

In vivo and in vitro validation of GATA-3 suppression for correcting impaired adipogenesis, restoring insulin sensitivity, and lowering risk of T2D

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Abstract

Purpose

Impaired adipogenesis is associated with development of insulin resistance and increased risk of type 2 diabetes (T2D). GATA Binding Protein 3 (GATA3) is implicated in impaired adipogenesis and the onset of insulin resistance. Therefore, we hypothesize that inhibition of GATA3 could promote adipogenesis, restore healthy fat distribution, and enhance insulin signaling.

Methods

BALB/c mice were treated with GATA3 inhibitor (DNAzyme hgd40) over a period of two weeks. Liposomes loaded with DNAzyme, pioglitazone, or vehicle controls were administered subcutaneously every two days, at the right thigh. At the end of the study, adipose tissues were collected and weighed from the site of injection, the opposite side, and the omental tissues. Anti-oxidant enzyme (SOD and catalase) activities were assessed in animals' sera, and gene expression was measured using well-established protocols. Primary human preadipocytes were treated with DNAzyme hgd40. Cell proliferation, adipogenic capacity, gene expression, and insulin signaling were measured following well-established protocols.

Results

In vivo GATA3 inhibition promoted adipogenesis at the site of injection and reduced MCP-1 expression. GATA3 inhibition also reduced omental tissue size and PPAR γ expression. Additionally, *in vitro* GATA3 inhibition induced adipogenesis of primary human preadipocytes, and enhanced insulin signaling through reduced expression of p70S6K.

Conclusions

These findings suggest that modulating GATA3 expression offers a potential therapeutic benefit by correcting impaired adipogenesis, promoting healthy fat distribution, restoring insulin sensitivity and lowering the risk of T2D.

Introduction

Obesity is at epidemic proportions with a steadily increasing prevalence that is expected to reach 300 million patients by 2025 (1). In addition, obesity is a significant risk factor and a prominent contributor in the development of many pathological conditions including cancer, cardiovascular diseases, diabetes (particularly type 2 (T2D)), liver and kidney diseases, and depression (2). Impaired adipogenesis was linked to adipose tissue dysfunction and it underlines the development of insulin resistance (3), and therefore T2D. During this process subcutaneous adipose tissues (SAT) often have

limited expandability, creating inappropriate adipocyte expansion, hypertrophic adipocyte feature, recruitment of inflammatory cells, and insulin-resistant phenotype (4, 5). Numerous factors and pathways, including transcription factors, epigenetic regulators, signaling pathways, and inflammatory pathways as well as others are involved in the processes of preadipocyte commitment and differentiation (6). Hence, abnormalities in these pathways can result in the development of adipocyte dysfunction, insulin resistance, leading to related comorbidities including T2D. Although the association between insulin resistance and obesity is well established (7, 8), the link between impaired adipogenesis and insulin resistance remains unknown in non-obese individuals (9). The dysfunction of SAT that includes adipocyte hypertrophy and impairment of adipogenesis may play a significant role in the development of insulin resistance in non-obese individuals as it leads to fat deposition in the liver, skeletal muscle, and other fat depots (3, 10, 11). Other studies have indicated that fat mass and distribution play a critical role in insulin resistance in non-obese individuals. In these studies, the large abdominal fat surface area was associated with an increased risk of insulin resistance, while the small size adipocytes was linked to the body's insulin sensitivity (12), although the omental fat remains the only depot that correlates significantly with the metabolic syndrome (13). The expression of high levels of cytokines by the hypertrophied adipocytes such as the monocyte chemoattractant protein-1 (MCP-1), IL-6, and IL-8 exacerbates the infiltration of macrophages into the adipose tissue and secretion of IL-1 β and TNF α . This in turn lowers the expression of insulin receptor substrate 1 (IRS-1) and glucose transporter type 4 (GLUT4) and leads to the development of insulin resistance (14, 15). Moreover, PPAR γ and CCAAT/enhancer-binding protein alpha (C/EBP α) represent the most critical players in maintaining adipocyte homeostasis, and their expression was found to be altered when impaired adipogenesis occurs (16). Therefore, identifying molecular mediators of insulin resistance in non-obese individuals may aid in reversing insulin resistance before the onset of T2D. Among the potential molecular targets underlying adipogenesis impairment, is the high expression of the anti-adipogenic transcription factor GATA3 (17). Previous studies showed that GATA3 suppresses the transition from preadipocytes to adipocytes by inhibiting the expression and activity of PPAR γ 2 and C/EBPs (18–23), however, most of these studies focused on the benefit of adipogenesis inhibition in reducing obesity. Other studies have suggested the GATA3's crucial function as a gatekeeper of terminal adipocyte differentiation (24), and that its inhibition may reverse the impaired adipogenesis and linked insulin resistance. Therefore, in a previous study, we investigated the inhibition of GATA3 using a new class of antisense molecules known as DNazymes (25), to rescue adipogenesis and improve insulin signaling. Our results indicated that such inhibition indeed improved adipocytes differentiation, modulated the cytokine profile, and improved insulin sensitivity in insulin resistant cells (26). In this study, we investigated the role of targeting GATA3 expression in fat depots both in vitro and in vivo in correcting the impaired adipogenesis and improving insulin sensitivity in non-obese healthy individuals. This is the first study that intended to utilize GATA3 to prevent and/or treated T2D.

