Article

Fine-Tuning of Adipogenesis is a Potential Mechanism for Improved Insulin Sensitivity in Human Adipose Tissue with Rosiglitazone Therapy

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Abstract

Back ground: Adipose tissue plays a crucial role in regulation of lipid and carbohydrate metabolism and many of these actions are regulated by insulin. **Objective:** The present study aims to detect potential novel protein targets for insulin sensitization in human adipose tissue by using an unbiased proteomic approaches. **Method:** Ten moderately obese, but otherwise healthy, subjects were treated with rosiglitazone 4 mg BD for 14 days. Protein profiles were obtained by two-dimensional gel (2-DG) electrophoresis and protein spots were identified using tandem mass spectrometry. Data were analyzed statistically by using student's paired t test. **Results:** Proteomic analysis revealed distinct up or down regulation ($\geq$ 2-fold) in 122 protein spots on the 2-D gel images between day 0 and day 14 adipose tissue samples. Treatment with rosiglitazone increased the expression of extracellular matrix proteins including collagens, galectin-1, nidogen-1 and laminin. Analysis revealed differential expression of several other proteins involved in redox regulation (ferritin light chain, heat-shock protein β-5, heat-shock protein β-1), endoplasmic reticulum (ER) stress (endoplasmin, 78 kDa glucose-regulated protein precursor), cytoskeletal reorganization (lamin A/C, vimentin, tropomyosin-3, tropomyosin-4, gelsolin, actin tubulin β-1 chain), signaling (calmodulin), and others (glyceraldehyde-3-phosphate dehydrogenase, α enolase). **Conclusion:** Such orchestrated changes in expression of multiple proteins provide insights into the mechanism undarlying the increased efficiency in adipocyte remodeling and differentiation and hence improvement of insulin sensitivity in response to rosiglitazone treatment.

Key words: Rosiglitazone, adipose tissue, proteomics, collagen, adipogenesis, insulin, electrophoresis, spectrometry.

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Introduction

One of the prime targets for the treatment of diabetes is to enhance the insulin sensitivity so that the tissues can precisely utilize glucose and keep its plasma level within physiological limit. Rosiglitazone, a member of the thiazolidinedione (TZD) class of antidiabetic agents, improves insulin sensitivity both in liver and peripheral tissues[1]. TZDs bind to and activate the peroxisome proliferator activated receptor (PPARã), a nuclear receptor and regulate the co ordinated expression of multiple genes that integrates the control of energy, glucose and lipid homeostasis, therefore contribute to increased insulin sensitivity[1-3]. PPARã is predominantly expressed in adipose tissue in both rodents and humans and a substantial number of studies have described changes in gene expression in adipose tissue in response to rosiglitazone treatment[2]. Stimulation of PPARã by fatty acids or TZD results in expression of markers associated with preadipocyte differentiation[4-6]. It has also been suggested that adipose tissue dysfunction and impaired adipogenesis is causally related to systemic insulin resistance[7,8]. In obesity and in non-obese insulin-resistant subjects,

adipogenesis is impaired which leads to an increased energy partitioning to existing adipocytes and consequently the fat cells are enlarged[8,9]. However, it is largely unclear how the functional consequences of PPARg activation lead to processes that can help to explain the insulin sensitization, especially *in vivo* in humans. In this context, the successful application of proteomic approaches is likely to identify some of the mechanisms behind the failure of insulin to act on adipocytes and development of type 2 diabetes. Using combined proteomic approaches we have previously shown that improved insulin sensitivity is coupled to upregulation of glucose uptake in human adipose tissue[10].

In the present comparative proteomic study global protein profiles of the human adipose tissue were obtained by 2D gel electrophoresis and differentially expressed proteins in response to rosiglitazone were identified by tandem mass spectrometry. We, for the first time, provide evidence that improvement of insulin sensitivity in response to rosiglitazone treatment is associated with simultaneous changes in expression of multiple proteins involved in differentiation of adipocytes, cytoskeletal rearrangement, calcium signaling, ER stress, lipid droplet stabilization and fatty acid metabolism in adipose tissue. The orchestrated changes in protein expression pattern in human adipose tissue primarily promote adipogenesis and thus improve insulin sensitivity.

Methods

Subjects: Ten healthy, in the range of being overweight or obese, male subjects aged 20 60 years participated in this study. Entry criteria included fasting plasma glucose of < 7.0 mmol/l and a BMI ranging between 25 45 kg/m^2. Patients with type 2 diabetes, heart failure and any with chronic disease that might interfere with outcome oral hypoglycemic agents were excluded, as were those taking any medication known to affect glucose metabolism. The study was approved by the Oxford Research Ethics Committee and all subjects gave their written informed consent.

Study design: Blood samples (antecubital venous, 20 ml blood per visit), blood pressure and anthropometric measurements were made first while the participants attended the Clinical Research Unit, OCDEM in the morning, after a 12 hour fast. Abdominal subcutaneous adipose tissue biopsies were taken with a 12 gauge needle under local anesthesia (1% lidocaine) in the fasted state at day 0 (baseline, without medication) and thereafter the participants were treated with rosiglitazone (Glaxo SmithKline) 4 mg twice daily. The volunteer returned for blood samples, anthropometric measurements and biopsies on day 14 after start of the medication. Fasting plasma triglyceride, NEFA, glucose and insulin concentrations were measured as described previously[11].

