Diabetologia (2013) 56:31–35
DOI 10.1007/s00125-012-2750-7

SHORT COMMUNICATION

Association of exercise-induced hyperinsulinaemic hypoglycaemia with *MCT1*-expressing insulinoma

J. Marquard · A. Welters · T. Buschmann · W. Barthlen · S. Vogelgesang · D. Klee · M. Krausch · A. Raffel · S. Otter · L. Piemonti · E. Mayatepek · T. Otonkoski · E. Lammert · T. Meissner

Received: 18 June 2012 / Accepted: 19 September 2012 / Published online: 17 October 2012

Abstract
Aims/hypothesis Exercise-induced hyperinsulinism (EIHI) is a hypoglycaemic disorder characterised by inappropriate insulin secretion following anaerobic exercise or pyruvate load. Activating promoter mutations in the *MCT1* gene (also known as *SCLA16A1*), coding for monocarboxylate transporter 1 (MCT1), were shown to associate with EIHI. Recently, transgenic *Mct1* expression in pancreatic beta cells was shown to introduce EIHI symptoms in mice. To date, MCT1 has not been demonstrated in insulin-producing cells from an EIHI patient.
Methods In vivo insulin secretion was studied during an exercise test before and after the resection of an insulinoma. The presence of MCT1 was analysed using immunohistochemistry followed by laser scanning microscopy, western blot analysis and real-time RT-PCR of *MCT1*. The presence of MCT1 protein was analysed in four additional insulinoma patients.

J. Marquard and A. Welters contributed equally to this work.

Electronic supplementary material The online version of this article (doi:10.1007/s00125-012-2750-7) contains peer-reviewed but unedited supplementary material, which is available to authorised users.

J. Marquard · A. Welters · E. Mayatepek · T. Meissner (✉)
Department of General Paediatrics,
Neonatology and Paediatric Cardiology,
University Children's Hospital Düsseldorf,
Moorenstrasse 5, 40225 Düsseldorf, Germany
e-mail: Thomas.Meissner@med.uni-duesseldorf.de

J. Marquard · A. Welters · T. Buschmann · S. Otter · E. Lammert
Institute of Metabolic Physiology,
Heinrich-Heine University,
Düsseldorf, Germany

W. Barthlen
Department of Paediatric Surgery, University Hospital Greifswald,
Greifswald, Germany

S. Vogelgesang
Department of Neuropathology, University of Greifswald,
Greifswald, Germany

D. Klee
Department of Diagnostic and Interventional Radiology,
University Hospital Düsseldorf, Medical Faculty,
Düsseldorf, Germany

M. Krausch · A. Raffel
Department of General, Visceral and Paediatric Surgery,
Heinrich-Heine-University,
Düsseldorf, Germany

L. Piemonti
Laboratory of Experimental Surgery,
San Raffaele Scientific Institute,
Milan, Italy

T. Otonkoski
Children's Hospital and Biomedicum Stem Cell Centre,
Program of Molecular Neurology, University of Helsinki,
Helsinki, Finland

E. Lammert
German Diabetes Centre,
Paul-Langerhans-Group of Beta Cell Research,
Düsseldorf, Germany

Results Clinical testing revealed massive insulin secretion induced by anaerobic exercise preoperatively, but not postoperatively. MCT1 protein was not detected in the patient's normal islets. In contrast, immunoreactivity was clearly observed in the insulinoma tissue. Western blot analysis and real-time RT-PCR showed a four- to fivefold increase in MCT1 in the insulinoma tissue of the EIHI patient compared with human pancreatic islets. MCT1 protein was detected in three of four additional insulinomas.
Conclusions/interpretation We show for the first time that an *MCT1*-expressing insulinoma was associated with EIHI and that MCT1 might be present in most insulinomas. Our data suggest that *MCT1* expression in human insulin-producing cells can lead to EIHI and warrant further studies on the role of MCT1 in human insulinoma patients.

Keywords Exercise-induced hyperinsulinism · Insulinoma · Monocarboxylate transporter 1

Abbreviations

EIHI	Exercise-induced hyperinsulinism
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
LSM	Laser scanning microscopy
MCT1	Monocarboxylate transporter 1

Introduction

Exercise-induced hyperinsulinism (EIHI) is a rare inherited hypoglycaemic disorder characterised by inappropriate insulin secretion following anaerobic exercise or pyruvate injection [1]. EIHI was first described in two children who responded to short-term intensive exercise with a massive burst of plasma insulin, resulting in severe hypoglycaemia [2]. Linkage analysis and sequencing of two affected families identified variants within the *MCT1* (also known as *SLC16A1*) promoter as a possible underlying cause of EIHI, with an autosomal dominant inheritance [3]. The variants are likely to result in an increase in levels of monocarboxylate transporter 1 (MCT1), as fibroblasts isolated from the EIHI patients displayed abnormally high *MCT1* transcript levels and the mutations activated *MCT1* transcription in reporter cell lines [3].

