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Prevention of hyperglycemia in Zucker diabetic fatty rats by exercise training: effects on gene expression in insulin-sensitive tissues determined by high-density oligonucleotide microarray analysis

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Abstract

Exercise training (ET) causes metabolic improvement in the prediabetic and diabetic states. However, only little information exists on the changes to ET at the transcriptional level in insulin-sensitive tissues. We have investigated the gene expression changes in skeletal muscle, liver, fat, and pancreatic islets after ET in male Zucker diabetic fatty (ZDF) rats. Eighteen ZDF rats (7 weeks old) were divided in a control and ET group. Exercise was performed using a motorized treadmill (20 m/min 1 hour daily for 6 days a week). Blood glucose, weight, and food intake were measured weekly. After 5 weeks, blood samples, soleus muscle, liver, visceral fat (epididymal fat pads), and islet tissue were collected. Gene expression was quantified with Affymetrix RG-U34A array (16 chips). Exercise training ameliorates the development of hyperglycemia and reduces plasma free fatty acid and the level of glucagon-insulin ratio ($P < .05$). In skeletal muscle, the expression of 302 genes increased, whereas that of 119 genes decreased. These changes involved genes related to skeletal muscle plasticity, Ca^{2+} signals, energy metabolism (eg, glucose transporter 1, phosphorylase kinase), and other signaling pathways as well as genes with unknown functions (expressed sequence tags). In the liver, expression of 148 genes increased, whereas that of 199 genes decreased. These were primarily genes involved in lipogenesis and detoxification. Genes coding for transcription factors were changed in parallel in skeletal muscle and liver tissue. Training did not markedly influence the gene expression in islets. In conclusion, ET changes the expression of multiple genes in the soleus muscle and liver tissue and counteracts the development of diabetes, indicating that ET-induced changes in gene transcription may play an important role en the prevention of diabetes.

1. Introduction

The development of type 2 diabetes is counteracted by lifestyle modifications such as weight loss, dietary changes, and exercise [1,2]. Exercise training (ET) is associated with an improvement of insulin sensitivity, amelioration of hyperlipidemia, and reduction in cardiovascular disease [3].

The mechanisms underlining the metabolic improvement during training have been studied previously. After ET, the glucose clearance and insulin action are increased in healthy [4] as well as in type 2 diabetic subjects [5]. Recent studies [6-8] have focused on the importance of gene expression modifications in the adaptation to ET, especially in skeletal muscle. However, the impact at the molecular level in the various insulin-sensitive tissues has only been scarcely described in diabetic and prediabetic states.

Prospective studies assessing the physiological and biochemical effects of physical training in humans can be difficult to carry out in a controlled setting. A suitable animal model may provide a unique research tool for such studies. The Zucker diabetic fatty (ZDF) rats have a mutation in the gene encoding the leptin receptor, which results in the expression of nonfunctional receptor, and

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develop obesity, insulin resistance, and type 2 diabetes. The progression into overt diabetes is quite similar to the development of human type 2 diabetes [9,10].

In the present study, we have investigated in an animal model of type 2 diabetes, using male ZDF rats, the effect of 5 weeks' training on the metabolism and gene expression in soleus muscle, liver, pancreatic islets, and adipose tissue using the oligonucleotide microarray gene chip technology.

Our hypothesis was that ET causes alterations at the gene expression level in insulin-sensitive tissues that lead to an improvement in the glucose metabolism in type 2 diabetes. The primary objective was to compare changes in the expression of genes involved in metabolic (glucose and lipid) and signaling pathways in skeletal muscles, liver, pancreatic islets, and visceral adipose. The second objective was to identify possible new genes involved in the prevention of type 2 diabetes after ET.

2. Research design and methods

2.1. Animals

Male ZDF (ZDF/Gmi-*fa/fa*) rats were purchased from Genetic Models (Indianapolis, IN). Animals were housed in individual cages in a vivarium, which maintains a constant temperature and an artificial 12-h light-dark cycle. Animals were fed with commercial chow (Purina Formulab 5008, Ralston Purina Corp, St Louis, MO).

2.2. Experimental protocol

After acclimatization, 18 animals at the age of 7 weeks were randomly divided into 2 groups: control (n = 9) and ET (n = 9). The animals were maintained on an ad libitum diet. The ET animals ran 1 hour daily at 20 m/min on a motorized treadmill (Columbus Instruments, Columbus, OH). The exercise was performed in the morning 6 days a week. In the first week, exercise intensity and duration were gradually increased to ensure compliance and to reduce stress and injury. The 2 groups were studied in parallel for a period of 5 weeks (from 7 to 12 weeks of age). All institutional guidelines for care and use of animals were followed. One ET animal was excluded and subsequently killed after injury in the second week. Animals were weighed in the morning once per week, and glucose was measured in blood from the tail vein after 4 hours without access to food. Food intake was measured. At the end of the fifth week, 24 hours after the last exercise bout and after 4 hours without access to food, the animals were anesthetized with pentobarbital (50 mg/kg IP), and capillary blood samples were obtained from the retro-ocular plexus using a 75-μL heparinized capillary tube. Successively, the pancreas, liver, skeletal muscle (soleus), and visceral fat tissues (epididymal fat pads) were isolated. Liver, muscles, and fat tissue were rapidly frozen and stored at -80°C. The remains of the animals were also stored at -800°C for further body composition measurements.

2.3. Biochemical measurements

The weekly control of blood glucose concentration was carried out using One Touch Instrument (Lifescan, Milpitas, CA). Other blood samples were placed on ice and centrifuged (10 minutes, 4°C, 4000 rpm). Total cholesterol was analyzed with the cholesterol CHOD-PAP method (Roche, Mannheim, Germany). Triglyceride was analyzed with the triglyceride GPO-PAP method (Roche). Free fatty acid was analyzed with the nonesterified fatty acid (NEFA) C kit, ACS ACOD method (Wako, Neuss, Germany). We analyzed plasma insulin and glucagon by radioimmunoassay kits (Linco Research, St. Charles, MO).

