Diabetologia (2014) 57:347–351
DOI 10.1007/s00125-013-3097-4

SHORT COMMUNICATION

Adipose tissue *INSR* splicing in humans associates with fasting insulin level and is regulated by weight loss

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Received: 5 August 2013 / Accepted: 9 October 2013 / Published online: 7 November 2013

Abstract
Aims/hypothesis The insulin receptor (INSR) has two protein isoforms based on alternative splicing of exon 11. INSR-A promotes cell growth whereas INSR-B predominantly regulates glucose homeostasis. In this study we investigated whether weight loss regulates *INSR* alternative splicing and the expression of splicing factors in adipose tissue.
Methods To determine the relative ratio of the *INSR* splice variants, we implemented the PCR-capillary electrophoresis method with adipose tissue samples from two weight-loss-intervention studies, the Kuopio Obesity Surgery study (KOBS, $n=108$) and a very low calorie diet (VLCD) intervention ($n=32$), and from the population-based Metabolic Syndrome in Men study (METSIM, $n=49$).
Results Expression of *INSR-B* mRNA variant increased in response to weight loss induced by both bariatric surgery ($p=1\times10^{-5}$) and the VLCD ($p=1\times10^{-4}$). The adipose tissue expression of *INSR-B* correlated negatively with fasting insulin levels in the pooled data of the three studies ($p=3\times10^{-22}$). Finally, expression of several splicing factors correlated negatively with the expression of the *INSR-B* variant. The strongest correlation was with *HNRNPA1* ($p=1\times10^{-5}$), a known regulator of *INSR* exon 11 splicing.
Conclusions/interpretation INSR splicing is regulated by weight loss and associates with insulin levels. The effect of weight loss on *INSR* splicing could be mediated by changes in the expression of splicing factors.

Keywords Adipose tissue · Alternative splicing · Insulin receptor · qPCR, Quantitative PCR · Weight loss

Abbreviations

INSR	Insulin receptor
KOBS	Kuopio Obesity Surgery study
METSIM	Metabolic Syndrome in Men study
qPCR	Quantitative PCR
VLCD	Very low calorie diet

Introduction

The insulin receptor (INSR) exists in two protein isoforms arising from inclusion (INSR-A) or skipping (INSR-B) of exon 11. Expression of the *INSR* gene variants depends on the tissue type and stage of tissue development. The INSR-A isoform is predominantly expressed in fetal cells and plays a

Electronic supplementary material The online version of this article (doi:10.1007/s00125-013-3097-4) contains supplementary material, which is available to authorized users.

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role in fetal development, whereas the INSR-B isoform is expressed in adult differentiated cells. These two protein isoforms have different functions: INSR-A promotes growth through its ability to bind IGF-II and proinsulin, whereas INSR-B is a highly specific receptor for insulin. It is expressed predominantly in insulin-sensitive tissues and thus regulates glucose homeostasis (reviewed in Belfiore et al [1]). *INSR* splicing has mostly been studied in skeletal muscle in relation to type 2 diabetes. In most, but not all, of the studies high skeletal muscle expression of the *INSR-B* variant has been associated with type 2 diabetes and insulin resistance (reviewed in Belfiore et al [1]). An increased level of *INSR-B* was detected in isolated adipocytes from ten patients with type 2 diabetes compared with 11 normoglycaemic patients [2]. The effect of weight loss on adipose tissue *INSR* splicing has not been studied.

We have previously shown that expression of several splicing factors is reduced in liver and skeletal muscle of obese individuals [3]. In addition, we have shown that the splicing of *TCF7L2* is regulated by weight loss in adipose tissue and liver [4]. Thus, we hypothesised that adipose tissue *INSR* splicing is modified by weight loss through changes in splicing factor gene expression levels. To test this hypothesis we determined the association of *INSR* splicing with metabolic variables and the expression of splicing factors in three independent studies (n = 189 combined): Kuopio Obesity Surgery study (KOBS, n = 108) [4], a very low calorie diet (VLCD) intervention (n = 32) [5] and the population-based Metabolic Syndrome in Men study (METSIM, n = 49) [4].