Based on these observations and overexpression studies of *MCT1* in vitro [4] it was proposed that overexpression of *MCT1* in beta cells, by allowing the entry of lactate and pyruvate into beta cells during exercise, could trigger insulin release even at low blood glucose concentrations by metabolism of pyruvate. This hypothesis was recently tested using transgenic mice that specifically overexpressed *Mct1* in pancreatic beta cells [5]. Importantly, in contrast to control islets, the islets isolated from these mice secreted insulin in response to pyruvate. Moreover, the transgenic mice secreted insulin in response to exercise, thus mimicking EIHI. However, to date, direct studies of *MCT1* overexpression in human islets have not been possible.

We now report a 16 year-old male patient with typical symptoms of EIHI and insulinoma tissue that overexpressed *MCT1*, and we suggest that *MCT1* might be expressed in most, but not all, insulinomas.

Methods

Exercise test The anaerobic exercise test was conducted on an electrically braked cycle ergometer after an overnight fast. The incremental test started with 50 watt workload, which was increased by 50 watt every 20 s until exhaustion after about 4 min. Blood sampling was performed just before exercise, immediately after exertion and at 4, 15, 25, 30, 45 min after exercise. Plasma lactate was measured with a peroxidase method and plasma glucose was measured with a hexokinase method. Serum insulin was measured with an electrochemiluminescence immunoassay.

Laser scanning microscopy For laser scanning microscopy (LSM), patient tissue was cryopreserved in 30% (wt/wt) sucrose (Sigma, St Louis, MO, USA), embedded in optimal cutting temperature (OCT) embedding medium (Thermo Fisher Scientific, Waltham, MA, USA), and 12 μm cryosections were made. The following antibodies were used: mouse anti-human MCT1 (Abcam, Cambridge, MA, USA), guinea pig anti-human insulin (Dako, Glostrup, Denmark) and rabbit anti-human glucagon (Santa Cruz Biotechnology, Santa Cruz, CA, USA). DAPI (Sigma) was used to stain cell nuclei. Secondary antibodies conjugated with AF488 (Molecular Probes, Eugene, OR, USA) and Cy3 (Jackson ImmunoResearch, West Grove, PA, USA) were used. LSM images were acquired with the same settings using a Zeiss LSM 710 confocal microscope (Zeiss, Jena, Germany).

Real-time RT-PCR Total RNA was extracted from insulinoma tissue from the EIHI patient and isolated human pancreatic islets from a control patient using peqGold TriFast (Peqlab, Erlangen, Germany), transcribed into cDNA (SYBR Green method; Stratagene, La Jolla, CA, USA), and used for real-time RT-PCR. For real-time RT-PCR, each sample was run in triplicate, and data were analysed according to the threshold cycle (C_t) method. The data were normalised to the expression of β-actin.

Primer sequences for real-time RT-PCR are shown in electronic supplementary material (ESM) Table 1.

Protein extraction and western blot analysis Human pancreatic islet preparations from multi-organ donors (one man and two women, 46–54 years of age) had a purity of >75%

and viability of 95%. Insulinoma tissue (from two men and two women, 25–79 years of age) was obtained from the Department of Neuropathology (University of Greifswald, Greifswald, Germany) and the Department for General, Visceral and Paediatric Surgery (Heinrich-Heine-University, Düsseldorf, Germany). The tissues were homogenised in radioimmunoprecipitation assay (RIPA) buffer. Total protein (up to 25 μg) was loaded on 4–12% pre-cast polyacrylamide gels (NuPage, Life Technologies, Carlsbad, CA, USA), separated by SDS page and blotted to a nitrocellulose transfer membrane (Whatman, Maidstone, UK). For detection, a mouse anti-MCT1 antibody (Abcam) was used, and the data were normalised to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) using rabbit anti-GAPDH antibody (Abcam).

Statistical analysis Statistical significance was determined by using the unpaired two-tailed Student's *t* test. Differences were considered significant with a p value <0.05. Quantified data are presented as means±SD.