2.4. Isolation of pancreatic islets

Islets were isolated by the collagenase digestion technique [11] with minor modifications. In brief, when the animals had become anesthetized, a midline laparotomy was performed. The pancreas was retrogradely filled through the pancreatic duct with 9 mL ice-cold Hanks balanced salt solution (Sigma Chemicals, St. Louis, MO) supplemented with 0.9 mg/mL collagenase P (Boehringer Mannheim, Mannheim, Germany). The pancreas was subsequently removed and incubated for 17 minutes at 37°C in a shaking water bath. After rinsing in ice-cold Hanks balanced salt solution, the islets were handpicked under a stereomicroscope and stored in 1.5 mL Trizol (Gibco BRL, Paisley, UK).

2.5. Messenger RNA preparation and analysis

2.5.1. RNA extraction and samples pool

Total RNA was isolated from the different tissues using Trizol. For gene chip analysis, an equal amount (10 μg) of total RNA from a given tissue was pooled in 2 subgroups (RNA from 4 to 5 animals in each subgroup).

2.5.2. Gene expression chip analysis

RNA labeling, array hybridization, and scanning were preformed as previously described [12] according to the Affymetrix (Santa Clara, CA) technical manual. All arrays were visualized using Affymetrix Genechip 5.0 software. The Rat Genome U34 A monitors the expression of more than 8000 genes and expressed sequence tag (EST) clusters. The present microarray has been largely evaluated and used in similar experiment [12-18].

2.5.3. Data analysis

A total of 16 gene chips were used, 2 biological replicas for each tissue and condition. After global scaling, the signal, the detection (P, present; A, absent), the signal log ratio (SLR; ie, the logarithm in base 2 of the fold change), and the different call change (I, increase; D, decrease; M, moderate I or D) were calculated. For every tissue, we compared the gene expression of the intervention groups vs the control group in a double-cross analysis (concordance analysis), for a total of 4 comparisons. Moreover, for every gene or EST, we calculated the concordance on the different call change (I + MI, increase and moderate increase; D +

MD, decrease and moderate decrease; or NC, no change), the average fold change, and the average SLR. A cutoff value was chosen as a concordance in the different call change of 75% or more. Finally, genes with a mean SLR of 0.8 or more or −0.8 or less (arbitrarily chosen) were considered up- or down-regulated, respectively, and were tentatively grouped with respect to the putative functions.

2.6. Statistical analysis

For metabolic measurements, statistical analysis was performed using 1-way analysis of variance or unpaired or paired Student *t* test, as appropriate. Data are expressed as the mean ± SEM or otherwise specified. Differences were considered significant at *P* values less than .05. Gene chip analyses were presented as specified above. Detection signal was only specified in the text if appropriate.

3. Results

3.1. Metabolic profiles

The metabolic results after 5 weeks are summarized in Table 1. At the start of the intervention study, no significant difference was measured in body weight and blood glucose between the groups. The time course of changes in the glucose level and the weight gain during the 5 weeks are presented in Fig. 1. After 5 weeks, the animals in the control group (n = 9) exhibited hyperglycemia (average blood glucose level, 12.6 ± 0.9 mmol/L) compared with the animals (n = 8) in the trained group (average blood glucose level, 5.7 ±1.3 mmol/L; *P* = .001). Furthermore, free fatty acid concentrations and the glucagon-insulin ratio were higher in control than in trained rats. The weight gain was similar in the 2 groups (Fig. 1B).

Table 1
Summary of metabolic measurements in ET and control groups of male ZDF rats after 5 weeks

	Control	ET
Fasting blood glucose (mmol/L)	12.6 ± 0.9	5.7 ± 1.3*
Weight gain (g)	132 ± 4.3	143 ± 6.2
Insulin (ng/mL)	9.6 ± 0.4	10.1 ± 0.5
Glucagon (pg/mL)	63 ± 6	50 ± 4.6
Glucagon-insulin ratio	6.9 ± 0.6	4.7 ± 0.8**
Triglycerides (mmol/L)	6.8 ± 0.3	6.5 ± 0.3
Free fatty acids (mmol/L)	2.3 ± 0.2	1.6 ± 0.03**
Cholesterol (mmol/L)	3.9 ± 0.2	3.2 ± 0.2
Urine glucose (mmol/L)	107 ± 20	58 ± 16**
Difference in food intake[a]	13.3 ± 1.6	9.6 ± 2.1

Values are means ± SE; n = 9 for control group and n = 8 for the ET group.

[a] Increment in food intake between the first and last day of the study period (g/d per rat).

* $P \leq .01$, significantly different from the control group.