Methods

Participants and clinical studies Subcutaneous adipose tissue samples were collected at the time of Roux-en-Y gastric bypass surgery from a total of 108 morbidly obese individuals participating in the ongoing KOBS study (45 with type 2 diabetes, 63 non-diabetic individuals). In addition, subcutaneous fat biopsies were taken 1 year after the surgery. Visceral biopsies (n = 81) were collected at the baseline [4]. Two independent study groups were used for the replication of the results. First, subcutaneous adipose tissue samples were taken from a study of 32 non-diabetic individuals recruited into a dietary intervention study consisting of a 7 week long VLCD followed by a 24 week weight-maintenance period. During the weight loss period the energy intake was 2, 510 kJ/day (600 kcal/day) [5]. Subcutaneous tissue biopsies were collected at all visits (baseline, 7 weeks and 24 weeks). Second, subcutaneous adipose tissue samples from a total of 49 men (21 with type 2 diabetes, 28 with normal glucose tolerance) were included from the population-based METSIM study [4] (Table 1). Diabetic status was determined using the ADA 2003 criteria. The study protocols were approved by the Ethics Committee of Northern Savo Hospital District and carried out in the accordance with the Helsinki Declaration.

Gene expression and splicing analysis A PCR-capillary electrophoresis method was used to determine the relative ratio of *INSR* splice variants (ABI Prism 3100 DNA Genetic Analyzer, Applied Biosystems, Foster City, CA, USA). Quantification of peak area was performed with Peak Scanner Software v1.0 (Applied Biosystems, Foster City, CA, USA). Total gene expression of *HNRNPA1*, *SF3A1*, *SFRS7*, *SFRS10* and *INSR* normalised to *RPLP0* was analysed by quantitative (q)PCR, using SYBR Green chemistry (KAPA SYBR FAST qPCR Kit, Kapa Biosystems, Woburn, MA, USA).

Statistical analysis Data are presented as mean ± SD. Data from the same individuals at different time points were

Table 1 Characteristics of the study groups

Characteristic	KOBS			VLCD					METSIM		
	Baseline (n = 108)	1 year Follow-up (n = 81)	p value	Baseline (n = 32)	7 weeks Follow-up (1) (n = 32)	24 weeks Follow-up (2) (n = 32)	p value (1)	p value (2)	NGT (n = 28)	T2DM (n = 21)	p value
Sex (male/female)	34/74	26/55		9/23	9/23	9/23			28/0	21/0	
Age (years)	47.1±9.1	47.6±9.0		48.8±8.7	49.0±8.8	49.5±8.8			56.7±5.3	56.3±5.5	0.803
BMI (kg/m^2)	45.0±6.3	34.6±5.8	2×10^{-37}	34.7±2.7	29.9±2.4	30.3±2.8	3×10^{-24}	2×10^{-16}	23.2±1.4	26.0±3.9	0.004
Fasting glucose (mmol/l)	6.7±1.7	5.6±0.9	2×10^{-8}	6.1±0.8	5.6±0.6	5.7±0.6	1×10^{-4}	6×10^{-5}	5.5±0.3	7.0±1.1	3×10^{-6}
Fasting insulin (pmol/l)	135.7±135.5	71.4±67.4	3×10^{-10}	82.8±46.2	40.8±19.2	51.0±27.0	3×10^{-6}	4×10^{-6}	27.6±8.4	65.4±47.4	0.002

Data are expressed as mean ± SD

Differences of variables between two groups were compared using paired t tests

NGT, normal glucose tolerance; T2DM, type 2 diabetic participants

compared using the two-tailed paired samples *t* test. Correlations were assessed using non-parametric Spearman's correlations and with partial correlations. Insulin levels were logarithmically transformed ($\log_{10}$) to obtain a normal distribution. The main effects of BMI, age, sex, fasting insulin, fasting glucose and study group on *INSR* splicing were evaluated by general linear model. A p value <0.05 was considered significant.

Results

The proportion of *INSR-B* mRNA differed in the study groups at baseline (Fig. 1a). The relative proportion of *INSR-B* mRNA in the subcutaneous fat increased by 9.1% in response to surgery ($p = 1\times10^{-5}$; Fig. 1b) and by 8.1% during the VLCD ($p = 1\times10^{-4}$; Fig. 1c). The relative proportion remained increased by 6.7% after the weight-maintenance period in the VLCD study ($p = 2\times10^{-4}$, Fig. 1c). No change in total gene expression of *INSR* was detected. Thus, the effects on splicing were independent of transcriptional regulation (electronic supplementary material [ESM] Fig. 1a). No difference in expression of *INSR-B* was observed between visceral and subcutaneous fat depots at baseline (ESM Fig. 1b).

