617.41±228.0
	After	677.06±203.54	671.90±272.3[§]
GIP (ng/ml)	Before	807.51±809.51	911.26±535.3
	After	437.04±284.1**	605.28±358.4^{§§}
UCN3 (ng/ml)	Before	6.68 (0.64 – 65.8)	9.49 (0.77 – 104.92)
	After	8.37 (0.76 – 72.8)*	10.56 (1.06 – 98.3)

Only statistically significant data are presented. Data are presented as the mean ± SD. VO₂ max (maximum oxygen consumption), and HbA1c (hemoglobin A1c). Sig (significant) of paired-t test. * $p < 0.05$, ** $p < 0.005$, *** $p < 0.0005$ where * is significance overweight non-diabetic before and after exercise. [§] $p < 0.05$, ^{§§} $p < 0.005$, ^{§§§} $p < 0.0005$, where [§] is significance between overweight diabetic before and after exercise.

Figures

Figure S1:

- (A) Representative confocal immunofluorescence images illustrating UCN3 expression in positive and negative controls using pancreas biopsies.
- (B) Representative confocal immunofluorescence images illustrating adiponectin abundance in SAT from normal-weight, overweight with and without people.