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Microarray analysis of pancreatic gene expression during biotin repletion in biotin-deficient rats

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ABSTRACT

Biotin is a B vitamin involved in multiple metabolic pathways. In humans, biotin deficiency is relatively rare but can cause dermatitis, alopecia and perosis. Low biotin levels occur in type II diabetes, and biotin supplementation with chromium may improve blood sugar control. The acute effect on pancreatic gene expression of biotin repletion following chronic deficiency is unclear, therefore we induced biotin deficiency in adult male rats via a 20% raw egg white diet for 6 weeks. Animals were then randomized: one group received a single biotin supplement and returned to normal chow lacking egg white, while the second group remained on the depletion diet. After one week, pancreata were removed from biotin-deficient (BD) and biotin-repleted (BR) animals and RNA isolated for microarray analysis. Biotin depletion altered gene expression indicative of inflammation, fibrosis and defective pancreatic function. Conversely, biotin repletion activated numerous repair and anti-inflammatory pathways reduced fibrotic gene expression and induced multiple genes involved in pancreatic endocrine and exocrine function. A subset of results was confirmed by qPCR analysis, as well as by biotin treatment of pancreatic AR42J cells. The results indicate that biotin repletion, even after lengthy deficiency, results in the rapid induction of repair processes in the pancreas.

Keywords: biotin, pancreas, microarray, deficiency, repletion

INTRODUCTION

The role of biotin as the prosthetic group of the four biotin-dependent carboxylases in higher organisms is well recognized. In view of the roles of these carboxylases in metabolism, the requirement of biotin for cell viability, growth and differentiation was established. However, biotin also appears to have a role in cell function other than as the prosthetic group of biotin enzymes. It influences processes such as proliferation of the mesenchyme and spermatogenesis (Dakshinamurti 2005, 2006). A direct effect of biotin at the transcriptional level has been shown for key enzymes of glucose metabolism such as glucokinase, a key glycolytic enzyme (Chauhan and Dakshinamurti 1991; Romero-Navarro et al. 1999) and phosphoenolpyruvate carboxykinase, a key gluconeogenic enzyme (Dakshinamurti and Li 1994). The effects of biotin depletion and repletion in three distantly-related eukaryotes, i.e. yeast (*Saccharomyces cerevisiae*), nematode (*Caenorhabditis elegans*) and rat (*Rattus norvegicus*), point to a strongly selected role of biotin in the control of carbon metabolism (Ortega-Cuellar et al. 2010).

In studies using pancreatic islets, the effect of *in vivo* biotin supplementation in the diet on rodent pancreatic islets was studied (Lazo de la Vega-Monroy et al. 2013). Biotin supplementation augmented the proportion of beta cells by enlarging islet size and increasing the mRNA expression of adhesion proteins participating in the maintenance of islet architecture. Thus, *in vivo* biotin supplementation augmented beta cell proportion as well as its function. Conversely, it has been reported that the circulating biotin level is lower in type 2 diabetic patients compared to normoglycemic individuals (Maebashi et al. 1993). Supplementation of biotin plus chromium has also been shown to improve glucose metabolism and glycemic control in clinical trials involving type 2 diabetic patients (Albarracin et al. 2008; Singer and Geohas 2006), and furthermore improves atherogenic risk factors and serum lipids in both diabetic and

non-diabetic individuals (Albarracin et al. 2007; Geohas et al. 2007; Revilla-Monsalve et al. 2006). Biotin thus appears to be a critical determinant of normal pancreatic function.

Using a well-established rat model, we demonstrate here that biotin repletion following chronic depletion results in myriad changes in pancreatic gene expression. These changes include induction of anti-inflammatory pathways, increases in endocrine and exocrine function, and reduced fibrosis marker expression. These results suggest that restoration of biotin to deficient individuals may improve pancreatic gene expression, even after a long period of deficiency, and that these reparative processes are induced relatively quickly. Similarly, treatment of AR42J pancreatic cells with biotin induced changes in gene expression congruent with the *in vivo* repletion studies within 24 to 48 hours.