Protein sample preparation and two dimensional gel electrophoresis: Adipose tissue protein was precipitated and isolated from the phenol ethanol supernatant obtained during RNA isolation according to the protocol (Molecular Research Center, Inc). The protein pellets were further treated with the Plus One 2 D Clean Up Kit (Amersham Biosciences) and later solubilized in buffer containing 7M urea, 2M thiourea, 0.5% Triton X 100, 4% CHAPS, 0.5% pharmalyte (pH 3 10), 0.1% NP 7, 5% glycerol, 10% isopropanol, protease inhibitor cocktail and 60 mM DTT. Protein concentration was determined using the 2 D Quant Kit (Amersham Biosciences) and 250 µg protein samples were loaded onto the immobiline dry strips, pH 3 11 NL (Amersham Biosciences). The rehydrated strips were focused on the Protean IEF Cell (Bio Rad) for about 12 kVh at a maximum of 6,000 V in rapid voltage ramping mode with a maximum current per strip of 30 µA.

Equilibration of the IPG strips was performed in two steps: reduction followed by alkylation[12,13]. The second dimension was run on 8 16% precast polyacrylamide SDS gels (8.7 × 13.3 × 0.1 cm; BioRad Dodeca cell) at room temperature with a constant voltage of 120 V for 10 min, followed by 200 V until the dye has migrated off the lower end of the gel.

Protein visualization and image analysis: The 2 D analytical gels (n = 12) were stained with fluorescent dye SYPRO Ruby (BioRad) according to the manufacturer. The 2 D preparative gels (n = 2) were visualized with Bio Safe Coomassie

stain (Bio Rad). The stained analytical gels were imaged using Molecular Imager FX Pro Fluorescent Imager (Bio Rad). Raw scans were processed by the 2 D gel analysis software, PDQuest version 8.0.1 (Bio Rad) as previously described[13]. For reliable matching 21 different landmark proteins were manually added in each gel. Significant changes between spots were determined using Student's *t* test for paired observations. Changes with a *p* value of < 0.05 were considered as being statistically significant.

Protein identification by tandem mass spectrometry: Protein spots with consistent presence in 2D analytical gels of replicate groups (day 0 and day 14) were selected and excised manually from preparative gels (n=2), and transferred to 1.5 mL Eppendorf tubes. Individual gel spots were subjected to in-gel trypsin digestion. Protein digests were analyzed by electrospray ionization liquid chromatography tandem mass spectrometry (ESI LC MS/MS) using a high capacity iontrap (HCTplus™, Bruker Daltonics, Bremen, Germany) tandem mass spectrometer[14]. Liquid chromatography was performed using an Ultimate™ (LC Packings, Dionex, Netherlands) system equipped with a Famos™ auto sampler. Samples were concentrated on a trapping column (300 µm i.d., 1 cm, LC Packings) at a flow rate of 25 µL/min. For the separation with a C18 PepMap column (75 µm i.d., 10 cm, LC Packings) a flow rate of 200 nL/min was used. For electrospray ionization, we used coated silica Picotips™ (New Objective, MA, USA). Instruments were controlled using HyStar™ 3.0 and EsquireControl™ 5.2 (Bruker Daltonics) software. The sample injection volume used in all experiments was 5 µL and tuning parameters of the mass spectrometer were kept as constant as possible.

Individual MS/MS spectra were searched against Swissprot/NCBInr using Mascot™ software (Matrixscience, London, UK). The interpretation and presentation of MS/MS data were performed according to published guidelines[15]. The peptide and fragment mass tolerances were 2.5 and 0.8 Da, respectively. Identification was based on the presence of at least two peptides and on Mascot scores of higher than 50.

Western blot analysis: Equal amounts of protein (10 µg) were separated by SDS PAGE (12.5%), transferred to a polyvinylidene difluoride membrane, and blotted with primary antibody containing 1% milk and 0.05% Tween 20. Primary antibodies used in this study were lamin A/C (1:1000; Santa Cruz Biotechnology, Santa Cruz, California), and protein disulfide isomerase (PDI; 1:1000; Santa Cruz Biotechnology). The membranes were incubated for 60 minutes with horseradish peroxidase–conjugated antimouse and antirabbit IgG (1:20,000). Immunodetection was performed using enhanced chemifluorescence detection according to the manufacturer's protocol (Amersham Biosciences). Briefly, chemifluorescence detection reagents were applied for 1 minute and chemifluorescence was detected using the AutoChemi System (UVP BioImaging, CA USA). Band intensities were analyzed using Quantity One, version 4.6.3 (Bio Rad).

Results

Rosiglitazone improves insulin sensitivity

Short term treatment with rosiglitazone 4 mg BD improved insulin sensitivity in the absence of any changes in body weight (Table I).