Materials And Methods

In vivo assessment of the GATA3 inhibition:

Liposomes preparation

Liposomes were prepared using the ethanol-based proliposome technology by adapting a previously published protocol (27). Briefly, 50mg of phospholipid was mixed with 100µl of absolute ethanol and dissolved at 70°C. Then 50mg of cholesterol was added to the previous mixture and dissolved at 70°C water bath. One ml of 0.1mg/ml DNAzyme “hgd40” was added to the phospholipid-cholesterol mixture with continuous vigorous mixing for 4 minutes. The resulting blend was left at room temperature for 2 hours followed by sonication for 10 minutes. The liposomes were centrifuged at 12000 rpm for 15 minutes to get rid of titanium particles released by the probe of the sonicator.

Animal care, experimental design and treatment:

Adult male (12-16 weeks old) BALB/c mice were provided by the Laboratory Animal Research Center (LARC) at Qatar University (QU). Animals were housed in individually ventilated cages (IVC) under standard husbandry conditions (room temperature 18-22°C, relative humidity 40-65% and 12/12 hrs light/dark cycle), provided with normal chow diet and drinking water *ad libitum*. All animal procedures were performed according to approved institutional ethical rules and regulations and were approved by Qatar University-Institutional Animal Care and Use Committee (QU-IACUC 024/2020). A total of 28 animals were used in this study and divided into three groups. (A) Vehicle Control Group with 8 animals that were treated with 100µl of DNAzyme-free liposomes, (B) Positive Control Group with 8 animals that were treated with liposome-loaded with 1µM of pioglitazone (40 mg/Kg), and (C) GATA3 inhibitor treated Group with 12 animals treated with liposome-loaded DNAzyme (10; µg/ml,hgd40). Treatments were administered subcutaneously to the right flank region (site of injection), twice a week for two weeks. The mice were housed under standard animal husbandry conditions with 12 hours dark and light cycle, were provided standard rodent chow and water *ad libitum*. Animals were weighed at the beginning and the end of the study. All the animals were euthanized as per AVMA guidelines and the subcutaneous adipose tissues from right flank (site of injection), left flank (opposite site) and omental were collected from scarified mice, weighed, and blood was drawn via a cardiac puncture.

Assessment of oxidative stress:

Oxidative stress was assessed by measuring the activity of anti-oxidant enzymes, including catalase and superoxide dismutase (SOD), in serum samples prepared from collected blood using the Catalase Assay Kit (Merck Millipore) and the SOD kit (Merck Millipore) following the manufacturer’s instructions. Measurements and data analysis was performed using the Cytation 5 Cell Imaging Multi-Mode Reader (BioTek, Germany).