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MATERIALS AND METHODS

Animal model of biotin depletion and repletion

Adult male Sprague-Dawley rats (~250 g; ten animals total) were fed a biotin-deficiency diet consisting of normal rodent chow supplemented with 20% egg white (ICN Biochemicals) using a well-established model (Dakshinamurti and Cheah-Tan 1968; Dakshinamurti and Mistry 1963). Animals were fed this diet for six weeks, then half (5 rats) were switched to a normal diet and injected with 10 mg/kg biotin I.P. (Biotin Repletion, BR) or were maintained on the biotin-deficiency diet and injected with saline vehicle (Biotin Depletion, BD). One week following injection, animals were humanely sacrificed using CO₂ asphyxiation euthanasia. Pancreata were collected then rinsed in sterile cold saline and frozen in liquid nitrogen until use. All animal studies were approved by the University of Manitoba Animal Care Committee in accordance with the guidelines of the Canadian Council on Animal Care.

Tissue isolation and RNA extraction

Pancreata were mechanically homogenized under Trizol using a Polytron, then total RNA isolated according to manufacturer's directions (Invitrogen), with the modification that a ratio of 4:1 Trizol:tissue was used. For long term storage, total RNA was stored in formamide. RNA concentration and purity was determined by absorbance at 260 and 280 nm.

Microarray analysis

Total pancreatic RNA samples from five animals in each group (BD or BR) were pooled to ensure equal contribution from each animal to the group RNA sample. Samples were prepared according to the Affymetrix GeneChip Expression 3'-Amplification Labeling kit directions, with

a modification that 5 µg total RNA was used as a starting point. Samples were hybridized to Affymetrix GeneChip Rat Genome 2.0 microarrays and analyzed using GCOS software, with post-analysis performed using BioConductor and AffyImGUI. For comparisons between samples, a cutoff of a 2-fold change in gene expression (with $P < 0.05$) was selected, and any genes called by GCOS as “absent” in both samples were ignored. We analyzed genes that exhibited expression changes of at least 2-fold, and that were statistically-significant and called as expressed in at least one sample, with the DAVID (Database for Annotation, Visualization and Integrated Discovery) Bioinformatics Resources 6.7. The results of this analysis identified enriched cell function pathways using ontological term identifiers (Huang et al. 2009a, 2009b). For DAVID analysis, default settings for Functional Annotation Clustering were used with medium classification stringency.

Cell culture and treatment

Rat pancreatic acinar AR42J cells (CRL 1492) were purchased from ATCC. Cells were maintained in F-12K medium (ATCC) supplemented with 20% FBS and 1% penicillin/streptomycin, at 37°C under humidified conditions of 5% CO₂. Alternatively, cells were maintained in biotin-free DME medium (Sigma-Aldrich) supplemented with 0.5% FBS and 1% penicillin/streptomycin under identical conditions of humidity, temperature and CO₂ concentration. Biotin (Sigma-Aldrich) was dissolved in sterile deionized water to yield a 5 mM stock solution. Biotin dilution for cell treatments was made using no-serum starvation media (either F-12K or DME as required). AR42J cells were treated with 50 pM biotin or vehicle for 24 or 48 hours.

RNA isolation and qPCR analysis

Total RNA was isolated from AR42J cells post-treatment using a commercially available purification kit as per manufacturer's instructions (Thermo Scientific). RNA concentrations were determined and purity confirmed using 260/280 nm absorbance ratio. RNA samples were assayed using an iQ5 multicolor real-time PCR thermocycler (Bio-Rad) using the qScript One-Step SYBR Green qRT-PCR kit for iQ (Quanta Biosciences). Gene-specific primers were synthesized commercially (Integrated DNA Technologies); primer sequences and qPCR cycle settings are listed in Supplementary Table S1. mRNA abundance was calculated using the $2^{-\Delta\Delta C_t}$ method and normalized to GAPDH.

Statistical analysis

For qPCR studies, results represent mean $\pm$ standard error of the mean for at least 3 independent experiments. Data was analyzed by two-tailed Student's *t* test, with $P < 0.05$ considered statistically significant.

RESULTS

As expected, six weeks of biotin depletion (BD) in rats resulted in spotty alopecia (not shown), indicative of the biotin deficient status of these animals. Pancreatic gene expression was analyzed by Affymetrix Rat Genome 2.0 microarrays, and a total of 972 transcripts exhibited differential expression of at least 2-fold between BR and BD groups (449 up-regulated in BR and 524 down-regulated in BR, representing ~3% of the transcripts on the array), with $P < 0.05$. The expression data was normalized using the Affymetrix GCOS software, and exhibited no indication of sample bias (Figure 1).