** $P \leq .05$, significantly different from the control group.

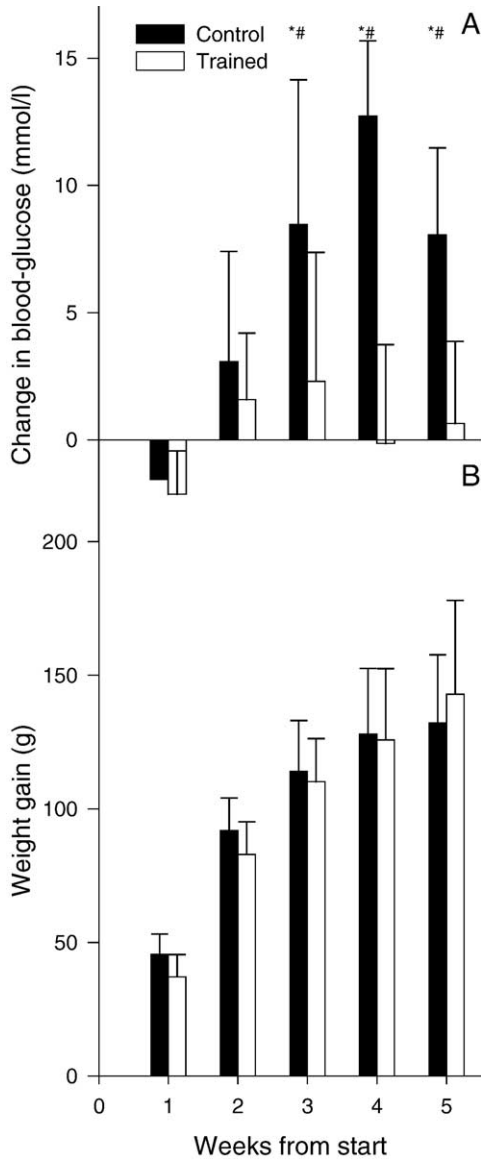


Fig. 1. Dynamic changes in blood glucose and weight gain. At the start of the experiment the blood glucose was 4.6 ± 0.3 mmol/L in the control group (n = 9) and 4.9 ± 0.6 mmol/L in the ET group (n = 8). At the start of the experiment, the weight was 222 ± 3 g in the control group and 231 ± 7 g in the ET group. Bars represent the difference from baseline and are expressed as means ± SD. **P* < .001 indicates significant difference from baseline; #*P* < .001, significant difference from corresponding value in trained group.

3.2. Gene chip analysis

We examined the possibility that changes in the expression of genes and ESTs represented on the Affymetrix Genechip RG-U34A might be associated with the beneficial effects of ET. In Tables 2 and 3, the messenger RNA (mRNA) expression changes, with a concordance in different call change of 75% or more and an SLR of 0.8 or more or −0.8 or less of genes related to lipid, glucose, and signaling pathways in soleus muscle are shown. Additional information and Supplementary Tables are available at the web site: http://www.mdl.dk/publications_sup.htm.

3.2.1. Skeletal muscle mRNA expression

Of all genes or ESTs surveyed in the oligonucleotide microarray, 7524 (86%) displayed a concordance of 75% or more. Of those, 302 (4.0%) increased expression, 119 (1.6%) decreased expression, and 7103 did not change expression among the ET group vs the control group. In Fig. 3, the number of genes as a function of SLR is shown for genes and ESTs with a concordance of 75% or more in I or D different call in comparison between trained and sedentary ZDF rats. There was a skewed bell-shaped distribution for the gene expression difference.

As described in Research design and methods, we grouped the genes that were up-regulated (SLR $\geq$ 0.8) or down-regulated (SLR ≤ -0.8) according to their putative function (Tables 2 and 3; Supplementary Tables 1 and 2).

We found that the expression of a number of genes involved in the formation of proteins, which are implicated in muscle contraction or in muscle differentiation and growth, was changed in soleus muscle in our intervention group. Thus, the mRNA expression of 4 components of myofibrils was increased. Interestingly, 4 transcription factors involved in muscle differentiation and tropism were increased and, in addition, other molecules involved in these processes were modified.

Table 2
Genes influencing the metabolism of glucose and lipids in the soleus muscle

Title	Seq. derived from	Gene symbol	SLR
Alcohol dehydrogenase family 3, subfamily A2	M73714	*Aldh3a2*	1.87
Lactate dehydrogenase B	U07181	*Ldhb*	1.29
Stearoyl-CoA desaturase 2	AA875269	*Scd2*	1.25
Transferrin receptor	M58040	*Trfr*	1.22
Epoxide hydrolase 1	M26125	*Ephx1*	1.16
ATPase Na^+/K^+ transporting β 1 polypeptide	AI230614	*Atp1b1*	1.04
Pyruvate dehydrogenase kinase, isoenzyme 1	L22294	*Pdk1*	0.95
NADH-cytochrome b_5 reductase	AA963839	*DIA1*	0.87
GLUT1	S68135	*Glut1*	0.81
Phosphorylase kinase (exons 2 and 3)	M98826	*PhK*	−0.81
Monocarboxylate transporter	U87627	*Mct3*	−0.89
Mitochondrial HMG-CoA synthase	M33648	*Mgcs*	−0.92
Glutathione *S*-transferase, mu type 2 (Yb2)	J03914	*Gstm2*	−0.95
Glutamine synthetase (glutamate-ammonia ligase)	AA852004	*Glul*	−1.11
Muscle fructose 1,6-bisphosphatase	AJ005046	*Fbp2*	−1.15
Phosphorylase kinase α subunit	M92919	*PhK-α-subunit*	−1.42
Pyruvate dehydrogenase kinase, isoenzyme 4	AF034577	*Pdk4*	−1.5
Phosphoglucomutase	U20195	*Pgm 1*	−1.61
Uncoupling protein 3	AF030163	*Ucp3*	−1.71
Phosphofructokinase-M	D21869	*PFK-M*	−2.07
Glycerol 3-phosphate dehydrogenase	AB002558	*Gpd3*	−3.69

Tables 2 and 3 list the up- and down-regulated genes in the exercise-trained ZDF rats as compared with the control rats. The first column lists the name of either the gene or the gene product. The second column lists GenBank accession number for the respective genes or ESTs. The second column lists the gene symbol. The fourth column lists the mean of the relative expression intensity values, expressed as SLR (see Research design and methods) of the exercise-trained group as compared with the control group.