***In vitro* effect of the GATA3 inhibition:**

Recruitment criteria of participants were previously described (17). Approvals of the Institutional Research Board (IRB) committees of Hamad Medical Corporation and Qatar University for the proposed project were sought before onset of research, (MRC-03-21-154, and QU-IRB 1548-EA/21). Five patients

undergoing maxillofacial surgeries were recruited, and information about the donors' gender and BMI were collected. SVFs were isolated from buccal fat pad (BFP) biopsies collected from the recruited subjects as described. Stromal vascular fraction (SVF) was re-suspended in stromal media containing DMEM-F12 with 10% FBS, 1% Antibiotic-Antimycotic solution and 1% L-Glutamine (200 mM) and plated at 4×10^4 /cm². The cells were then maintained in a humidified incubator at 37°C with 5% CO₂. The media was changed every 2–3 days until the cells achieve 80–90% confluence. When confluent, cells were either harvested or induced to differentiate by changing the medium into differentiation medium (DMEM-F12, 3% FBS, 1% Antibiotic-Antimycotic solution, 1% L-Glutamine (200 mM), 1 μM dexamethasone, 0.25 mM IBMX, 0.2 μM Insulin, Biotin (66 μM), Rosiglitazone (PAPARγ agonist) (5 μM)) for 3-7 days, followed by 9-10 days in maintenance medium containing the same components as the differentiation medium except IBMX and rosiglitazone as we described previously (28). To investigate the effect of GATA3 inhibition; cells were grown as above and treated with GATA-3 inhibitor as mentioned previously (29). Briefly, 24 hours after seeding, the cells were transfected with hgd21 (human GATA3 mRNA specific DNase) and Lipofectamine 3000 transfection reagent. The cells were incubated for 6-8 hours, followed by changing media to induce adipogenic differentiation.

Assessment of cell viability and adipogenic capacity

Cells were fixed with 4 % Formaldehyde (Thermo Scientific, 28908) and stained with DAPI (Molecular prods by life technonolies, D1306) and Lipidtox (Invetrogen, H34476) as previously described. Total number of nuclei (DAPI-positive) and differentiated adipocytes (Lipidtox-positive) were automatically scored in twenty five fields/well by Cytation 5 Cell Imaging Multi-Mode Reader (BioTek, Germany). While adipogenic capacity was assessed by calculating the percentage of Lipidtox-positive cells to total number of nuclei.

Assessment of gene expression from both in vitro and in vivo experiments:

For the *in vivo* experiments, stromal Vascular Fractions (SVFs) were isolated from adipose tissue biopsies collected from right/left thighs and omental depots using established protocol (30-32). Briefly, the collected adipose tissue biopsies (0.5g) were homogenized using gentleMACS™ Dissociator (Miltenyi Biotec) then digested using collagenase solution (0.1% collagenase I/1% BSA in PBS) for one hour at 37°C. Samples were then centrifuged at 1500 rpm for 5 minutes to separate SVF. The resulting cell pellet was then washed with 1% BSA, followed by erythrocyte lysis buffer for 10 minutes. TRizol reagent (Invitrogen) was added to the pellet for the RNA extraction using the TRizol method according to manufacturer's instructions. For the *in vitro* experiments, RNA was extracted from preadipocyte cultures prior and post induction of differentiation using TRizol method (Invitrogen) according to manufacturer's instructions. Three μg of the resulting RNA from *in vitro* and *in vivo* experiments was used for First-strand cDNA synthesis using Superscript III first strand synthesis super mix kit (Invitrogen) according to manufacturer's instructions. Real-time PCR was carried out for gene expression analysis using 10 ng of the produced cDNA with the listed primers (Table I) using 7500 Real Time PCR System from Applied Biosystem. The PCR conditions were as follows: 1 cycle of 95 °C for 10 minutes, 45 cycles of 95 °C for 15

seconds, 55 °C for 40 s and 72 °C for 30 cycles and finally 60 °C for 15 seconds. Real-time PCR was carried out in triplicate and the GAPDH was used as a housekeeping gene for normalization of the amplified signals of the target genes. The data analysis was performed using the $\Delta\Delta C_t$ based calculations (33).