The Affymetrix identifiers of all 972 differentially-expressed genes were uploaded to the DAVID Bioinformatics resource for ontological analysis. These genes were mapped to 754 equivalent DAVID identifiers and used for further analysis. DAVID employs a modified Fisher Exact P -Value (the EASE score) to assess whether transcripts in the list of up/down-regulated transcripts belong to specific gene ontology categories that are represented in the list more frequently than would be expected by random chance. The resulting Enrichment Score provides a relative indication of how enriched these gene ontology categories are in the transcript list. Ontology terms sharing an enrichment score are also related by the member genes populating those terms. Using this approach, the most enriched annotation cluster corresponded to extracellular matrix ontology terms (Table 1), indicative of alterations in extracellular matrix gene expression. Other noteworthy enriched clusters mapped to signal/stimulus response (cluster 2), carbohydrate/pattern binding (cluster 3), endocrine pancreas function (cluster 4) and secretion (cluster 6). An additional cluster 12, with an Enrichment Score of 2.75, included the GO terms vitamin binding, vitamin B6 binding, pyridoxal phosphate binding and cofactor binding ($P < 0.05$ for all terms; not shown), fitting with the variant biotin status of these animals.

We further analyzed gene expression patterns using DAVID to identify Functional Annotation Clustering by KEGG (Kyoto Encyclopedia of Genes and Genomes) pathways. Using this approach, the most significantly altered pathway by EASE score was PPAR signaling (Table 2). Other notable pathways exhibiting alteration between BR and BD animals included ECM (extracellular matrix)-receptor interaction, maturity onset diabetes of the young, and insulin signaling. These findings were consistent with the GO term changes previously noted, including indicators of altered extracellular matrix expression and pancreatic function.

Examination of gene expression changes following biotin repletion revealed a variety of noteworthy features. Numerous markers of inflammation were significantly elevated in BD samples compared to BR (Table 3). Similarly, various collagens were expressed at significantly higher levels in the depleted BD samples, in agreement with the gene ontology analysis results indicative of enrichment of extracellular matrix genes and fibrosis, while angiotensinogen and BNIP3 were further suggestive of pancreatic injury (Table 4).

Biotin repletion appears to result in induction of pancreatic repair processes, as noted by Annotation Cluster 4 highlighting pancreatic development (Table 1). Congruent with this finding, we noted induction of several mediators of pancreas repair including PapI/Reg3b, PapII/Reg3a and osteopontin (Table 5). These regulators have been previously noted to be induced during acute pancreatitis (Duseti et al. 2000).

Given the expression alterations highlighted by Annotation Cluster 6, suggestive of alterations in pancreatic secretory function, transcripts involved in endocrine and exocrine function were examined. Expression of transcripts encoding glucagon and α -amylase 1A were higher in BR samples, while insulin exhibited a modest although significant decrease (Table 6).

Transcriptional regulators of pancreatic secretory functions, including HNF6, FoxA2, Mist1 and pancreas-specific transcription factor 1a were also higher following biotin repletion.

We confirmed a subset of microarray-identified gene expression changes using quantitative PCR analysis of biotin-depleted and biotin-repleted samples. The injury/fibrosis-related transcript Bnip3 was approximately 60% lower in biotin repleted samples compared to biotin depleted samples (Figure 2A). Col5a1 showed a similar trend although the results were not statistically significant ($P=0.0564$). Similarly, the repair transcripts PapI and PapII were dramatically higher in repleted samples, approximately 200-fold higher than in the biotin depleted samples (Figure 2B).