Table 3
Genes influencing signal pathways in the soleus muscle

Title	Seq. derived from	Gene symbol	SLR
Mitogen-activated protein kinase–activated protein kinase	AI011376		−1.27
Phosphodiesterase	L27059	*Pde4d*	−1.88
Protein phosphatase 3, regulatory subunit B, α isoform (calcineurin B, type I)	D14568	*Ppp3r1*	−0.82
Ras-related protein p23	X12535	*Rras*	−1.19
SAP kinase-3	X96488	*Sapk3*	−1
IGF1	M81183	*Igf1*	1.34
Annexin 1 (p35) (Lipocortin 1)	AI171962	*Anxa1*	0.98
S100-related protein, clone 42C	J03627	*S100A10*	0.84
S100 calcium-binding protein A4	X06916	*S100a4*	0.95
14-3-3 Protein γ subtype	AI236721	*Ywhag*	1.21
Calsequestrin 2	U33287	*Casq2*	2.21

The level of mRNA expression of 7 enzymes related to glucose metabolism and of 5 proteins related to fat metabolism or energy metabolism was changed. Moreover, we found changes in mRNAs for proteins related to signaling pathways (eg, via Ca^{2+}).

3.2.2. Liver mRNA expression

Of all genes or ESTs surveyed in the oligonucleotide microarray, 8108 (92%) displayed a concordance of 75% or more. Of those, 148 (1.8%) increased expression, 199 (2.6%) decreased expression, and 7761 did not change expression. We found that most of the changes in mRNA expression were related to metabolic pathways (Supplementary Tables 3 and 4; Fig. 3B). Especially, genes involved in lipid metabolism were differentially regulated. Exercise training up-regulated particularly key enzymes involved in lipogenesis (eg, malic enzyme, fatty acid synthase), whereas a number of enzymes involved in lipid oxidation were down-regulated (short branched-chain acyl-coenzyme A [CoA] dehydrogenase precursor, mitochondrial long-chain 3-ketoacyl-CoA thiolase β subunit of mitochondrial trifunctional protein). Moreover, 6 enzymes that could be involved in detoxification and/or fat catabolism, with formation and metabolism of epoxide compounds, were down-regulated in the exercise-trained group compared with the control group, whereas others were up-regulated after training (Supplementary Tables 3 and 4).

3.2.3. Visceral adipose tissue mRNA expression

Of all genes or ESTs surveyed in the oligonucleotide microarray, 77% displayed a concordance of 75% or more, and of those, 192 increased expression, whereas 278 decreased expression (Fig. 2). Based on our criteria, 51 were up-regulated, whereas 278 were down-regulated (Supplementary Table 6).

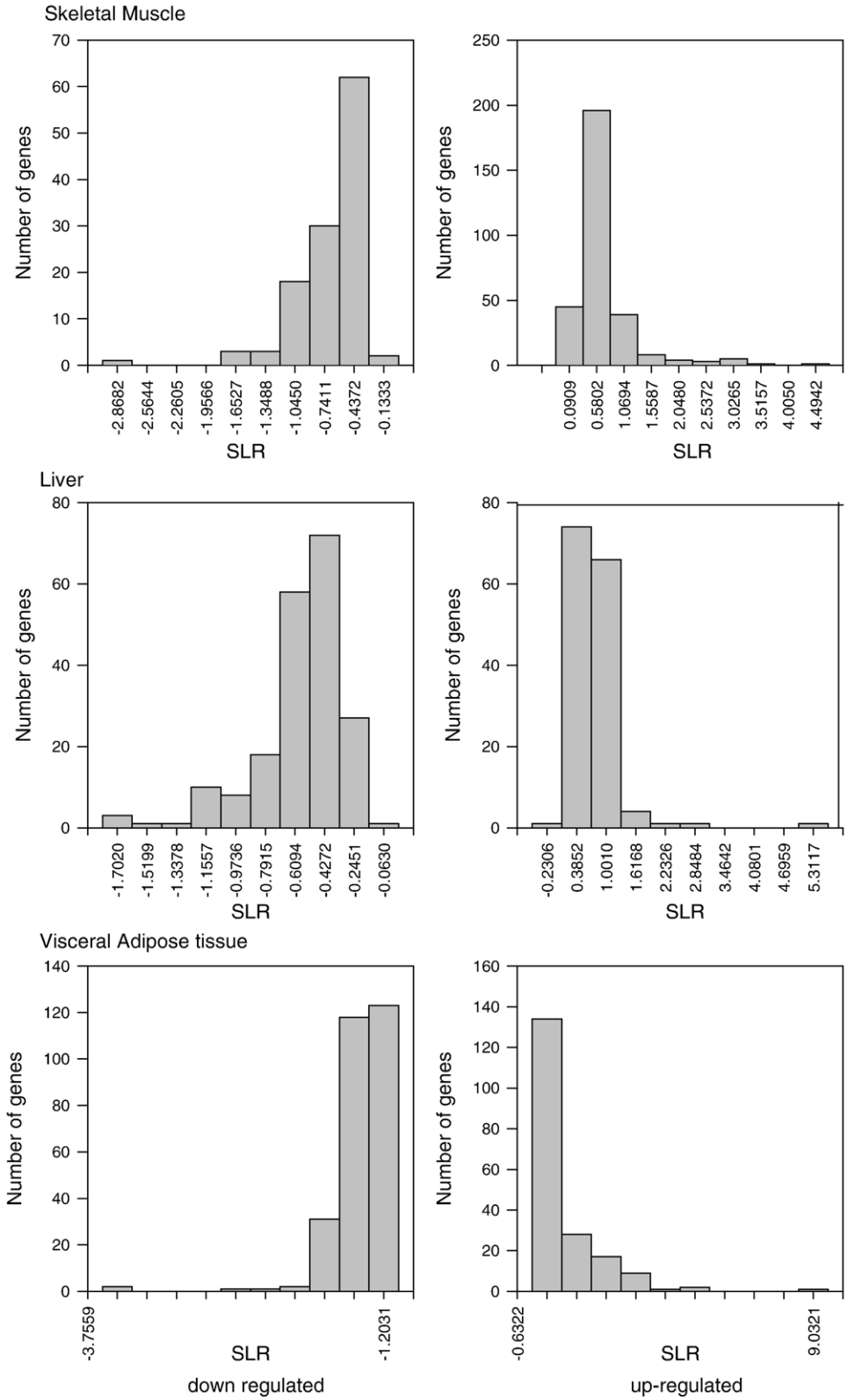


Fig. 2. Relative changes in mRNA expression in exercise-trained vs control in skeletal muscle, liver, and visceral adipose tissue. The number of genes up- or down-regulated with a concordance of 75% or more is plotted to show the distribution of the magnitude of change. SLR indicates average SLR.