Table I: Effects of rosiglitazone on fasting plasma glucose, insulin, NEFA and triglyceride (TG) (n = 10).

Parameter	Day 0	Day 14	*p* value
Glucose (mM)	4.9 ± 0.1	4.7 ± 0.1	0.04
Insulin (mU/l)	12.1 ± 2.2	9.7 ± 1.5	0.04
NEFA (mM)	567 ± 42	379 ± 51	0.01
TG (mM)	1.2 ± 0.1	1.5 ± 0.1	0.04
Weight (kg)	94.8 ± 4.4	95.0 ± 4.3	ns

Rosiglitazone treatment produced significant ($p < 0.05$) decreases in fasting plasma glucose (FPG) and fasting insulin concentrations compared with baseline. Non esterified fatty acids showed significant reduction after rosiglitazone ($p = 0.01$); conversely significant elevation in triglyceride levels were observed in subjects taking rosiglitazone for 14 days. However, there were no significant changes in the BMI after rosiglitazone treatment.

Rosiglitazone induced changes in human adipose tissue proteome

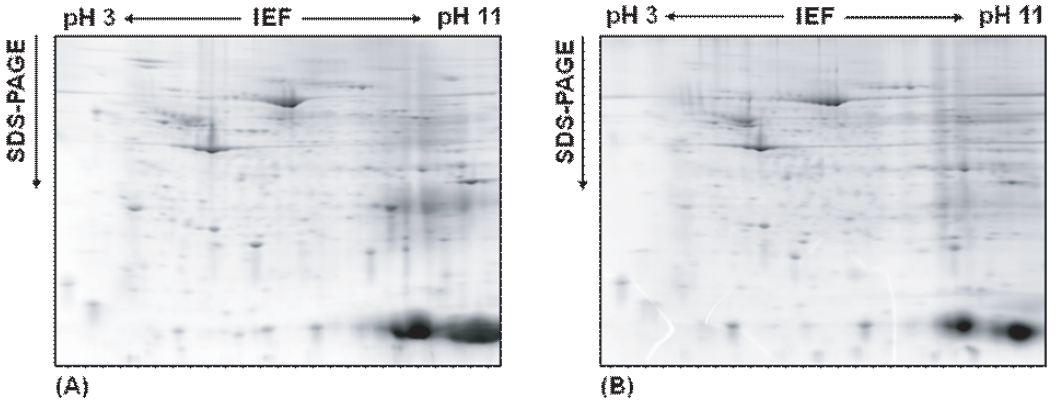


Figure 1: Representative 2-D gels of six separate experiments of control (A) and rosiglitazone treated (B) human adipose tissue. Approximately 250 µg of adipose tissue proteins were separated on 11 cm IPG strip (pH 3-11 NL), followed by 8-16% SDS-PAGE. Proteins were detected by SYPRO ruby staining and image analysis of scanned gels was carried out using the PDQuest software.

Treatment with rosiglitazone for 14 days modulated expression of multiple proteins spots present on analytical 2 D gel images. Figure-1 represents 2 D protein patterns of the control and rosiglitazone treated human adipose tissue samples.

When gel replicates of the control or treated adipose tissue were matched by PD Quest, a correlation coefficient of 0.75 or more was obtained. Typical of 2 DE images, several proteins appeared at multiple positions on the gels reflecting post translational modifications. When comparing the protein spots on the gels from control samples with that of 14 days treated samples, about 122 spots showed differential expression. Further qualitative analysis of the 122 spots revealed that 5 of these spots were barely visible in the control gels but had at least 10 fold greater densities than the minimum detectable spots in gels of the 14 days treated adipose tissue samples.

Identification of differentially expressed proteins

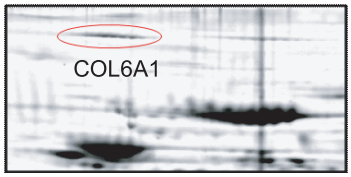
In total 71 unknown spots were selected and excised manually from gels of both control and treated samples for identification by mass spectrometry. Sixty six spots, which corresponded to 52 distinct proteins, have been characterized by LC ESI IT MS tandem MS/MS. In the remaining 5 spots no significant identification was obtained. The excised spots were selected among the differentially expressed proteins with consistent changes but also among other proteins. Our gel spot identification yield was 92% with several proteins appearing at multiple positions on the gels.

Characterization of adipogenic proteins correlated with improved insulin sensitivity

Rosiglitazone induced changes in expression levels of multiple proteins and their fold changes were calculated by dividing the average spot quantities recorded in adipose tissue exposed to rosiglitazone for 14 days with quantities recorded in control samples. Among the proteins which showed significant (p<0.05) higher expression level, when rosiglitazone treated adipose tissue samples were compared to untreated control adipose tissue, collagen a-1(VI) chain precursor (COL6A1), nidogen-1 precursor (entactin),

laminin gamma-1 chain precursor (laminin B2 chain) and collagen a-2(VI) chain precursor (COL6A2) showed >4 fold changes. Figure-2 shows differential expression of COL6A1 in human adipose tissue in response to rosiglitazone.