Assessment of insulin signaling

Insulin signaling was measured by assessing the phosphorylation levels of IRS-1, GSK3B, IGF1R, Akt, Mtor, p70s6k, IR, PTEN, GSK3, TSC2, and RPS6, using a commercial Bio-Plex Pro™ Cell Signaling Akt Panel (Bio-Rad) using Luminex 200 technology (Thermo Fisher Scientific, USA) following manufacturer's instructions.

Statistical analysis

Comparisons were performed using t-test, One-way ANOVA, Two-way ANOVA or Linear regression models as appropriate using GraphPad prism. Data are presented as mean \pm SEM.

Results

Effect of GATA3 inhibition on total animal and tissue weight

Twenty-eight animals were divided among three groups; Vehicle Control (N=8), Positive Control (N=8), and GATA3 inhibitor Treated (N=12) Group. Repeated treatment of animals with 10 μ g/0.1ml of DNazyme over a period of two weeks showed a significant increase in animal total weight at the end of the study compared to the animal weight at the beginning of the study (Figure 1) from the three groups (Vehicle Control, Positive Control, and GATA3 inhibitor Treated). Results indicated a significant increase in the total animal weight at the end of the study among the three groups. Control group showed 1.04 fold (P-value 0.020246) increase in animal weight, with positive and treated groups showing 1.03 fold (P-value 0.049796), and 1.04 fold (P-value 0.000469) increase respectively.

When comparing the weight difference between right (treatment site) and left (opposite control side) SAT, there was a significant increase in the right adipose tissue weight from the GATA3 inhibitor treated group of 1.2458 fold (P-value 0.0191), but not in the control group (P-value 0.422941). The positive control group showed a significant decrease in the weight of the right adipose tissue of 0.8973 Fold (P-value 0.004076) (Figure 2).

There was a statistically significant reduction in the omental adipose tissue weight between the GATA3 inhibitor treated group and the vehicle control groups of 0.5567 Fold (P-value 0.003), and the GATA3 inhibitor treated and positive control groups of 0.7345 Fold (P-value 0.0156) (Figure 3). However, there was no statistically significant difference in the Omental weight between the vehicle control and positive control groups (Figure 3).

Effect of GATA3 inhibition on SOD and Catalase levels from serum

There was a significant increase in SOD activity in the GATA3 inhibitor treated group compared to vehicle control group (Figure 4A). However, no significant difference was detected between the positive control and vehicle control groups (Figure 4A). Moreover, there was no significant difference in the catalase levels among the three groups (Figure 4B).

Effect of GATA3 inhibition on the gene expression levels from different adipose tissues (right, left, and omental sites)

In terms of gene expression from the SVF derived from the different adipose tissue sites, we found that GATA3 inhibition decreased the expression levels of the MCP-1 in the right (treatment site) (Figure 5) compared to opposite site (Figure 5). Additionally, the inhibition of the GATA3 caused a reduction of the PPAR γ expression levels from the omental in the GATA3 inhibitor treated group compared to the vehicle control group (Figure 6). No difference in gene expression was seen between the right (treatment site) and left (opposite control side) tissues dissected from the control and positive groups (Data not shown).

Effect of GATA3 inhibition on preadipocyte proliferation, adipogenic capacity and gene expression

GATA3 inhibition was further validated in primary human preadipocytes isolated from BFP biopsies. Results showed that treating preadipocytes with GATA3 inhibitor caused an increase in the cell number (Figure 7A) and adipogenic capacity with more mature adipocytes (1.797 Fold increase) in the GATA3 inhibitor treated group compared to the untreated group (Figure 7B). Moreover, a significant increase in the expression levels of the adipogenic genes (PPAR γ , CEPB β) from the preadipocytes treated with the GATA3 inhibitor was observed (Figure 8).