To begin to examine whether gene expression changes may be directly regulated by biotin, we treated AR42J pancreatic cells with 50 pM biotin for 24 or 48 hours. In preliminary dose-dependency studies ranging from picomolar to micromolar concentrations of biotin (not shown), we observed maximal PapI and PapII gene expression response at this concentration. PapI and PapII were rapidly up-regulated in a time-dependent fashion by biotin treatment (Figure 3A). This induction was observed regardless of whether the cells were grown in the normal growth medium for the cells, F-12K which contains ~300 nM biotin, or in biotin-free DME medium, suggesting that exogenous biotin could alter gene expression independently of the presence of endogenous biotin. The repair transcript osteopontin/Spp1 exhibited a non-significant trend to a decrease at 24 hours in F-12K but an induction in DME, and was significantly induced at 48 hours in either medium. The injury/fibrosis-related transcripts Bnip3, Col1a1 and Col5a1 were significantly down-regulated by biotin treatment at both 24 and 48 hours (Figure 3B). The secretory function of these cells may also be altered by biotin treatment: similar to the results in the *in vivo* study, Glut4 expression was reduced by biotin at both time points and in both media

(Figure 3C). Conversely, HNF6, which was induced in the biotin repleted pancreas, exhibited a biphasic response in F-12K medium, with a significant reduction at 24 hours of biotin treatment but a 3-fold induction at 48 hours. In contrast, HNF6 expression was induced by biotin in DME medium at both 24 and 48 hours.

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DISCUSSION

The present data suggests that biotin repletion following depletion resembles the repair processes that occur during pancreatitis. A number of damage markers, including signs of inflammation and fibrosis, were elevated in BD pancreata compared to BR (Tables 1 to 4, Figure 2A), suggesting that damage had occurred in the depleted organs and was being ameliorated following repletion. These changes suggest that chronic biotin depletion may have more substantive negative effects on pancreatic function than previously assumed.

The induction of genes associated with tissue repair following biotin repletion (Table 5, Figure 2B) suggests that damage to the pancreas may be transient and/or reversible to some degree. Furthermore, the induction of these genes after biotin supplementation suggests that biotin may, itself, trigger repair processes. This finding is consistent with previous reports that biotin supplementation can improve pancreatic function in diabetes, at least in part by regulating glucokinase expression (McCarty 2007; Zhang et al. 1997). The altered expression of genes associated with pancreatic endocrine and exocrine function (Table 6) following biotin repletion further suggest that biotin may exert direct effects on pancreatic function.

Of particular note is the very high degree of induction of the pancreatitis-associated proteins PapI/Reg3b and PapII/Reg3b by biotin. The Pap family consists of three related C-type lectins, including PapIII, and all three members have been reported to be secreted after the induction of pancreatitis (Frigerio et al. 1993; Keim and Loffler 1986). Indeed, PapI has been reported to be induced in multiple tissues in response to insult, including both acute and chronic pancreatitis, hypoxia, toxin exposure and diabetes, as well as following organ transplant, in Crohn's diseases, ulcerative colitis and animal models of inflammatory bowel disease (Baeza et al. 2001; Chen et al. 1996; Dieckgraefe et al. 2000; Lawrance et al. 2001; Masciotra et al. 1995; McKie et al. 1996;

Orelle et al. 1992; van der Pijl et al. 1997). Pap expression is induced in the brain tissue of Alzheimer patients and in injured sensory and motor neurons, and is also elevated in adenocarcinoma and hepatocarcinoma (Averill et al. 2002; Livesey et al. 1997; Ozturk et al. 1989; Simon et al. 2003; Xie et al. 2003). Pap induction thus appears to be a general response to tissue damage or disease.

It has been suggested that the induction of Pap gene expression may form part of the normal response to inflammatory stress (Closa et al. 2007). The pancreatic acinar cells are the primary source of Pap in pathological conditions, and serum factors from rats with acute pancreatitis, but not from healthy rats, could induce endogenous PapI gene expression in the acinar cell line AR-42J (Bodeker et al. 1998; Dusetti et al. 1994). PapI may behave as an anti-inflammatory similar to IL-10, both of which inhibit activation of neutrophils and macrophages (Moore et al. 2001). Both also inhibit IL-6 and TNF α synthesis and block translocation of NF κ B (Vasseur et al. 2004). The IL-10-related cytokine IL-22 has been shown to activate the expression of PapI in epithelial cells (Aggarwal et al. 2001). Dexamethasone exerts an anti-inflammatory effect in acute pancreatitis at least in part via up-regulation of Pap gene expression, possibly through a glucocorticoid response element in the Pap gene promoter (Dusetti et al. 1996; Kandil et al. 2006). That biotin status may influence inflammatory diseases is indicated by findings that biotin deficiency in mice up-regulates TNF α production and biotin repletion down-regulates TNF α production (Kuroishi et al. 2008).