CONTROL



ROSI TREATED

Figure 2: Selected regions of the 2-D gels showing significant ($p < 0.05$) changes in expression of collagen a-1 (VI) chain (COL6A1) in human adipose tissue in response to rosiglitazone (ROSI) (n = 6).

Other adipogenic proteins showing significant changes in expression in response to rosiglitazone includes cytoskeletal proteins (lamin A/C, vimentin, tropomyosin 3, tropomyosin 4, gelsolin, actin, tubulin â-1 chain), extracellular matrix proteins (galectin-1, integrin a-IIb precursor, integrin â-3 precursor), signaling proteins (calmodulin), antioxidants (ferritin light chain, heat-shock protein â-5, heat-shock protein â-1), ER stress proteins (endoplasmin, 78 kDa glucose-regulated protein precursor) and others (glyceraldehyde-3-phosphate dehydrogenase, a enolase). The increased expression of lamin A/C and protein disulfide-isomerase (PDI) was further verified by Western blotting (Figure-3).

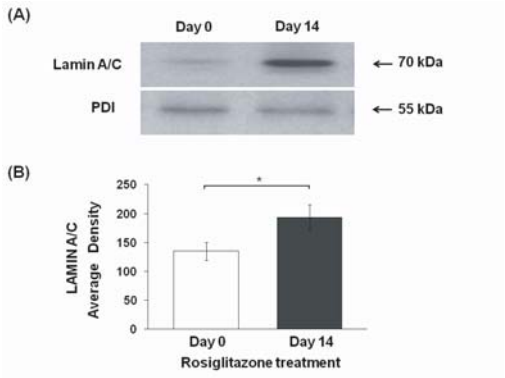


Figure 3: Rosiglitazone up-regulates expression of lamin A/C in human adipose tissue. **(A)** Representative Western blots of lamin A/C (n=6), performed on human adipose tissue before (day 0) and after 14 days of treatment with rosiglitazone. As a loading control and for normalization, the blot was immunostained with antibody against protein disulfide-isomerase (PDI). **(B)** Bars show the changes in average density of the proteins in response to rosiglitazone treatment. Values are expressed as means ± SEM. *$p < 0.05$.

Discussion

The identification of novel biomarkers associated with insulin resistance is an essential research goal in diabetes research. In the present study, we have taken advantage of the 2 D gel based proteomics approaches to identify such targets using rosiglitazone as a tool for insulin sensitizer. We have identified clusters of proteins exhibiting altered expression during the process of amelioration of insulin resistance driven by the potent TZD, rosiglitazone. Our report provides a molecular snapshot of the mechanism of action of the TZD class of insulin sensitizers regulating adipogenesis in human adipose tissue.

In humans, the onset of the antidiabetic action of rosiglitazone is slow and it is commonly believed that a treatment period of 3 months is needed. However, insulin sensitization has been

demonstrated using pioglitazone within three weeks as well[16,17]. We observed a modest but significant decrease in plasma glucose and insulin after 14 days of treatment with rosiglitazone. Plasma NEFA was significantly reduced after rosiglitazone treatment, likely to represent acute reduction in the release of NEFAs from adipose tissue depots, with only minor changes in plasma TG concentrations[16]. Correlating to these biochemical findings we have identified multiple adipogenic proteins showing differential expression in response to rosiglitazone treatment.

PPARã is the master regulator of adipogenesis and a direct role of extracellular matrix (ECM) in regulating gene expression and promoting adipocyte differentiation has been proposed[6,18]. The ECM of adipose tissue interconnects adipocytes and gives rise to fat cell clusters *in vitro* and to fat lobules of adipose tissue *in vivo*. During adipocyte differentiation drastic changes occur in cell morphology, cytoskeletal components, and the level and type of secreted ECM components. An early ultrastructural change seen in *in vivo* adipocyte differentiation is the deposition of collagen at the cell-ECM border and extracellular basement membrane biogenesis[6,19]. It has been reported that inhibition of collagen synthesis prevented the preadipocytes from differentiation[20]. All types of collagen were secreted from adipocytes at any time point during differentiation but with a different profile[21]. During the differentiation of adipocytes, the expressions of type I and type III collagens decreased, while the synthesis and secretion of type IV collagen increased[19,22-24]. The a2 chain of type VI collagen increased at confluence and post confluence and then gradually decreased at a time when mRNAs encoding lipogenic enzymes increased dramatically[9,25], while extracellular nidogen 1 and laminin (LN) increased[22,26]. Laminin expression is tightly coupled with lipid accumulation in adipocytes and unilocular rounded or three dimensional morphology of adipocytes[23,27] and our study shows a >3 fold changes in expression of laminin B2 (LAMB2) chain in response to rosigltazone. Another extracellular protein, galectin 1 is an adipocyte specific secreted protein with pleotropic functions and showed unambiguous up regulation during 3T3 L1 adipocyte differentiation[21]. Adipocyte differentiation is also regulated by intermediate filaments, such as lamin A/Cs, which form a meshwork of filaments on the inside of the nuclear membrane and are encoded by the *LMNA* gene. Recent studies have reported association between variation at the *LMNA* gene and type 2 diabetes in ethnically diverse populations[28,29]. In human subcutaneous adipose tissue, expression of lamin A/C protein increases during preadipocyte differentiation[30]. Over expression of the earliest marker of adipocyte differentiation, a2(VI) collagen along with a1(VI) collagen, galectin 1, nidogen 1, lamin A/C and LAMB2 suggests that one of the mechanisms behind insulin sensitization of rosiglitazone may involve differentiation and remodeling of human adipocytes with concurrent increase in lipid storage. Remodeling and expansion of adipose tissue by the TZDs, which offers 'storage space' for lipids, is believed to offset ectopic lipid accumulation in muscle and liver, thereby contributing to insulin sensitivity[31,32]. The increase in fat mass in response to TZD occurs due to increased number of small adipocytes in fat depots, enhanced lipogenic activity in white adipose tissue and depot-specific redistribution resulting in a remodeling of the adipose depots, especially the visceral adipose depots. The higher lipid storage capacity of the new, small, insulin-sensitive adipocytes likely serves to lower circulating plasma FFA levels and visceral triglyceride levels, which possibly accounts for the decrease in insulin resistance[33,34].

