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Effects of Glypican-4 Protein on INS1E Cell Viability and Insulin Signaling

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BACKGROUND

Glypican-4 is a heparan sulfate proteoglycan, released from human and rodent adipose tissue and shown to regulate insulin signaling through direct interaction with the insulin receptor, enhancing its activation and downstream signaling.[1] Because of its positive effect on insulin sensitivity, glypican-4 might play a role in the development of obesity, insulin resistance and type 2 diabetes (T2D).[2,3] Glypican-4 serum levels are associated with obesity and insulin resistance.[2,3] Several studies revealed a positive correlation between glypican-4 and obesity-related parameters, e.g. weight, BMI, WHR, body fat content.[3,4,5]

Glypican-4 is an interesting new adipokine and might link obesity and T2D. Because of the fact that studies concerning this protein, specifically measuring circulating levels in lean and obese children, are rare, we found it interesting to investigate this heparan sulfate proteoglycan.
We hypothesized: Glypican-4 plays an essential role in β-cell function and went to answer the following questions:

1. Do INS1E cells express glypican-4?
2. Does extracellular glypican-4 influence β-cell function?
3. Does glypican-4 link obesity to T2D?

Methods

1. Expression	2. β-Cell Function	3. Clinical Relevance
Cell Culture: INS-1E = insulin-secreting rat insulinoma cell line		ELISA Kit Validation
Western Blot	WST1 Assay (Cell Viability)	Serum Sample Measurement (LIFE Child cohort)
TaqMan qPCR	Stimulation Experiments	Statistical Analysis

1. Ussar, S., Bezy, O., Blüher, M. & Kahn, C.R. Glypican-4 enhances insulin signaling via interaction with the insulin receptor and serves as a novel adipokine. *Diabetes* 61, 2289–2298 (2012). **2.** Flehmig, G. *et al.* Identification of adipokine clusters related to parameters of fat mass, insulin sensitivity and inflammation. *PloS one* 9, e99785 (2014). **3.** Li, K. *et al.* Glypican-4 is increased in human subjects with impaired glucose tolerance and decreased in patients with newly diagnosed type 2 diabetes. *Acta diabetologica* 51, 981–990 (2014). **4.** Zhu, H.J. *et al.* The changes of serum glypican4 in obese patients with different glucose metabolism status. *J Clin Endo Metab* 99, E2697-701 (2014). **5.** Leelalertlauw, C. *et al.* Serum glypican 4 level in obese children and its relation to degree of obesity. *Clin Endo* 87, 689–695 (2017).

CONCLUSION

1. INS1E cells express both glypican-4 protein and mRNA. The expression of glypican-4 mRNA is high in the metabolic organs kidney, muscle, pancreas, and liver. In contrast, glypican-4 protein was found in high amounts in lung, muscle, heart, and pancreas.
2. Exogenous glypican-4 seems to have no effect on INS1E cell function, as cell viability and insulin signaling were not significantly influenced after stimulation with recombinant glypican-4 protein.
3. To measure glypican-4 concentration in serum samples of the LIFE Child cohort, we validated two different human glypican-4 ELISA kits. One of them has not measured any glypican-4. Validation results of the other kit are pending.