3.2.4. Islets mRNA expression

Gene expression in pancreatic islets was only slightly affected. Of the genes or ESTs surveyed in the oligonucleotide microarray, 7 increased expression, 88 decreased expression, and 8790 did not change expression in the ET group compared with the control group. Only 14 RNA sequences among the 8800 compared satisfied our criteria (Supplementary Table 5).

4. Discussion

The major findings in the present study are that ET is able to abolish the development of hyperglycemia in ZDF rats, and this effect is accompanied by changes in mRNA expression in skeletal muscle and in other insulin-sensitive tissues in specific pathways. Furthermore, new genes that may be associated with mechanisms able to control diabetes have been pointed out.

High-density microarray (gene chip) analysis was applied to examine the adaptive responses of the ZDF rat, an animal model of type 2 diabetes, to 5 weeks of ET. This enabled us to test the transcriptional expression of more than 8000 genes or ESTs from 4 tissues (skeletal muscle, liver, visceral adipose tissue, and pancreatic islets). The data demonstrated that training differentially modified the transcription of several genes related to different functions in various tissues. Our data indicate that regulation of gene expression represents one cause of tissue adaptations to ET. This mechanism may be involved in the prevention or treatment of type 2 diabetes by exercise.

4.1. Metabolic profiles

Hyperglycemia, increased levels of free fatty acids, and relative hypoinsulinemia and hyperglucagonemia characterize type 2 diabetes. Regular exercise counteracts the development of diabetes in ZDF rats. The training program improved blood glucose, NEFA, and the glucagon-insulin ratio, corroborating previous reports [2,19]. It is worth noting that body weight changed in parallel in the 2 groups. This corroborates previous reports [20,21] showing that muscle mass increased and fat mass decreased after training. Unfortunately, we did not directly measure total fat and skeletal muscle mass in our animals. The levels of NEFA and glucose in plasma were significantly lowered in the training group. This may reflect the fact that peripheral tissue, presumably skeletal muscle, uses free fatty acids (NEFA) and glucose as fuel during exercise. Reduction in NEFA and glucose levels is a major metabolic improvement induced by ET, which may ameliorate glucotoxicity and lipotoxicity in the ZDF rat [22].

It is important to emphasize that in the present study, samples were obtained in a fasting state and several hours after the last exercise session, enabling an extrapolation of the effects of the training to everyday life conditions.

4.2. Gene chip

Gene chip represents a novel method to study gene expression. We found that the concordance was 86% to 92% in the 4 pairwise comparisons in each of the 4 tissues. Many of the changes were relatively modest (Fig. 2). However, because the criteria (75% concordance and SLR ≥ 0.8 or SLR ≤ -0.8) chosen for defining the differences in gene expression were rather severe, the detected differences are possibly part of the overall program of change. Similar amplitudes in gene expression changes have been described by other reports where gene chip technology was used to investigate gene expression in diabetic [23] and energy-restricted mice [24]. The relative importance of transcriptional (mRNA synthesis) and translational (mRNA stability or translational efficiency) mechanisms for the training-induced increases in skeletal muscle protein abundance remains to be fully elucidated [6]. It is even so that an increase in mRNA expression may not result in an increase in protein or enzyme activity. At the moment, the cutoff values in gene expression that define a concomitant increase in protein levels after gene activation have not yet been defined [25]. However, modest changes in gene expression of certain genes may have pronounced physiological impact.

In the present study, the requirement of a high numerical SLR value to avoid false-positive response implies missing physiologically significant changes. Thus, it is a common finding that concentration of glucose transporter (GLUT) 4 and glycogen synthase are increased in muscle after training [4,5,26], but in the present study, the observed GLUT4 mRNA found was not considered significant (SLR, 0.8; concordance, $<75\%$), and neither did glycogen synthase mRNA increase significantly.

Because samples from whole muscle, liver, adipose tissue, and pancreatic islets were the source of RNA for this study, a variety of different cell types may contribute to the changes seen in gene expression. For instance, skeletal muscle tissue includes skeletal muscle cells, smooth muscle cells, satellite cells, endothelial cells, nerve cells, blood cells, adipocytes, and fibroblasts. However, the isolation of a single cell type from a tissue is often complicated and may influence the impact of the conditions that were under study, in present case ET. For this reason, we find it important as an initial step to study changes in mRNA expression as a response to training in intact tissues of diabetic rats. In addition, because a single cell type dominates each of the tissues studied, it is a fair assumption that clear changes in mRNA levels reflect changes in those cells.

Our observations provide the first comprehensive assessment of the impact of ET in a diabetic animal model on gene expression and underscore the utility of high-density oligonucleotide microarray analysis in the study of complex biological phenomena, such as the interaction between exercise and type 2 diabetes. Moreover, oligonucleotide microarray analysis appears to be a unique method to search for new genes that could be associated with type 2 diabetes

or with adaptations of the body to ET (see ESTs in the tables) and help to answer questions not resolved. However, although we, in the present study, have investigated more than 8000 sequences, in the whole genome, more than 30 000 genes are present.