Written informed consent to analyse insulinoma tissue and islet preparations for research purposes was obtained from patients.

Results

The male patient presented with a history of recurrent episodes of drowsiness and impaired consciousness since the age of 10 years. At 15 years, an episode of severe hypoglycaemia (1.2 mmol/l blood glucose) was documented. During a subsequent hypoglycaemic episode (2.3 mmol/l blood glucose), serum insulin was found to be elevated (576 pmol/l). At that time, the patient was diagnosed as suffering from hyperinsulinism. Diazoxide was started (3.7 $mg kg^{-1} day^{-1}$), which reduced the occurrence of hypoglycaemia. We saw the patient for the first time and performed standardised exercise testing. Retrospectively, the recurrent episodes of dizziness could be attributed to physical exercise, as they developed during sporting activities. The family history of hypoglycaemia and/or seizures was negative.

During anaerobic exercise the plasma lactate concentration increased (Fig. 1a) and the serum insulin concentration became highly elevated (Fig. 1b). The plasma glucose concentration declined correspondingly (Fig. 1b). The test was terminated 15 min after exercise because of severe symptomatic hypoglycaemia (0.8 mmol/l blood glucose). Magnetic resonance images were subsequently taken and revealed a focal lesion in the head of the pancreas (Fig. 1c), suggesting the presence of an insulinoma. Endoscopic ultrasonography confirmed this finding (Fig. 1d). After surgical resection of the lesion the pathological examination revealed a well-circumscribed tumour, 17 mm× 8 mm in size (data not shown), with histology typical of insulinoma (Fig. 1e). The tumour cells were stained for insulin in immunohistochemistry of sections through the tumour tissue (Fig. 1f).

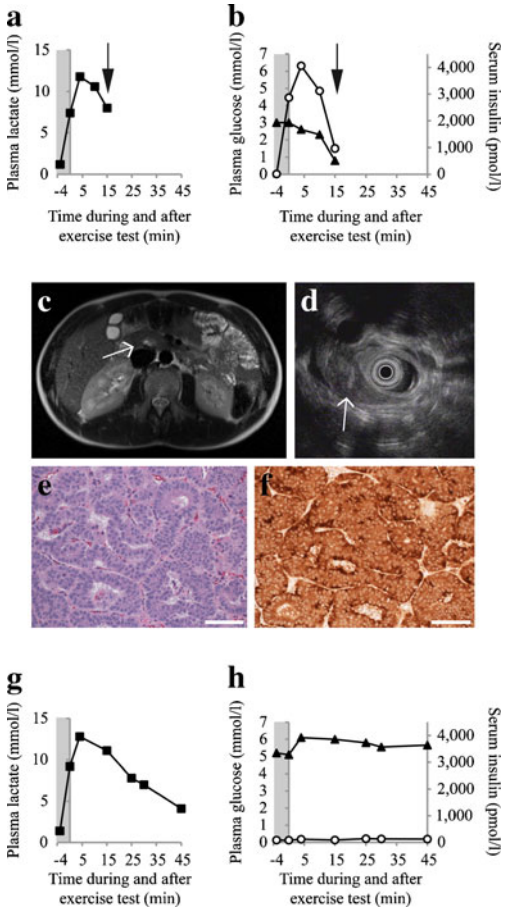
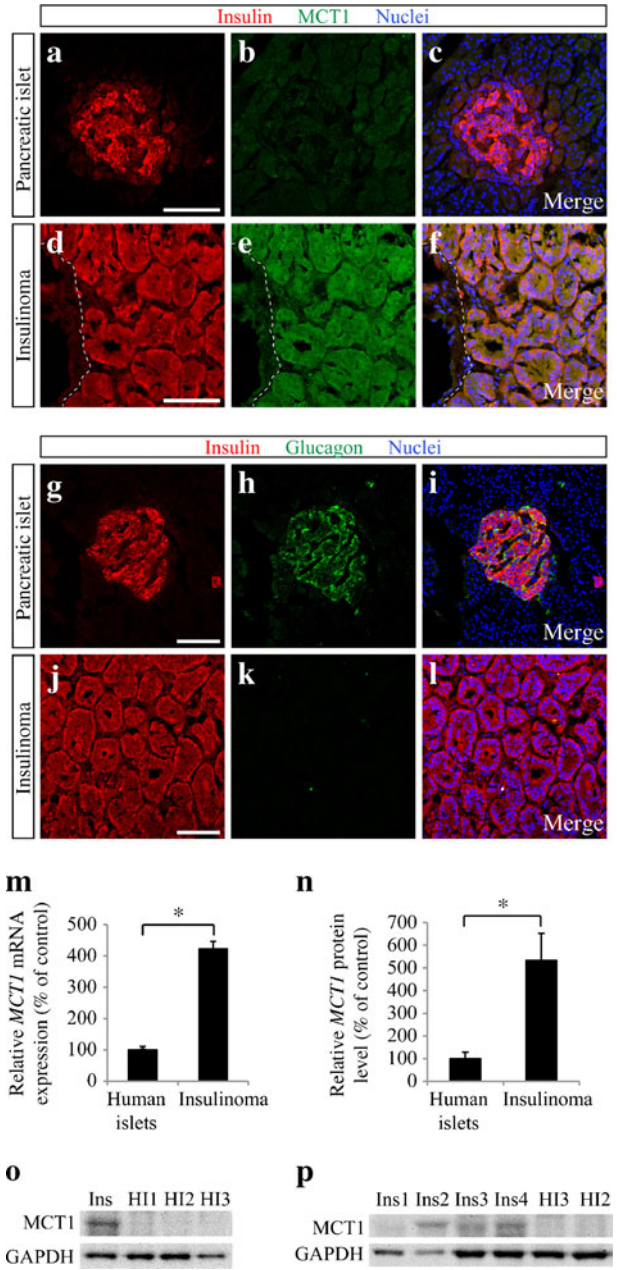