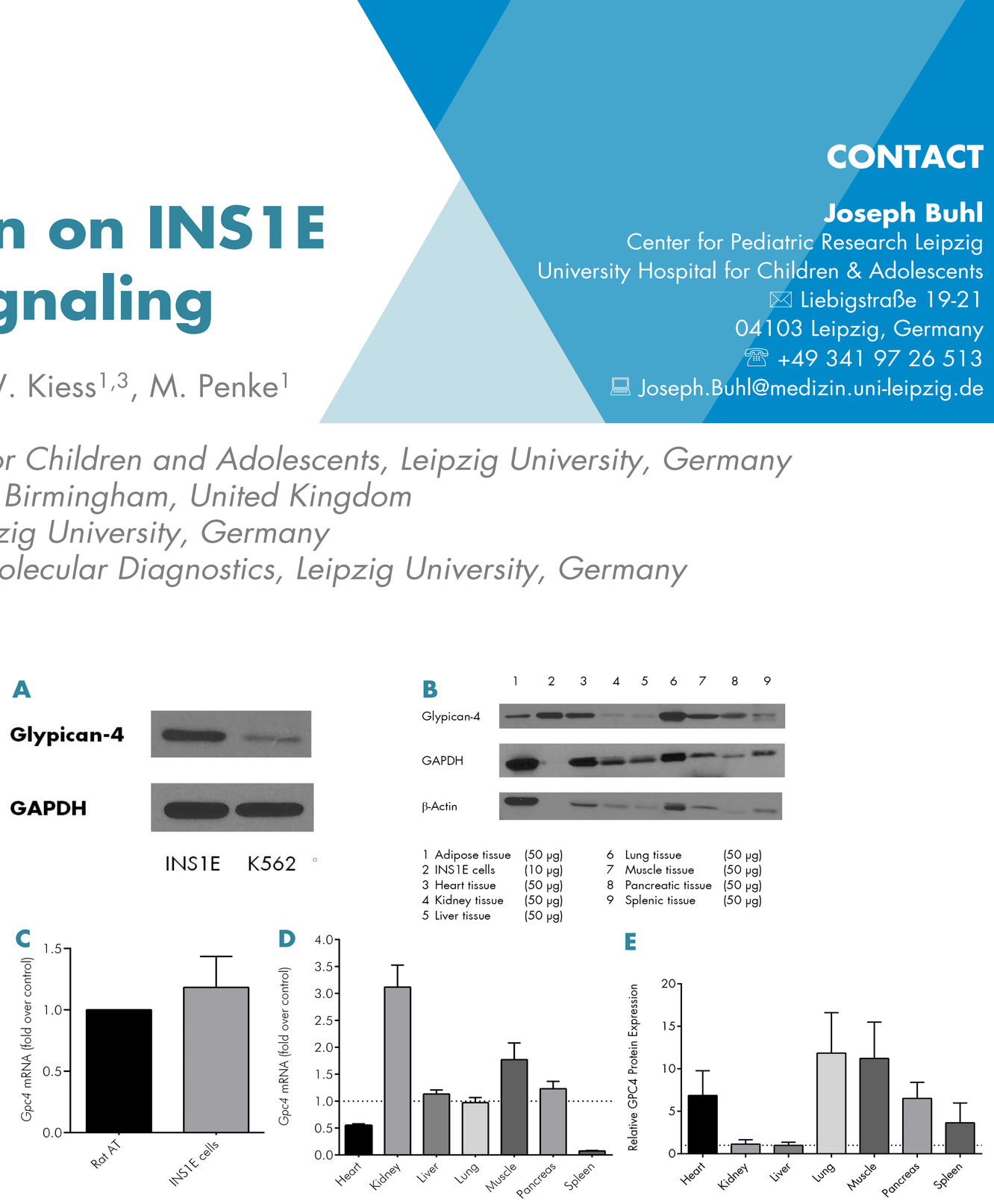
FUNDED BY

DFG Deutsche Forschungsgemeinschaft
German Research Foundation

SFB 1052
Obesity Mechanisms

RESULTS

Figure 1. INS1E cells express glypican-4.
A, B Western Blot detection of glypican-4 protein in INS1E cells and different rat tissues. Positive control: K562 cell lysate (A), rat adipose tissue (B). Loading control: GAPDH (A, B), β-Actin (B). **C, D** TaqMan qPCR detection of *Glypican-4* mRNA in INS1E cells and different rat tissues. Each sample was measured in triplicates (n=3 independent experiments). As control rat adipose tissue (Rat AT) was used and arbitrarily set 1. Data are shown as mean ± SEM, normalized to rat *Hprt1* and rat *Tbp* and analyzed using the ΔΔCt method. **E** Densitometrical analysis of B.



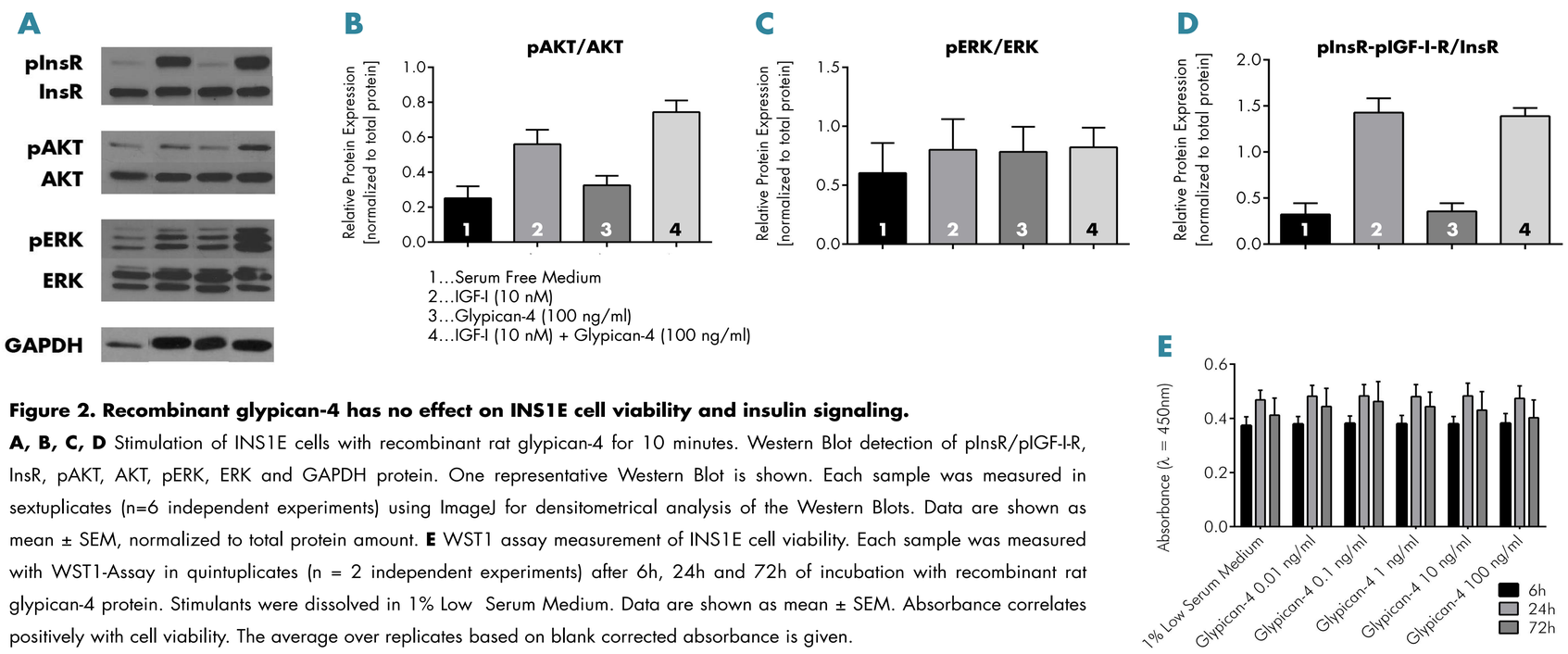
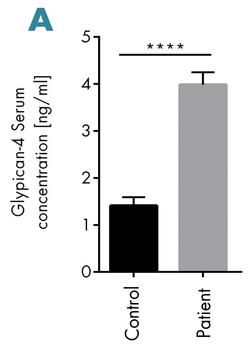