4.2.1. Type 2 diabetes development and gene expression

Because the pathophysiology of type 2 diabetes is complex, it is likely that a detailed analysis of gene expression patterns will reveal both organ-specific alterations and alterations that are common in many tissues. Glucotoxicity and lipotoxicity are considered 2 important factors in the development of type 2 diabetes and the late complication of diabetes [22,27,28]. There is evidence indicating an increase in non–adipose tissue lipogenesis in obese subjects [29] and in animal models of type 2 diabetes [30-32]. However, only when the capacity to accumulate fatty acids in the liver is impaired does a diabetic phenotype develops [30].

Intracellular increase in reactive oxygen species (ROS) mediates the glucotoxicity and possibly the lipotoxicity in beta cells and in other tissues [28,33,34]. ROS induce the activation of stress-inducible genes, leading to organ failure and apoptosis [34]. Exercise training lessened the side of glucotoxicity and lipotoxicity by lowering plasma glucose and fatty acid levels (Table 1, Fig. 1). In line with this, training was able to counteract the increase in mRNA expression of components previously known to be associated with oxidative stress failure (eg, Sap kinase-3, c-*fos*, and activating transcription factor 3 in skeletal muscle) (Table 3) [35]. mRNA of uncoupling protein 3, which may reduce the formation of ROS from NEFA [36,37], was also decreased in soleus muscle of trained animals (Table 2, Fig. 3A). In liver, mRNAs encoding detoxification enzymes (eg, catalase) were up-regulated, suggesting an increased protection against ROS in trained animals. Moreover, in liver, genes related to lipogenesis (eg, malic enzyme 1, fatty acid synthase, and adenosine triphosphate citrate lyase) were up-regulated in trained ZDF rats (Supplementary Table 4, Fig. 3B), a finding agreeing with the hypothesis that a high capacity to accumulate fatty acids in liver tissue protects against development of a diabetic phenotype [30].

4.2.2. Skeletal muscle metabolism after ET

mRNAs for enzymes and molecules favoring glycolysis (eg, glycerol-3-phosphate dehydrogenase, phosphoglucomutase, phosphofructokinase, and monocarboxylate transporter) were down-regulated upon training in soleus muscle (Table 2, Fig. 3A) in accordance with what has generally been found at the protein level in skeletal muscle. This does not provide an explanation for the training-induced decrease in glucose levels seen in intact animals. However, the findings were not unambiguous. Thus, after training, the mRNA for the synthesis of the B form of glycolytic enzyme lactate dehydrogenase was up-regulated, and fructose 1,6-bisphosphatase, which facilitates reverse glycolysis, was down-regulated. Furthermore, mRNA for the GLUT1 glucose transporter was up-regulated and mRNA for phosphorylase kinase was down-regulated, and these changes tend to enhance glucose storage in skeletal muscle, which is probably essential for the improved glucose metabolism after training [4]. Increases in the GLUT4 transporter [26] and in glycogen synthase [4,5] may also be important for enhancing glucose storage, although such changes were not detected in the present study. The time of sampling in relation to exercise bouts may be critical for detection of mRNA changes, and this may explain discrepancies between the present and previous findings of mRNA and protein changes [38]. Furthermore, although the soleus muscle has been the most studied rodent muscle, responses of this muscle to training may quantitatively differ from responses of muscles with a different fiber-type composition [39].

4.2.3. Muscle-specific mRNA expression (muscle plasticity)

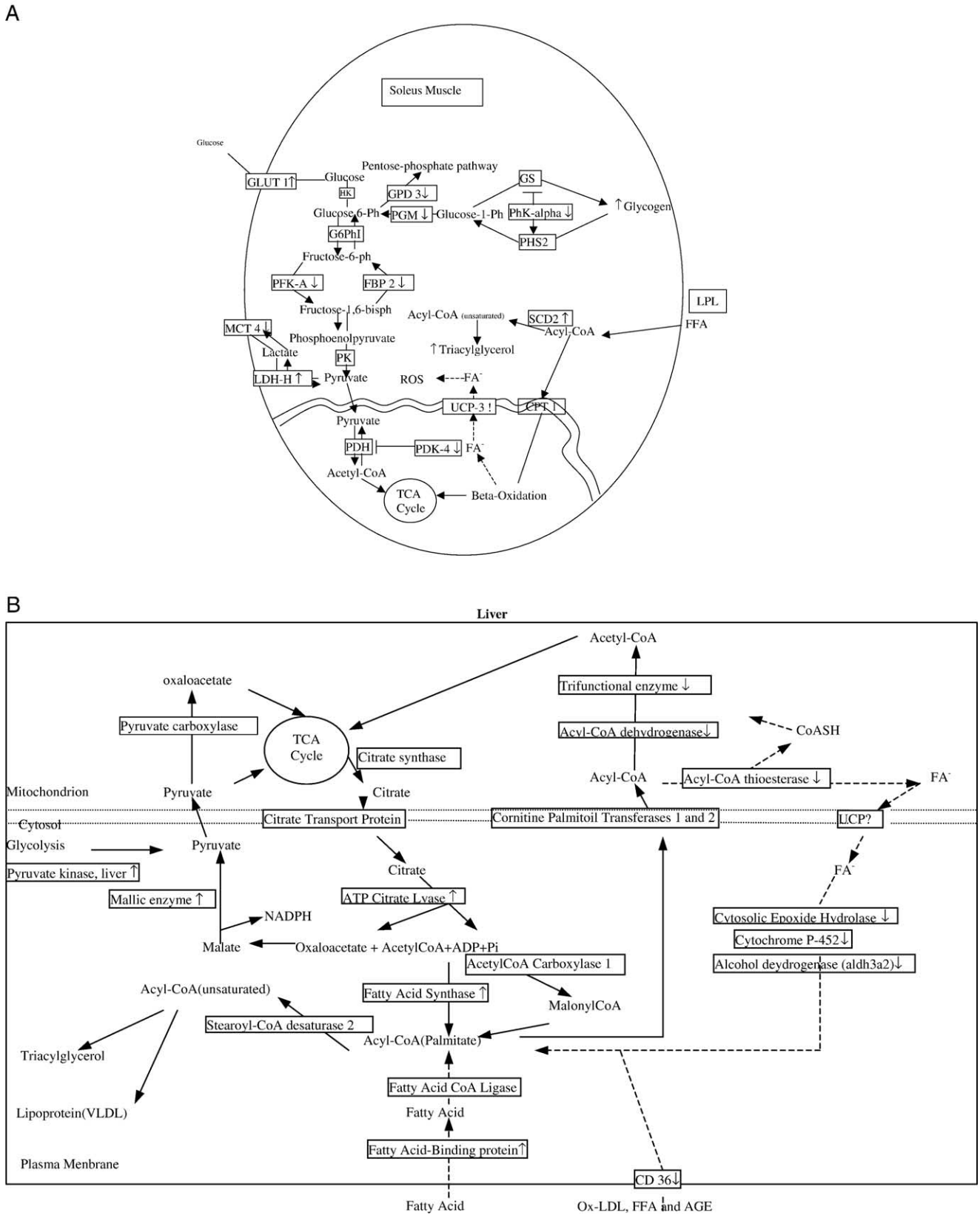
Skeletal muscle is a remarkably adaptive tissue able to sustain regeneration, hypertrophy, and metabolic adaptation [40]. One of the most powerful stimuli for inducing skeletal muscle cellular reorganization (plasticity) is ET [41]. During muscle hypertrophy, the sarcoplasma-myonuclei ratio remains constant [42]. The requirement for additional nuclei to support hypertrophy appears to be met via the proliferation and differentiation of satellite cells. We found that the expression of a number of genes involved in synthesis of proteins implicated in muscle differentiation and growth (eg, myogenin) as well as of myofibrillar protein (eg, sarcomeric myosin heavy chain) was changed in soleus muscle after 5 weeks of training (Supplementary Tables 1 and 2).