Next, we investigated clinical variables that associate with *INSR* splicing. Fasting glucose correlated negatively with *INSR-B* splicing in the KOBS study ($r = -0.261$, $p = 0.010$, ESM Table 1). *INSR-B* expression was 7.1% higher in normoglycaemic individuals compared with patients with type 2 diabetes in the METSIM study ($p = 5\times10^{-4}$; Fig. 1d). However, the most consistent finding was a negative correlation between fasting insulin levels and *INSR* splicing in subcutaneous fat in all three studies, KOBS ($r = -0.348$, $p = 5\times10^{-4}$), VLCD ($r = -0.417$, $p = 0.020$) and METSIM ($r = -0.522$, $p = 1\times10^{-4}$). Significant correlation was also observed between insulin levels and *INSR* splicing in visceral fat (KOBS n=81, $r = -0.338$, $p = 8\times10^{-4}$; ESM Table 1). Additionally, negative correlation was observed between HOMA-IR and *INSR-B* at baseline in all three studies (ESM Table 2).

In pooled data from the KOBS, VLCD and METSIM studies ($n = 189$) *INSR-B* variant correlated strongly with fasting insulin levels ($r = -0.649$, $p = 3\times10^{-22}$; Fig. 1e). Insulin was the strongest determinant of *INSR-B* splicing ($p = 9\times10^{-9}$) followed by BMI (5×10^{-7}), age ($p = 0.009$) and sex ($p = 0.036$) in a multivariate general linear model. After controlling for study group, insulin remained the strongest determinant of *INSR-B* splicing ($p = 7\times10^{-7}$) (ESM Table 3). Fasting glucose level did not associate with *INSR-B* splicing.

Expression of *HNRNPA1*, *SF3A1*, *SFRS7* and *SFRS10* has been shown to be lower in the skeletal muscle and liver of obese individuals compared with lean individuals [3]. Therefore, we analysed the effect of weight loss on the adipose tissue expression of these genes and the association with *INSR* splicing. The expression of *HNRNPA1*, a known regulator of *INSR* splicing [6], was increased in response to surgery-induced weight loss ($p = 0.001$, data not shown) and its expression correlated negatively with *INSR-B* expression in the KOBS ($r = -0.427$, $p = 1\times10^{-5}$), but not significantly in the VLCD study ($r = -0.222$, $p = 0.238$). However, after pooling adipose tissue samples from all time points of the KOBS and VLCD studies and after controlling for insulin levels the correlation between *INSR-B* and *HNRPNA1* was significant in both KOBS and VLCD studies ($r = -0.226$, $p =$

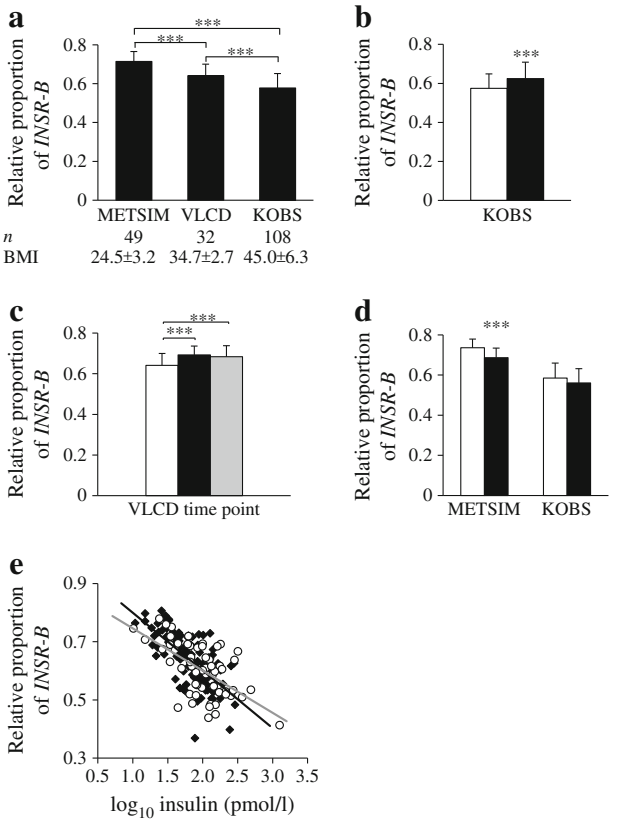

Fig. 1 The relative proportion of *INSR-B* in subcutaneous fat (**a**) in the METSIM, VLCD and KOBS studies at baseline and in response to (**b**) obesity surgery in the KOBS study (white bar, baseline; black bar, 1 year post surgery) and (**c**) to a 7 week VLCD followed by a 24 week weight-maintenance period (white bar, baseline; black bar, 7 weeks VLCD; grey bar, 24 weeks weight maintenance). (**d**) Relative proportion of the *INSR-B* variant in individuals with and without type 2 diabetes in the METSIM and KOBS studies. White bars, non-diabetic participants; black bars, participants with type 2 diabetes. (**e**) Scatter plot demonstrating the correlation of *INSR-B* with logarithmically transformed fasting insulin levels in pooled samples from the KOBS, VLCD and METSIM studies ($r = -0.649$, $p = 3\times10^{-22}$). Black diamonds, non-diabetic participants ($n = 123$, $r = -0.676$, $p = 4\times10^{-16}$); white circles, participants with type 2 diabetes ($n = 66$, $r = -0.601$, 3×10^{-7}). Mean ± SD shown. $^{***}p < 0.001$ (**b**,**c**) vs baseline of same study (**d**) and vs non-diabetic participants of same study