Figure 2. Recombinant glypican-4 has no effect on INS1E cell viability and insulin signaling.
A, B, C, D Stimulation of INS1E cells with recombinant rat glypican-4 for 10 minutes. Western Blot detection of pInsR/pIGF-I-R, InsR, pAKT, AKT, pERK, ERK and GAPDH protein. One representative Western Blot is shown. Each sample was measured in sextuplicates (n=6 independent experiments) using ImageJ for densitometrical analysis of the Western Blots. Data are shown as mean ± SEM, normalized to total protein amount. **E** WST1 assay measurement of INS1E cell viability. Each sample was measured with WST1-Assay in quintuplicates (n = 2 independent experiments) after 6h, 24h and 72h of incubation with recombinant rat glypican-4 protein. Stimulants were dissolved in 1% Low Serum Medium. Data are shown as mean ± SEM. Absorbance correlates positively with cell viability. The average over replicates based on blank corrected absorbance is given.



B

Experiment	Serum Sample Control	Serum Sample Patient
Detected Glypican-4 [ng/ml]	1.1 – 1.4	3.0 – 3.6
Intra-Assay CV [%] (<10%)	11.4	6.2
Inter-Assay CV [%] (<12%)	9.3	9.9
Freeze-Thaw-Cycles [%]	100 > 109 > 85	100 > 99 > 76
Spiking Recovery [%]	0	0

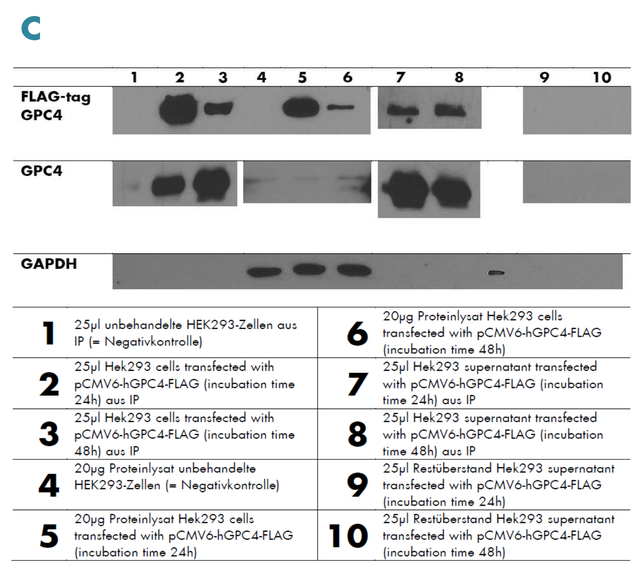


Figure 3. Validation of two different human Glypican-4 ELISA kits.
A Tenfold detection (analysis of intra-assay variation) of glypican-4 protein with human glypican-4 ELISA kit (Cloud Clone Corp.) in control serum and serum of an obese, diabetic patient. We did not detect glypican-4 in any form (serum, recombinant, recombinant spiked in serum) with the other ELISA kit (Signalway Antibody). **B** Validation results of Cloud Clone Corp. human glypican-4 ELISA kit. We could not detect any signal when using recombinant human glypican-4 expressed in E. coli in spiking experiments. Since a potential reason is incorrect folding because of the heterologous expression, we expressed glypican-4 in a mammalian system (HEK293) for use in validation experiments. **C** Immunoprecipitation and Western Blot detection of human recombinant glypican-4 protein expressed in HEK293 cells.