In addition to the ECM proteins, our proteomic data confirm rosiglitazone-induced differential expression of several other proteins that regulate adipogenesis. Recent evidences suggest that

increased oxidative stress in accumulated fat is an early indicator of metabolic syndrome and the redox state in adipose tissue is a potentially useful therapeutic target for obesity-associated metabolic syndrome[35,36]. In the present study, ferritin light chain appeared as new spot after treatment with rosiglitazone for 14 days but was barely visible in control samples. It has been previously shown that over expression of ferritin exert an antioxidant function and limit iron toxicity during adipocyte differentiation[37]. Other upregulated chaperones with antioxidative function include a crystallin B chain and HSP27. The induction of these proteins has been reported during adipogenesis of human adipose derived stem cells[38]. In addition, aB crystallin expression has been found to decrease in adipose tissue of diabetic rat[39]. An intriguing function of HSP27 and a crystallin B chain is the ability to increase the resistance of cells to oxidative injuries. The expression of these chaperones correlates with decreased levels of reactive oxygen species (ROS) and nitric oxide[40]. In adipocytes from obese mice ROS can induce enoplasmic reticulum (ER) stress coupled with PKRlike eukaryotic initiation factor 2 (eIF2a) kinase (PERK) activation, eIF2 phosphorylation, and GRP78 induction[41,42]. Recently it has been shown that ER stress, evidenced by induction of GRP78, suppresses adipocyte differentiation[43]. ER stress also induces expression of endoplasmin (GRP94) in adipocytes[44]. Considering the fact that ER stress inhibits adipogenesis and reduces insulin sensitivity, our findings of decreased expression of GRP78 and endoplasmin in response to rosiglitazone treatment suggests reduction of ER stress and consequently improved adipogenesis and enhanced insulin sensitivity in human adipose tissue.

In our 2 D gel system the spots corresponding to tropomyosin 3 and tropomyosin 4 showed decreased expression in response to rosiglitazone. Other proteomic studies showed that the expression of tropomyosin changes during adipocyte differentiation suggesting an important role for tropomyosin in maintaining cell shape[45,46]. Tropomyosins increase filament stiffness, stabilize actin filaments by protecting them against the severing action of gelsolin and cofilin and more importantly, influence myosin mechanochemistry[47,48]. Gelsolin, an actin binding protein, respond to Ca^{2+} and phosphatidylinositol 4,5 bisphosphate (PIP_2) and control actin organization by severing filaments, capping filament ends and nucleating actin assembly[49-51]. During adipogenesis expression of actin and tubulin decreases while levels of vimentin increase[52,53]. It has also been shown that during adipose conversion, the extended wavy, fibrillar organization of vimentin in undifferentiated cells is altered[54,55].

Calmodulin showed a > 2 fold increase in expression level in response to rosiglitazone. In addition to the fundamental role of Ca^{2+}/calmodulin in GLUT4 vesicle docking and fusion in 3T3 L1 cells[56], calmodulin exert a positive regulatory role in adipogenesis through phosphorylation of calmodulin-dependent protein kinase II[57,58]. With respect to glycolytic enzymes, the increased expression of GAPDH and enolase is in agreement with enhanced glycolytic activity and also fatty acid synthesis during adipocyte differentiation[59]. Alpha enolase is a multifunctional enzyme[60] and has been reported as secreted protein from 3T3 L1 adipocytes with a single transmembrane helix[21]. The cell surface protein nature of the a enolase may render it to serve as a receptor for plasminogen and there is evidence that the plasminogen cascade is required for adipocyte differentiation[60,61].