Fig. 1 Characterisation of the EIHI patient before and after surgical removal of an insulinoma. (**a**) Plasma concentrations of lactate, and (**b**) plasma glucose and serum insulin were measured just before anaerobic exercise, immediately after exertion (bicycle ergometer) and at 4, 15, 25, 30 and 45 min after exercise. The concentrations were determined before surgical resection of the insulinoma. The arrow denotes termination because of symptomatic hypoglycaemia. (**c**) T2-weighted magnetic resonance image demonstrating a lesion (white arrow) in the head of the pancreas. (**d**) Endoscopic ultrasonography showing an inhomogeneous lesion (approximately 17×8 mm in size, white arrow) in the head of the pancreas. (**e**) Haemotoxylin and eosin staining and (**f**) insulin staining of cryosections through the resected lesion. Scale bars, 100 μm. (**g**, **h**) Same test as in (**a**, **b**), but 4 weeks after surgical resection of the insulinoma. The period of exercise in (**a**), (**b**), (**g**) and (**h**) is shown by the grey bar. Black squares, lactate; black triangles, glucose; white circles, insulin

Postoperatively, the patient was constantly normoglycaemic and an exercise test was repeated 6 weeks after surgery (Fig. 1g, h). During anaerobic exercise plasma lactate concentration increased to a similar extent as observed before surgery (compare Fig. 1a and g). However, during and after exercise, the concentrations of both plasma glucose and serum insulin did not change (compare Fig. 1h and b). In addition, to date, 9 months after surgical resection of the



◀ **Fig. 2** High protein and mRNA expression of MCT1 in the insulinoma tissue of the EIHI patient and several insulinoma patients. (**a**–**l**) Representative LSM images of cryosections through healthy pancreatic tissue harbouring a pancreatic islet (**a**–**c**, **g**–**i**) and insulinoma tissue (**d**–**f**, **j**–**l**), both isolated from the EIHI patient. Staining in (**b**) and (**e**) shows that MCT1 (green) is abundant in the insulinoma tissue (**e**) compared with the healthy tissue containing a pancreatic islet (**b**). Co-staining (**c**, **f**) is shown for MCT1 (green), insulin (red) and nuclei (DAPI, blue). A dashed white line (**d**–**f**) indicates the transition from healthy pancreatic tissue to insulinoma tissue. Scale bars, 100 μm. (**g**–**l**) Insulin (red), glucagon (green) and nuclear (DAPI, blue) staining is shown on cryosections through healthy pancreatic tissue (**g**–**i**) and insulinoma tissue (**j**–**l**), both isolated from the EIHI patient. Scale bars, 100 μm. (**m**) Real-time RT-PCR showing relative expression of *MCT1* mRNA in human pancreatic islets of a non-diabetic individual and in insulinoma tissue from the EIHI patient. Number of experiments: $n=3$; Student's t test, $^{*}p<0.05$. Data shown are mean±SD. (**n**) Western blot analysis showing relative levels of MCT1 protein in control pancreatic islets and in insulinoma tissue of the reported EIHI patient. Number of experiments: $n=3$. Student's t test, $^{*}p<0.05$. Data shown are mean±SD. (**o**) Representative protein bands on western blots of insulinoma tissue from the reported EIHI patient (Ins) and pancreatic islets from three non-diabetic human individuals (HI1 to HI3), probed with anti-MCT1 and anti-GAPDH. (**p**) Representative protein bands on western blots of insulinoma tissue from four insulinoma patients (Ins1–4) and pancreatic islets from two non-diabetic individuals (HI 2 and 3), probed with anti-MCT1 and anti-GAPDH