Effect of GATA3 inhibition on preadipocyte insulin signaling

In order to assess the effect of GATA3 inhibition on insulin signaling, we measured the phosphorylation levels of different insulin response-associated kinases in human primary preadipocytes. Results showed that there was a significant reduction in the expression of p70S6K from the GATA3 inhibitor treated group compared to the untreated group (Figure 9).

Discussion

Various studies have suggested that non-obese individuals could equally become insulin resistant and develop T2D if left untreated, with the term metabolically obese apparently healthy individuals being used to describe insulin resistant non-obese individuals (3, 8, 34). Modulators including GATA3 were shown to be highly expressed in insulin resistant tissues and to be responsible for preventing adipogenesis. Despite its potential role in obesity prevention, such an approach has a great risk of preventing adipogenesis, which is required to maintain adipose tissue homeostasis and insulin sensitivity (35). GATA3 associated impaired adipogenesis affects lipid homeostasis contributing to body-fat distribution, such disturbance causes the deposition of ectopic fat in the liver, kidney, and skeletal muscles triggering insulin resistance and increasing the risk of T2D (35). In this study, we investigated the effect of GATA3 inhibition in

rescuing impaired adipogenesis, correcting fat deposition, and enhancing insulin signaling. Our findings demonstrate that there was an increase in the total animal weight at the end of the experiment from the GATA3 inhibitor treated group. The increase in the animal weight was accompanied by an increase in the weight of the tissues dissected from the right site (Injection site) compared to the left site tissues. Inhibiting GATA3 in the injection sites might have enhanced mobilization, recruitment, and differentiation of adipocyte progenitor cells to the right site, therefore promoting adipogenesis and causing an increase in the tissue weight. In contrast, GATA3 inhibitor treated group showed a reduction in the omental adipose tissue weight compared to the other groups, perhaps accounting for the increased weight of fat tissue at the injection site in treated animals. Such reduction was triggered by decrease in the expression levels of PPAR γ . This suggests a protective effect associated with GATA-3 inhibition, which goes along with previous studies associating the omental adipose tissue mass/size with the amplified inflammatory status, and insulin resistance (36). Moreover, other studies correlated omental adipose tissue size with the degree of insulin resistance (13). Impaired adipogenesis is characterized by having an imbalance between the oxidative and anti-oxidative markers. SOD, a major antioxidant enzyme that protects adipocytes during proliferation and differentiation was found to be elevated in tissues and cells undergoing active adipogenesis and cellular differentiation (37). Impaired adipogenesis causes a reduction in the SOD levels increasing the oxidative stress on the adipose tissues, hence, having an antioxidant modulator might restore the balance between the oxidative and anti-oxidative markers. Our results showed that GATA3 inhibition caused an increase in the SOD levels measured from the animal's serum, which correlates with the increase in the animal's total weight indicating the presence of active adipogenesis (37).

The pro-inflammatory profile of dysfunctional adipose tissue plays a critical role in lowering the adipogenic capacity of preadipocytes, leading to a reduction of the lipid storage space and elevation in ectopic lipid accumulation (38). To validate this point we measured to what extent GATA3 inhibition could account for the expression of MCP-1(38). MCP-1 was the first discovered and most extensively studied CC chemokine shown to be linked to etiologies of obesity related insulin resistance and therefore T2D (39). Studies showed that MCP-1 is overexpressed in obese and insulin resistant animals, suggesting that the elevation in the MCP-1 levels could reduce adipocyte differentiation, alternatively causing metabolic abnormalities associated with obesity, and hyperinsulinemia (e.g. T2D) (40). In addition, MCP-1 is a well-known potent inflammatory chemokine that recruits macrophages, thus, targeting it could prevent macrophage-induced inflammation in adipose tissue (39). Our results showed that expression levels of MCP-1 at the site of GATA3 inhibitor injection were significantly lower than at the opposite side. Suggesting that GATA3 inhibition has the potential to reduce macrophage infiltration associated with adipose tissue inflammation seen in impaired adipogenesis.