Conclusion

In conclusion, the present study for the first time shows that improved insulin sensitivity in human individuals induced by short term treatment with rosiglitazone corresponds to differential expression of multiple adipogenic proteins between control and treated adipose tissue

samples. Treatment of obese subjects with rosiglitazone for 14 days results in a marked reduction of plasma glucose, insulin and NEFA without body weight gain. Regulation of extracellular matrix proteins, redox proteins and chaperones, and proteins involved in lipid, carbohydrate and iron metabolism might result in the improvement of adipogenesis and thus insulin sensitivity. Furthermore, modulation of proteins involved in maintaining cellular and cytoskeleton structure of the adipocyte and signaling pathways can be associated with the remodeling of the adipose depots.

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References

1. Rosen ED, Spiegelman BM. PPARg: a nuclear regulator of metabolism, differentiation, and cell growth. *J Biol Chem* 2001 Oct 12; 276(41): 37731-4.
2. Berger J, Moller DE. The mechanisms of action of PPARs. *Annu Rev Med* 2002;53:409-35.
3. Lehrke M, Lazar MA. The many faces of PPARg. *Cell* 2005 Dec 16;123(6):993-9.
4. Tontonoz P, Hu E, Graves RA, Budavari AI, Spiegelman BM. mPPARg2: tissue-specific regulator of an adipocyte enhancer. *Genes Dev* 1994 May 15;8(10):1224-34.
5. Schoonjans K, Staels B, Auwerx J. Role of the peroxisome proliferator-activated receptor (PPAR) in mediating the effects of fibrates and fatty acids on gene expression. *J Lipid Res* 1996 May;37(5): 907-25.
6. Gregoire FM, Smas CM, Sul HS. Understanding adipocyte differentiation. *Physiol Rev* 1998 Jul;78(3):783-809.
7. Smith U. Impaired ('diabetic') insulin signaling and action occur in fat cells long before glucose intolerance—is insulin resistance initiated in the adipose tissue? *Int J Obes Relat Metab Disord* 2002 Jul;26(7):897-904.
8. Yang X, Jansson PA, Nagaev I, Jack MM, Carvalho E, Sunnerhagen KS, et al. Evidence of impaired adipogenesis in insulin resistance. *Biochem Biophys Res Commun* 2004 May 14;317(4):1045-51.
9. Avram MM, Avram AS, James WD. Subcutaneous fat in normal and diseased states 3. Adipogenesis: from stem cell to fat cell. *J Am Acad Dermatol* 2007 Mar;56(3):472-92.
10. Ahmed M, Neville MJ, Edelmann MJ, Kessler BM, Karpe F. Proteomic Analysis of Human Adipose Tissue After Rosiglitazone Treatment Shows Coordinated Changes to Promote Glucose Uptake. *Obesity (Silver Spring)* 2009 Jun 25.
11. Evans K, Burdge GC, Wootton SA, Clark ML, Frayn KN. Regulation of dietary fatty acid entrapment in subcutaneous adipose tissue and skeletal muscle. *Diabetes* 2002 Sep;51(9):2684-90.
12. Ahmed M, Forsberg J, Bergsten P. Protein profiling of human pancreatic islets by two-dimensional gel electrophoresis and mass spectrometry. *J Proteome Res* 2005 May-Jun;4(3):931-40.
13. Ahmed M, Bergsten P. Glucose-induced changes of multiple mouse islet proteins analysed by two-dimensional gel electrophoresis and mass spectrometry. *Diabetologia* 2005 Mar;48(3):477-85.
14. Batycka M, Inglis NF, Cook K, Adam A, Fraser-Pitt D, Smith DG, et al. Ultra-fast tandem mass spectrometry scanning combined with monolithic column liquid chromatography increases throughput in proteomic analysis. *Rapid Commun Mass Spectrom* 2006;20(14):2074-80.
15. Taylor GK, Goodlett DR. Rules governing protein identification by mass spectrometry. *Rapid Commun Mass Spectrom* 2005;19(23):3420.

16. Tan GD, Fielding BA, Currie JM, Humphreys SM, Desage M, Frayn KN, et al. The effects of rosiglitazone on fatty acid and triglyceride metabolism in type 2 diabetes. *Diabetologia* 2005 Jan;48(1):83-95.

17. Hammarstedt A, Sopasakis VR, Gogg S, Jansson PA, Smith U. Improved insulin sensitivity and adipose tissue dysregulation after short-term treatment with pioglitazone in non-diabetic, insulin-resistant subjects. *Diabetologia* 2005 Jan;48(1):96-104.

18. Otto TC, Lane MD. Adipose development: from stem cell to adipocyte. *Crit Rev Biochem Mol Biol* 2005 Jul-Aug;40(4):229-42.

19. Nakajima I, Muroya S, Tanabe R, Chikuni K. Extracellular matrix development during differentiation into adipocytes with a unique increase in type V and VI collagen. *Biol Cell* 2002 Jun;94(3):197-203.

20. Ibrahimi A, Bonino F, Bardon S, Ailhaud G, Dani C. Essential role of collagens for terminal differentiation of preadipocytes. *Biochem Biophys Res Commun* 1992 Sep 30;187(3):1314-22.