insulinoma, no further episodes of hypoglycaemia have been reported.

Immunohistochemistry and LSM were used to detect the MCT1 protein (Fig. 2a–l). No MCT1 immunoreactivity was detected in the patient's pancreatic islets (Fig. 2a–c), and, in contrast to rat exocrine pancreatic tissue [6], there was no MCT1 in the exocrine pancreas of the patient (Fig. 2a–c; and data not shown). Importantly, MCT1 protein was observed in the insulinoma (Fig. 2d–f). In contrast to the islets, the insulinoma contained only insulin- rather than glucagon-expressing endocrine cells (Fig. 2g–l). Real-time RT-PCR showed that *MCT1* mRNA expression was approximately fourfold higher in the patient's insulinoma compared with control pancreatic islets (Fig. 2m), Using western blots, MCT1 protein levels were also shown to be about fivefold higher in the patient's insulinoma compared with the islets from three multi-organ donors (Fig. 2n, o). The results therefore show that the patient's insulinoma tissue specifically overexpressed *MCT1* at the mRNA and protein level. Moreover, a western blot of lysates from four additional insulinomas revealed MCT1 in three insulinomas (Fig. 2p).

Discussion

In summary, we report a patient suffering from exercise-induced hypoglycaemia over several years, subsequently shown to be associated with an insulinoma that expresses *MCT1*. Furthermore, we show that MCT1 protein occurs in other human insulinomas. The presence of MCT1 in the tumour cells is likely to be only one of numerous changes in this tissue. However, taken together with evidence showing that MCT1 production is downregulated in beta cells in order to prevent inappropriate insulin release by exogenous metabolites [3–6], it appears likely that the high MCT1 levels in the insulinoma tissue induced EIHI in our patient. Lactate and pyruvate are transported into the cell by MCT1, and pyruvate may then directly trigger insulin secretion via increased ATP

production. Although pyruvate levels were not measured in our patient, a high lactate level normally correlates well with a high pyruvate level [1, 7, 8].

Three insulinoma patients reported so far showed an inability to suppress insulin secretion during exercise [8], whereas in our patient, as in other EIHI patients, a massive stimulation of insulin secretion was found. Interestingly, MCT1 protein could be detected in three of four additional insulinomas, suggesting that the inability to suppress insulin secretion during exercise in EIHI and insulinoma patients might be associated with the expression of *MCT1* in the transformed pancreatic beta cells. Our data thus warrant future studies to find out whether *MCT1* expression and insulinoma formation are independent events or whether MCT1 contributes to the transformation and proliferation of human pancreatic beta cells.

In conclusion, our data suggest that *MCT1* expression in human islets is associated with EIHI symptoms and motivate studies on the role of MCT1 in beta cell proliferation and transformation.

Funding J. Marquard was funded by the Research Commission (9772455) of the Heinrich-Heine University (Düsseldorf, Germany). T. Buschmann and S. Otter were funded by the Collaborative Research Centre 974 (SFB 974, A01, DFG) and the graduate school 'vivid' of the Heinrich-Heine University, respectively. E. Lammert was funded by the German Centre for Diabetes Research (DZD e.V.) of the Federal Ministry for Education and Research (BMBF). None of the other authors received funding.

Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

Contribution statement JM, AW, TM, TB, SO, LP, SV, MK, AR, DK and WB contributed to the acquisition of data. JM, AW, TM, EM, TO and EL made substantial contributions to the analysis and interpretation of data. EL, TM, TO, JM and AW contributed to the conception and design of the study. JM, AW, TM, TO and EL wrote the manuscript with critical input from EM, TB, SO, LP, DK, SV, MK, AR and WB. All authors approved the final version.

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