After studying the effect of GATA3 inhibition *in vivo*, we moved on to study the effect of the GATA3 inhibition on primary adipocytes isolated from adipose tissue of different human subjects. Recent reports suggested that both preadipocytes and mature adipocytes play an equally important role in the maintenance of adipose tissue hemostasis and the development of insulin resistance when dysfunctional (41). These results were consistent with our finding showing an increase in the cell number

and adipogenic capacity in GATA3 inhibition group, which indicates the presence of active adipogenesis and differentiation of the preadipocyte to mature adipocytes. These results support the *in vivo* findings, particularly with previous studies showing the effect of GATA3 on preventing preadipocyte differentiation. To elucidate the roles of adipogenesis in the early development of insulin resistance, we characterized the gene expression profile of human primary adipocytes. Previous studies reported that down-regulation of PPAR γ /CEPB β was observed in preadipocytes isolated from insulin resistant individuals (42). Other studies showed that adipocyte differentiation was compromised under these conditions, thus, using modulators such as GATA3 inhibitor could improve adipogenesis and correct insulin signaling in non-obese individuals. Our results indicated that PPAR γ and CEPB β genes were differentially expressed in these cells, with high expression levels seen from the cells in the GATA3 inhibition group compared to the cells in the untreated group.

Insulin resistance is commonly linked with obesity; however, studies showed evidence of insulin resistance in non-obese apparently healthy individuals. Thus, determining the insulin sensitivity in these individuals could be used as an indicator of their risk of prediabetes. Impaired adipogenesis could affect the levels of different proteins and kinases involved in the insulin signaling pathway. Our results showed a reduction in the phosphorylation of the p70S6K from primary adipocytes treated with the GATA3 inhibitor. p70S6K (ribosomal protein S6 kinase 1, p70S6K,) is a serine kinase that was reported to inhibit the function of IRS-1 by facilitating its degradation, thus inhibiting insulin signaling. Previous *in vivo* studies showed that knocking-out p70S6K in mice protected them from diet-induced insulin resistance. Moreover, obese mice were shown to have elevated p70S6K activity in the adipose tissue, skeletal muscles, and liver, which are strong indicators that could contribute to insulin resistance (43). These findings together highlighted the important role GATA3 plays in the development of impaired adipogenesis and insulin resistance, therefore blocking GATA3 could reverse these mechanisms and enhance both adipogenesis and insulin signaling.

In conclusion, our *in vivo* and *in vitro* data indicate that inhibiting GATA-3 expression restores adipogenesis and fat distribution, improves adipocytes differentiation, and enhances insulin signaling. Our data, therefore, suggest the potential utilization of GATA-3 modulation for preventing the development of insulin resistance in non-obese as well as obese individuals. However, despite holding a great potential and being a promising modulator, such approach requires further investigation and validation.