We confirmed that Ras related associated with diabetes (GenBank accession no. U12187), previously associated with type 2 diabetes [43], is positively associated with muscle plasticity [44]. Eukaryotic initiation factors (eIFs), eukaryotic translation elongation factors, and eIF-binding proteins (or PHAS) are members of a newly discovered family of proteins that regulates translation initiation and protein synthesis [45]. In our study, mRNA for PHAS-I, an inhibitor of translation factor eIF-4 [46], was down-regulated possibly reflecting an increase in protein synthesis. On the other hand, eIF 2 was down-regulated.

Insulin-like growth factor 1 (IGF1) is involved in both skeletal muscle regeneration and differentiation after muscle damage and training [40]. IGF1 stimulates glucose uptake. IGF1 mRNA was increased in soleus muscle and also in the liver (Table 3 and Supplementary Table 3) of trained ZDF rats.

4.2.4. Other changes

We found that mRNA of CD36, a newly discovered receptor that binds oxidized low-density lipoprotein, free fatty acid, and advanced glycation end products, was down-regulated in islets as well as in soleus muscle, liver, and adipose tissue (Supplementary Tables 2, 4, 5, and 6) from trained ZDF rats. Increased levels of CD36 have been found in human atherosclerotic lesions [47]. In islets, the presence and the function of CD36 have not been investigated so far.


A
Soleus Muscle
Glucose
GLUT 1↑
Glucose
HK
Pentose-phosphate pathway
GPD 3↓
Glucose 6-Ph
PGM ↓
Glucose-1-Ph
GS
PhK-alpha ↓
↑Glycogen
PHS2
G6PhI
Fructose-6-ph
PFK-A ↓
FBP 2 ↓
Fructose-1,6-bisph
LPL
FFA
Acyl-CoA (unsaturated)
SCD2 ↑
Acyl-CoA
MCT 4 ↓
Phosphoenolpyruvate
↑Triacylglycerol
Lactate
PK
LDH-H ↑
Pyruvate
ROS
FA-
UCP-3 ↑
CPT ↑
Pyruvate
PDH
PDK-4 ↓
FA-
Acetyl-CoA
TCA Cycle
Beta-Oxidation
B
Liver
Acetyl-CoA
oxaloacetate
Trifunctional enzyme ↓
Pyruvate carboxylase
TCA Cycle
Citrate synthase
Acyl-CoA dehydrogenase↓
CoASH
Mitochondrion
Pyruvate
Citrate
Acyl-CoA
Acyl-CoA thioesterase ↓
FA-
Cytosol
Citrate Transport Protein
Cornitine Palmitoil Transferases 1 and 2
UCP?
Glycolysis
Pyruvate
Citrate
FA-
Pyruvate kinase, liver ↑
Mallic enzyme ↑
ATP Citrate Lyase ↑
NADPH
Cytosolic Epoxide Hydrolase ↓
Cytochrome P-452↓
Malate
Oxaloacetate + AcetylCoA+ADP+Pi
AcetylCoA Carboxylase 1
Alcohol deydrogenase (aldh3a2)↓
Acyl-CoA(unsaturated)
Fatty Acid Synthase ↑
MalonylCoA
Stearoyl-CoA desaturase 2
Acyl-CoA(Palmitate)
Triacylglycerol
Fatty Acid CoA Ligase
Lipoprotein(VLDL)
Fatty Acid
Fatty Acid-Binding protein↑
Plasma Menbrane
CD 36↓
Fatty Acid
Ox-LDL, FFA and AGE

The spontaneously hypertensive rat does not express CD36 protein and develops a syndrome similar to the human dysmetabolic syndrome [48,49]. One may speculate that CD36 increases as a protective mechanism when blood NEFA, blood glucose, or advanced glycation end product increases.

4.2.5. Pancreatic islet mRNA expression

Reports suggest that islets of trained rats undergo metabolic modifications [50-52]. Our finding of identical insulin levels in the face of lower glucose levels in trained compared with untrained rats agrees with a beneficial effect of training in beta-cell function in diabetes [53].