0.005 and $r = -0.330$, $p = 0.012$ respectively) (ESM Table 4). In addition, we observed negative correlations of *INSR-B* expression with *SF3A1* and *SFRS7* expression in the KOBS study (ESM Table 4). No correlation between examined splicing factors and fasting insulin levels was detected (ESM Table 5).

Discussion

In this study we report, for the first time, that weight loss regulates alternative splicing of *INSR* in adipose tissue. The proportion of *INSR-B* mRNA variant was increased in response to weight loss induced by both obesity surgery and VLCD (Fig. 1b, c). Interestingly, insulin levels correlated strongly with *INSR* splicing (Fig. 1e) in both subcutaneous and visceral fat (ESM Table 1), suggesting common regulatory mechanisms related to insulin action. Finally, we observed a correlation between alternatively spliced *INSR* variants and expression of *HNRNPA1*, a known regulator of *INSR* splicing [6].

Weight loss resulted in higher expression of *INSR-B*, the more active isoform in insulin signalling [7]. Accordingly, we detected a strong negative correlation between *INSR-B* and fasting insulin levels, suggesting an association between *INSR-B* splicing and insulin action. Multivariate analysis of the pooled samples from all three studies ($n = 189$) revealed that the main determinant of the expression of *INSR* splice variants in subcutaneous adipose tissue was fasting insulin. This is consistent with a previous study in monkeys, including an analysis of human data, suggesting a link between hyperinsulinaemia and *INSR* splicing [8, 9].

There are two potential mechanisms for an association of *INSR* splicing with fasting insulin levels. First, lower insulin levels in relation to *INSR-B* may reflect better peripheral insulin sensitivity as expected for this isoform. However, an increase in *INSR-B* in response to weight loss could also be secondary to lower insulin levels, as insulin is a known regulator of splicing factor activity through phosphorylation [10] and because hyperinsulinaemia associates with lower expression of splicing factors [3]. We found a correlation between *INSR* splicing and expression of *HNRNPA1*, *SF3A1* and *SFRS7* in the KOBS study (ESM Table 4). The strongest negative correlation was with *HNRNPA1*, previously reported to inhibit exon 11 inclusion in HepG2 and HEK293 cells [6]. We acknowledge that other regulators of *INSR* splicing exist [6], and they may also be modified by weight loss. One limitation of this study is that INSR protein isoforms created by alternative splicing could not be detected because the difference between the protein isoforms is only 1 kDa (data not shown).

In conclusion, we demonstrated that *INSR* splicing correlates strongly with fasting insulin and is regulated by weight loss. These changes may associate with changes in splicing factor activity.

Acknowledgements The authors thank T. Sistonen (Department of Medicine, University of Eastern Finland, Kuopio, Finland), P. Turunen, M. Laitinen, K. Juvonen, A. Jääskeläinen and E. Holma (Institute of Public Health and Clinical Nutrition, University of Eastern Finland, Kuopio, Finland) for assistance with tissue biopsies and laboratory analyses.

Some of the data were presented as an abstract at the 73rd Scientific Sessions of the ADA in 2013.

Funding This study was supported by grants from the Academy of Finland (contracts 124243 to ML and 120979 and 138006 to JP), the Emil Aaltonen Foundation (to JP), the Finnish Diabetes Research Foundation (to ML and JP), the Finnish Cultural Foundation (to JP), the Finnish Heart Foundation (to ML) and Tekes–the Finnish Funding Agency for Technology and Innovation (contracts 1510/31/ 06 to ML and 40100/07 to LK).

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

Contribution statement DK acquired, analysed and interpreted splicing and qPCR data and wrote the manuscript. MH acquired qPCR data. PK, SV and HG acquired data in the KOBS study. PM, II, K-HH, MK and LK acquired data in the VLCD study. LK is the principal investigator responsible for the VLCD study. ML, JK and HC acquired data in the METSIM study. ML is the principal investigator responsible for the METSIM study. JP is the principal investigator responsible for the KOBS study, designed the study, contributed to the discussion, and reviewed and edited the manuscript. JP is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. All authors critically revised the manuscript for intellectual content and approved the final version to be published.

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