21. Wang P, Mariman E, Keijer J, Bouwman F, Noben JP, Robben J, et al. Profiling of the secreted proteins during 3T3-L1 adipocyte differentiation leads to the identification of novel adipokines. *Cell Mol Life Sci* 2004 Sep;61(18):2405-17.

22. Aratani Y, Kitagawa Y. Enhanced synthesis and secretion of type IV collagen and entactin during adipose conversion of 3T3-L1 cells and production of unorthodox laminin complex. *J Biol Chem* 1988 Nov 5;263(31):16163-9.

23. Kubo Y, Kaidzu S, Nakajima I, Takenouchi K, Nakamura F. Organization of extracellular matrix components during differentiation of adipocytes in long-term culture. *In Vitro Cell Dev Biol Anim* 2000 Jan;36(1):38-44.

24. Nakajima I, Muroya S, Tanabe R, Chikuni K. Positive effect of collagen V and VI on triglyceride accumulation during differentiation in cultures of bovine intramuscular adipocytes. *Differentiation* 2002 May;70(2-3):84-91.

25. Nakajima I, Yamaguchi T, Ozutsumi K, Aso H. Adipose tissue extracellular matrix: newly organized by adipocytes during differentiation. *Differentiation* 1998 Aug;63(4):193-200.

26. Patrick CW, Jr., Wu X. Integrin-mediated preadipocyte adhesion and migration on laminin-1. *Ann Biomed Eng* 2003 May;31(5):505-14.

27. Hausman GJ, Richardson RL. Newly recruited and pre-existing preadipocytes in cultures of porcine stromal-vascular cells: morphology, expression of extracellular matrix components, and lipid accretion. *J Anim Sci* 1998 Jan;76(1):48-60.

28. Owen KR, Groves CJ, Hanson RL, Knowler WC, Shuldiner AR, Elbein SC, et al. Common variation in the LMNA gene (encoding lamin A/C) and type 2 diabetes: association analyses in 9,518 subjects. *Diabetes* 2007 Mar;56(3):879-83.

29. Mesa JL, Loos RJ, Franks PW, Ong KK, Luan J, O'Rahilly S, et al. Lamin A/C polymorphisms, type 2 diabetes, and the metabolic syndrome: case-control and quantitative trait studies. *Diabetes* 2007 Mar;56(3):884-9.

30. Lelliott CJ, Logie L, Sewter CP, Berger D, Jani P, Blows F, et al. Lamin expression in human adipose cells in relation to anatomical site and differentiation state. *J Clin Endocrinol Metab* 2002 Feb;87(2):728-34.

31. de Souza CJ, Eckhardt M, Gagen K, Dong M, Chen W, Laurent D, et al. Effects of pioglitazone on adipose tissue remodeling within the setting of obesity and insulin resistance. *Diabetes* 2001 Aug;50(8):1863-71.

32. Rogers PM, Mashtalir N, Rathod MA, Dubuisson O, Wang Z, Dasuri K, et al. Metabolically favorable remodeling of human adipose tissue by human adenovirus type 36. *Diabetes* 2008 Sep;57(9):2321-31.

33. Sharma AM, Staels B. Peroxisome proliferator-activated receptor g and adipose tissue - understanding obesity-related changes in regulation of lipid and glucose metabolism. *J Clin Endocrinol Metab* 2007 Feb;92(2):386-95.

34. Kintscher U, Law RE. PPARg-mediated insulin sensitization: the importance of fat versus muscle. *Am J Physiol Endocrinol Metab* 2005 Feb;288(2):E287-91.

35. Evans JL, Goldfine ID, Maddux BA, Grodsky GM. Oxidative stress and stress-activated signaling pathways: a unifying hypothesis of type 2 diabetes. *Endocr Rev* 2002 Oct;23(5):599-622.

36. Furukawa S, Fujita T, Shimabukuro M, Iwaki M, Yamada Y, Nakajima Y, et al. Increased oxidative stress in obesity and its impact on metabolic syndrome. *J Clin Invest* 2004 Dec;114(12):1752-61.

37. Festa M, Ricciardelli G, Mele G, Pietropaolo C, Ruffo A, Colonna A. Overexpression of H ferritin and up-regulation of iron regulatory protein genes during differentiation of 3T3-L1 pre-adipocytes. *J Biol Chem* 2000 Nov 24;275(47):36708-12.

38. DeLany JP, Floyd ZE, Zvonic S, Smith A, Gravois A, Reiners E, et al. Proteomic analysis of primary cultures of human adipose-derived stem cells: modulation by Adipogenesis. *Mol Cell Proteomics* 2005 Jun;4(6):731-40.

39. Kumar PA, Haseeb A, Suryanarayana P, Ehtesham NZ, Reddy GB. Elevated expression of aA- and aB-crystallins in streptozotocin-induced diabetic rat. *Arch Biochem Biophys* 2005 Dec 15;444(2): 77-83.