Apparently, compared with soleus muscle, liver tissue, and adipose tissue, islets were only influenced to a limited degree at the level of gene expression by ET. On the other hand, the number of changes in the 4 tissues cannot be precisely compared. The tissues were differently treated. Muscle, adipose tissue, and liver were directly frozen after isolation, whereas islets had to be treated with collagenase and subsequently conserved in Trizol. Gene expression in pancreatic islets differs between prediabetic and diabetic ZDF male rats compared with lean Zucker fatty male rats [10]. These differences presumably contribute to the abnormalities in insulin secretion seen in ZDF rats. Exercise training did not influence the gene transcription of, for example, glucokinase and mitochondrial glycerol-3-phosphate dehydrogenase, which was modified in the above study [10]. Apparently, the genetic modifications present in islets of male ZDF rats cannot be corrected by ET. However, direct comparison between the present and the previous [10] study is impaired by the fact that different methodology, that is, gene chip microarray vs real-time polymerase chain reaction, was used.

Mitochondrial 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) synthase is the first and rate-limiting enzyme for the production of ketone bodies. Interestingly, we found that in pancreatic islets after training, mRNA for HMG-CoA synthase was down-regulated together with the mRNA of the second enzyme involved in the production of ketone bodies (HMG-CoA lyase). In rats, HMG-CoA synthase has been found in different organs, such as in the liver, testis, and colon, however, only in a very low amount in pancreas. It is also known that fasting, insulin, and circulating NEFA regulates the gene coding for HMG-CoA synthase. Classically, the liver organ has been considered the organ that is involved in the formation of ketones. The presence of HMG-CoA synthase mRNA and the possible activation of ketone body formation in pancreatic islets and skeletal muscle are puzzling. Ketones can directly influence the release of insulin and somatostatin from the endocrine pancreas [54]. Thus, ketones produced in islets may play a direct physiological as well as a pathophysiological role.

Inducible carbonyl reductase (CBR) is able to metabolize aromatic ketones and other substrates, which generally result in detoxication or inactivation of more chemical reactive carbonyl groups, so the reaction may induce the formation of ROS [55]. CBR mRNA was down-regulated in trained ZDF rats. The production of ketones and ROS might induce toxicity in the pancreatic islets and cell death.

4.2.6. Visceral adipose tissue mRNA expression

In adipose tissue mRNA for CD36, lipoprotein lipase, heart fatty acid–binding protein, and oxidized low-density lipoprotein, all involved in the kinetics of lipid, were down-regulated. mRNA of molecules involved in signaling was mainly reduced in adipose tissue of trained ZDF rats. Transcripts coding for proteins involved in signaling were mainly reduced in adipose tissue of trained ZDF rats. In adipose tissue, there was a change in expression of mRNAs coding for molecules associated with inflammation and immune response in trained animals. The present observations are similar to the finding in adipose tissue [30] and in isolated adipocytes [56] of leptin-deficient mice. However, the findings were not unambiguous. No genes specifically coding for enzymes involved in fatty acid synthesis (eg, fatty acid synthase) were up- or down-regulated. Various genes considered housekeeping genes were differentially regulated (eg, β-actin, glyceraldehyde 3-phosphate dehydrogenase, aldolase A) within the trained compared with the untrained group (Supplementary Table 6). Thus, we confirm the strong association between inflammation and the development of diabetes, whereas we could not confirm a differential expression of specific genes involved in fatty acid metabolism in diabetic and prediabetic animals in visceral adipose tissue [30]. In the present study, because a whole fat pad was used to isolate RNA, it is not clear whether the elevated expression of inflammatory genes in control rats is caused by an enrichment of macrophages and other proinflammatory cells in the adipose tissue or is a result of elevated expression in adipocytes.

5. Conclusion

In summary, the present study has demonstrated that ET prevents the development of hyperglycemia in ZDF rats. The prevention of type 2 diabetes after ET is associated with changes in muscle in expression of genes involved in

Fig. 3. The metabolic pathways in the soleus muscle (A) and liver (B) illustrating the changes in gene expression after ET in ZDF rats. In skeletal muscle, the gene changes support previous findings where glycogen and possibly triacylglycerol are stored after ET. Uncoupling protein 3 was also down-regulated in parallel with a decrease of plasma free fatty acid level. This is in accordance with the hypothesis that UCP-3 could play a role in the regulation of fatty acid ion (FA^-) in the mitochondrion. → indicates stimulation; –·–, possible pathway; ⊣, inhibition; ↑, genes up-regulated; ↓, genes down-regulated; UCP-3, uncoupling protein 3, GS, glycogen synthase; PHS2, glycogen phosphorylase; HK, hesokinase; G6PhI, glucose 6-phosphate isomerase; PK, pyruvate kinase; PDH, pyruvate dehydrogenase; LPL, lipoprotein lipase; CPT1, carnitine palmiotyl transferase. GenMapp (www.genmapp.org) was used to construct the figure. Other abbreviations are listed in Tables 2 and 3.

skeletal muscle plasticity (differentiation and growth), Ca^{2+} signaling, and in other signaling pathways (eg, IGF1) as well as of gene with unknown function. Alterations involved in energy metabolism were present especially in some of the genes encoding kinases that indirectly regulate the rate-limiting steps in glycolysis. In liver, genes involved in fatty acid synthesis were increased, confirming the hypothesis that decreased lipogenesis in the liver is associated with a diabetic phenotype. In islets, training did not markedly influence the gene expression. However, the major finding in islets was that HMG-CoA synthase was down-regulated in trained animals. Exercise training also modified gene expressions in visceral fat tissue, however, without an equivocal pattern.

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Appendix A. Supplementary materials

Supplementary data associated with this article can be found, in the online version, at http://www/mdl.dk/publications_sup.htm.

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