Pap may also work by attenuating apoptosis, as Pap expression confers significant resistance to apoptosis induced by TNF α or oxidative stress (Christa et al. 1994; Malka et al. 2000; Ortiz et al. 1998). Conversely, administration of anti-Pap antibodies increased the severity of pancreatitis (Zhang et al. 2004). Similarly, expression of all three Pap isoforms has been knocked down via

delivery of anti-sense oligodeoxyribonucleotides introduced by intrapancreatic injection. When delivered two days prior to induction of acute pancreatitis, the pathology was exacerbated: serum levels of amylase and c-reactive protein increased, as did pancreas wet weight indicative of edema (Vasseur et al. 2004; Zhang et al. 2004). Pancreata also exhibited increased signs of leukocyte infiltration, and peripheral blood mononuclear cells showed increased expression of IL-1 and IL-4. In contrast, induction of Pap expression prior to the triggering of necrotizing pancreatitis significantly improved animal survival (Fiedler et al. 1998).

The exact mechanism by which Pap expression is induced in response to disease is unclear. As noted above, dexamethasone may act directly at the gene promoter, but whether a similar mechanism holds true for other inducers of Pap remains to be seen. Our data suggests that PapI and PapII gene expression rises in response to biotin repletion, but the mechanism responsible remains to be determined. We cannot rule out that Pap expression rises indirectly, after biotin repletion has already induced other repair processes in the pancreas. However, given the critical role of Pap in normal pancreatic function, and the improvements seen in pancreatitis following Pap administration, it seems more likely that Pap induction by biotin may be a directly regulated process. Using the rat pancreatic acinar AR42J cell line, we found that PapI and PapII expression was rapidly and potently up-regulated in response to biotin treatment (Figure 3A), supporting the possibility that altered Pap gene expression upon biotin repletion *in vivo* represents a biotin-induced event, and not merely a generalized response to stress. Intriguingly, we also found that biotin induced a reduction in the expression of Bnip3, associated with cell injury, and the fibrotic collagens Colla1 and Col5a1 (Figure 3B). Glut4 and HNF6 expression were also altered following biotin treatment similar to the changes observed following biotin repletion *in vivo*. Intriguingly, we observed a better fit of gene expression data in biotin-treated AR42J cells with

the *in vivo* repletion data when the cells were maintained in biotin-free DME medium compared to biotin-containing F-12K medium. This is perhaps not surprising since the *in vivo* studies compared biotin repletion to depletion, thus the addition of biotin to DME may better model the *in vivo* situation. Together, these results suggest that biotin may exert direct positive and negative effects on a variety of gene targets, consistent with improved cell health and function although the precise mechanism remains unknown.

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Table 1. Functional annotation clustering by Gene Ontology terms.

The top seven functional annotation clusters are given following DAVID analysis of 754 transcripts differentially expressed between BR and BD at least 2-fold. GO ID, gene ontology identification number; count indicates the number of transcripts (out of 754) mapping to a given GO ID with corresponding percentage listed; EASE, Modified Fisher Exact P Value.