40. Arrigo AP, Simon S, Gibert B, Kretz-Remy C, Nivon M, Czekalla A, et al. Hsp27 (HspB1) and alphaB-crystallin (HspB5) as therapeutic targets. *FEBS Lett* 2007 Jul 31;581(19):3665-74.

41. Marciniak SJ, Ron D. Endoplasmic reticulum stress signaling in disease. *Physiol Rev* 2006 Oct;86(4):1133-49.

42. Hotamisligil GS. Role of endoplasmic reticulum stress and c-Jun NH_2-terminal kinase pathways in inflammation and origin of obesity and diabetes. *Diabetes* 2005 Dec;54 Suppl 2:S73-8.

43. Shimada T, Hiramatsu N, Okamura M, Hayakawa K, Kasai A, Yao J, et al. Unexpected blockade of adipocyte differentiation by K-7174: implication for endoplasmic reticulum stress. *Biochem Biophys Res Commun* 2007 Nov 16;363(2):355-60.

44. Song H, Kim H, Park T, Lee DH. Characterization of myogenic differentiation under endoplasmic reticulum stress and taurine treatment. *Adv Exp Med Biol* 2009;643:253-61.

45. Rodriguez Fernandez JL, Ben-Ze'ev A. Regulation of fibronectin, integrin and cytoskeleton expression in differentiating adipocytes: inhibition by extracellular matrix and polylysine. *Differentiation* 1989 Dec;42(2):65-74.

46. Ben-Ze'ev A. Application of two-dimensional gel electrophoresis in the study of cytoskeletal protein regulation during growth activation and differentiation. *Electrophoresis* 1990 Mar;11(3):191-200.

47. Gunning PW, Schevzov G, Kee AJ, Hardeman EC. Tropomyosin isoforms: divining rods for actin cytoskeleton function. *Trends Cell Biol* 2005 Jun;15(6):333-41.

48. Pittenger MF, Kazzaz JA, Helfman DM. Functional properties of non-muscle tropomyosin isoforms. *Curr Opin Cell Biol* 1994 Feb;6(1):96-104.

49. Silacci P, Mazzolai L, Gauci C, Stergiopulos N, Yin HL, Hayoz D. Gelsolin superfamily proteins: key regulators of cellular functions. *Cell Mol Life Sci* 2004 Oct;61(19-20):2614-23.

50. Revenu C, Athman R, Robine S, Louvard D. The co-workers of actin filaments: from cell structures to signals. *Nat Rev Mol Cell Biol* 2004 Aug;5(8):635-46.

51. dos Remedios CG, Chhabra D, Kekic M, Dedova IV, Tsubakihara M, Berry DA, et al. Actin binding proteins: regulation of cytoskeletal microfilaments. *Physiol Rev* 2003 Apr;83(2): 433-73.

52. Smas CM, Sul HS. Control of adipocyte differentiation. *Biochem J* 1995 Aug 1;309 (Pt 3):697-710.

53. Teichert-Kuliszewska K, Hamilton BS, Roncari DA, Kirkland JL, Gillon WS, Deitel M, et al. Increasing vimentin expression associated with differentiation of human and rat preadipocytes. *Int J Obes Relat Metab Disord* 1996 Mar;20 Suppl 3:S108-13.

54. Brandes R, Arad R, Gaathon A, Bar-Tana J. Induction of adipose conversion in 3T3-L1 cells is associated with an early phosphorylation of a protein partly homologous with mouse vimentin. *FEBS Lett* 1993 Oct 25;333(1-2):179-82.

55. Franke WW, Hergt M, Grund C. Rearrangement of the vimentin cytoskeleton during adipose conversion: formation of an intermediate filament cage around lipid globules. *Cell* 1987 Apr 10;49(1):131-41.

56. Whitehead JP, Molero JC, Clark S, Martin S, Meneilly G, James DE. The role of Ca^{2+} in insulin-stimulated glucose transport in 3T3-L1 cells. *J Biol Chem* 2001 Jul 27;276(30):27816-24.

57. Wang H, Goligorsky MS, Malbon CC. Temporal activation of Ca^{2+}-calmodulin-sensitive protein kinase type II is obligate for adipogenesis. *J Biol Chem* 1997 Jan 17;272(3):1817-21.

58. Szabo E, Qiu Y, Baksh S, Michalak M, Opas M. Calreticulin inhibits commitment to adipocyte differentiation. *J Cell Biol* 2008 Jul 14;182(1): 103-16.

59. Renes J, Bouwman F, Noben JP, Evelo C, Robben J, Mariman E. Protein profiling of 3T3-L1 adipocyte differentiation and (tumor necrosis factor a-mediated) starvation. *Cell Mol Life Sci* 2005 Feb;62(4):492-503.

60. Pancholi V. Multifunctional a-enolase: its role in diseases. *Cell Mol Life Sci* 2001 Jun;58(7):902-20.

61. Selvarajan S, Lund LR, Takeuchi T, Craik CS, Werb Z. A plasma kallikrein-dependent plasminogen cascade required for adipocyte differentiation. *Nat Cell Biol* 2001 Mar;3(3):267-75.