Annotation Cluster 1; Enrichment Score: 8.18				
GO ID	Term	Count	%	EASE
GO:0044421	extracellular region part	72	9.5	5.60E-11
GO:0031012	extracellular matrix	38	5.0	2.23E-10
GO:0005578	proteinaceous extracellular matrix	34	4.5	1.24E-09
GO:0044420	extracellular matrix part	15	2.0	1.19E-04
Annotation Cluster 2; Enrichment Score: 4.57				
GO ID	Term	Count	%	EASE
GO:0010033	response to organic substance	68	9.0	5.00E-06
GO:0009725	response to hormone stimulus	43	5.7	1.69E-05
GO:0043434	response to peptide hormone stimulus	24	3.2	5.00E-05
GO:0009719	response to endogenous stimulus	44	5.8	1.19E-04
Annotation Cluster 3; Enrichment Score: 3.36				
GO ID	Term	Count	%	EASE
GO:0030246	carbohydrate binding	31	4.1	6.55E-05
GO:0001871	pattern binding	15	2.0	3.02E-04
GO:0030247	polysaccharide binding	15	2.0	3.02E-04
GO:0005539	glycosaminoglycan binding	13	1.7	1.01E-03
GO:0008201	heparin binding	10	1.3	2.77E-03
Annotation Cluster 4; Enrichment Score: 3.14				
GO ID	Term	Count	%	EASE
GO:0031016	pancreas development	10	1.3	2.95E-05
GO:0031018	endocrine pancreas development	6	0.8	3.61E-04
GO:0035270	endocrine system development	8	1.1	3.57E-02
Annotation Cluster 5; Enrichment Score: 3.03				
GO ID	Term	Count	%	EASE
GO:0042803	protein homodimerization activity	28	3.7	3.24E-04
GO:0042802	identical protein binding	43	5.7	3.77E-04
GO:0046983	protein dimerization activity	35	4.6	6.68E-03
Annotation Cluster 6; Enrichment Score: 2.95				
GO ID	Term	Count	%	EASE
GO:0030141	secretory granule	22	2.9	4.59E-04
GO:0031982	vesicle	48	6.4	5.57E-04
GO:0016023	cytoplasmic membrane-bounded vesicle	41	5.4	7.81E-04
GO:0031410	cytoplasmic vesicle	45	6.0	9.54E-04
GO:0031988	membrane-bounded vesicle	42	5.6	9.68E-04
GO:0044433	cytoplasmic vesicle part	16	2.1	1.14E-02
Annotation Cluster 7; Enrichment Score: 2.88				
GO ID	Term	Count	%	EASE
GO:0001944	vasculature development	23	3.1	3.55E-04
GO:0001568	blood vessel development	22	2.9	5.98E-04
GO:0048514	blood vessel morphogenesis	16	2.1	1.07E-02

Table 2. Functional annotation clustering by KEGG terms.

Differentially-expressed transcripts (>2-fold) were analyzed by DAVID as in Table 1, with functional clustering according to KEGG terms.

KEGG ID	Term	Count	%	EASE
rno03320	PPAR signaling pathway	13	1.7	2.30E-05
rno04512	ECM-receptor interaction	13	1.7	8.85E-05
rno04510	Focal adhesion	20	2.7	3.15E-04
rno00564	Glycerophospholipid metabolism	10	1.3	7.93E-04
rno00260	Glycine, serine and threonine metabolism	7	0.9	0.001565
rno04950	Maturity onset diabetes of the young	6	0.8	0.003453
rno00565	Ether lipid metabolism	6	0.8	0.011225
rno04640	Hematopoietic cell lineage	9	1.2	0.013236
rno04270	Vascular smooth muscle contraction	11	1.5	0.015961
rno04920	Adipocytokine signaling pathway	8	1.1	0.018192
rno00620	Pyruvate metabolism	6	0.8	0.019725
rno04610	Complement and coagulation cascades	8	1.1	0.022652
rno04144	Endocytosis	15	2.0	0.033278
rno04910	Insulin signaling pathway	11	1.5	0.041346

Table 3. Expression of inflammation-related transcripts.

Relative transcript expression is given as fold change in BR samples compared to BD, thus a negative number is indicative of higher expression in BD. All listed transcript expression changes are P<0.05. AffyID, Affymetrix identifier.

AffyID	Gene name	Gene symbol	BR/BD (fold)
1387796_at	Arachidonate 12-lipoxygenase	Alox12	-2.1
1370405_at	Mast cell protease 1 precursor	RMCP1	-2.3
1387173_at	Mast cell chymase 1	Cma1	-2.3
1387902_a_at	Similar to NGF-binding Ig light chain	LOC500183	-2.8
1388275_at	T cell receptor beta chain	LOC100912777	-2.0
1371016_at	T cell receptor active alpha-chain C-region	LOC290071	-2.3
1370218_at	Lactate dehydrogenase B	Ldhb	-2.6
1388166_at	Immunoglobulin heavy chain 6	Igh6	-2.6

Table 4. Expression of injury/fibrosis-related transcripts.

Expression fold-change is listed as in Table 3.

AffyID	Gene name	Gene symbol	BR/BD (fold)
1387811_at	Angiotensinogen	Agt	-2.8
1387805_at	Bcl2 adenovirus E1B 19 kDa-interacting protein 3	Bnip3	-2.3
1370864_at	Collagen I α 1	Col1a1	-2.0
1369955_at	Collagen V α 1	Col5a1	-2.1
1368347_at	Collagen V α 3	Col5a3	-2.5
1390846_at	Collagen XVI α 1	Col16a1	-2.1

Table 5. Expression of pancreatic repair transcripts.

Expression fold-change is listed as in Table 3. Expression in acute pancreatitis is derived from a previously published cDNA profile (Duseti et al. 2000).

AffyID	Gene name	Gene symbol	BR/BD (fold)	Acute pancreatitis
1368238_at	Pancreatitis-associated protein 1 (PapI) / Regenerating islet-derived 3 beta	Reg3b	+34.3	+13.3
1387930_at	Pancreatitis-associated protein 2 (PapII) / Regenerating islet-derived 3 alpha	Reg3a	+14.9	+8.5
1367581_a_at	Osteopontin / Secreted phosphoprotein 1	Spp1	+2.5	+3.6

Table 6. Expression of endocrine and exocrine transcripts.

Expression fold-change is listed as in Table 3.

AffyID	Gene name	Gene symbol	BR/BD (fold)
1371034_at	Hepatocyte Nuclear Factor 6 / One cut homeobox 1	HNF6	+3.5
1387760_a_at	Hepatocyte Nuclear Factor 6 / One cut homeobox 1	HNF6	+3.0
1368711_at	Hepatocyte Nuclear Factor 3 β / Forkhead box A2	FoxA2	+3.5
1369888_at	Glucagon	Gcg	+3.0
1387815_at	Insulin	Ins1	-1.3
1370359_at	α -amylase 1A	Amy1a	+3.0
1387228_at	Glucose transporter 2 / Slc2a2	Glut2	+2.1
1367989_at	Glucose transporter 4 / Slc2a4	Glut4	-3.5
1387212_at	Mist1 transcription factor	Bhlha15	+2.1
1387660_at	Islet amyloid polypeptide	Iapp	+3.0
1369787_at	Cholecystokinin A receptor	Cckar	+2.1
1387700_at	Cholecystokinin A receptor	Cckar	+2.0
1369803_at	Pancreas-specific transcription factor 1a	Ptf1a	+2.0
1369410_at	Cis-Golgi p28	p28	-2.5

FIGURE LEGENDS

Figure 1. Quality analysis of microarray data. Microarray data was analyzed using GCOS, BioConductor and AffyImGUI software. (A) Scatter plot of all transcript intensities for BD (x axis) and BR (y axis) samples, demonstrating a linear relationship without evidence of bias. (B) MA plot of microarray data following data normalization, with BR/BD signal intensity ratio (M) plotted against the average signal intensity (A). The distribution around M=0 reveals a lack of difference in expression of most transcripts between the two samples, as expected, and indicates successful normalization of the data.

Figure 2. qPCR analysis of gene expression following biotin repletion. Total RNA was isolated from BD or BR rat pancreata used for the microarray study and mRNA expression of injury/fibrosis transcripts Bnip3 and Col5a1 (A) or repair transcripts PapI and PapII (B) were analyzed by qPCR. Results were normalized to GAPDH and to the corresponding BD sample. Results represent mean $\pm$ SEM for n=4-5; *P<0.05 vs. BD sample.

Figure 3. Biotin-mediated regulation of gene expression in AR42J cells. AR42J pancreatic acinar cells were grown in either biotin-containing F-12K medium or biotin-free DME medium and treated with vehicle or 50 pM biotin for 24 or 48 hours. Total RNA was isolated and assayed by qPCR for expression of repair transcripts PapI, PapII and Spp1 (A), for injury/fibrosis transcripts Bnip3, Col1a1 and Col5a1 (B), or for secretory function transcripts Glut4 and HNF6 (C). Results were normalized to GAPDH and to the corresponding vehicle control. Results represent mean $\pm$ SEM for n=3-5; *P<0.05 vs. vehicle control.

Figure 1

254x874mm (300 x 300 DPI)

Figure 2

268x893mm (300 x 300 DPI)

Figure 3

268x536mm (300 x 300 DPI)