Abbreviations

T2D: Type 2 Diabetes

GATA3: Transcription Factor of the GATA family – binding protein

BALB: Bagg and Albino

DNAzyme: Deoxyribozyme

SOD: Superoxide Dismutase

PPAR γ : Peroxisome Proliferation-Activator gamma

MCP-1: Monocyte Chemoattractant Protein-1

SAT: Subcutaneous Adipose Tissue

IL-6: Interleukin-6

IL-8: Interleukin-8

IL-1 β : Interleukin-1 beta

IRS-1: Insulin receptor substrat-1

TNF α : Tumor necrotic factor alpha

GLUT4: Glucose transporter factor 4

C/EBP α : CCAAT/ enhancer binding protein alpha

LARC: Laboratory Animal Research Center

IVC: Individual Ventilated cages

QU: Qatar University

IACUC: Institutional Animal Care and Use Committee

IBC: Institutional Biosafety Committee

mg: milligrams

Kg: kilograms

μ l: microliters

μ M: micro molar

ml: milliliter

AVMA: American Veterinary Medical Association

IRB: Institutional Research Board

BMI: Body mass index

SVF: Stromal vascular fraction

BFP: Buccal fat pad

DMEM-F12: Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12

FBS: Fetal bovine serum

CO₂: Carbon dioxide

mM: millimolar

IBMX: 3-Isobutyl-1-methylxanthine

DAPI: 4',6-Diamidino-2'-phenylindole dihydrochloride

LipidTox: Deep Red neutral lipid stain

BSA: Bovine serum albumin

RNA: Ribonucleic acid

PCR: Polymerase chain reaction

cDNA: Complementary DNA

GAPDH: Glyceraldehyde 3-phosphate dehydrogenase

$\Delta\Delta$ CT: Delta Delta cycle threshold

PGC-1 α : Peroxisome proliferator-activated receptor gamma coactivator 1-alpha

Nrf2: Nuclear factor-erythroid factor 2-related factor 2

KEAP-1: Kelch-like ECH-associated protein 1

GSK3B: Glycogen synthase kinase 3 beta

IGF1R: Insulin-like growth factor 1 receptor precursor

Akt: A group of enzymes involved in several processes related to cell growth and survival

Mtor: mammalian target of rapamycin

PTEN: Phosphatase and tensin homolog

GSK3: Glycogen synthase kinase-3

TSC2: Tuberous sclerosis 2

RPS6: Ribosomal protein S6

ANOVA: Analysis of Variance

SEM: Standard Error Mean

ns: not significant

UT: untreated

Declarations

This is a declaration that the manuscript titled : *“In vivo and in vitro validation of GATA-3 suppression for correcting impaired adipogenesis, restoring insulin sensitivity, and lowering risk of T2D”* is our original work and is not published earlier and we have not submitted this manuscript for publication elsewhere. We have cited the names of the authors whose published materials are used in the manuscript(s).

Yours sincerely,

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Ethics approval

All participants provided informed consent. Human protocols were approved by Institutional Review Board (IRB) of Hamad Medical Corporation and Qatar University (MRC-03-21-154, and QU-IRB 1548-EA/21). Animal protocols were approved by Qatar University Institutional Animal Care and Use Committee (QU-IACUC 024/2020).

Consent for publication

Not applicable.

Availability of data and material

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request

Competing interests

The authors declare a competing interest (US Patent App. 16/909,755)

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Authors' contribution

HJ, NAA, SM, MA, NA, LM and MAE carried out experiments and contributed to experimental design, data analysis and manuscript writing. VKG and HAN carried out animal experiments and contributed to experimental design and animal ethics approvals. ST, JJ and SH recruited patients and contributed to experimental design and human ethics approvals. AA and AE contributed to experimental design. All authors reviewed and approved the final version of the paper. MAE is responsible for the integrity of the work as a whole.

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Tables

Table 1. List of primers used for RT-PCR experiments.

Gene	Primers sequences (5' to 3')
<i>GATA-3</i>	<i>f</i> : GAACCGGCCCTTATCAAG <i>r</i> : ACAGTTCGCGCAGGATGTC
<i>GAPDH</i>	<i>f</i> : AGGTCGGTGTGAACGGATTTG <i>r</i> : TGTAGACCATGTAGTTGAGGTCA
<i>IL-6</i>	<i>f</i> : TAGTCCTTCTACCCCAATTTCC <i>r</i> : TTGGTCCTTAGCCACTCCTTC
<i>IL-10</i>	<i>f</i> : GCTCTTACTGACTGGCATGAG <i>r</i> : CGCAGCTCTAGGAGCATGTG
<i>PPAR-γ</i>	<i>f</i> : GGCTTCCACTATGGAGTTCA <i>r</i> : GATCCGGCAGTTAAGATCAC
<i>PGC1 - α</i>	<i>f</i> : TGCAGCCAAGACTCTGTATG <i>r</i> : ATTGGTCGCTACACCACTTC
MCP-1	<i>f</i> : GCTACAAGAGGATCACCAGCAG <i>r</i> : GTCTGGACCCATTCTTCTTGG
NrF2	<i>f</i> : CTGAACTCCTGGACGGGACTA <i>r</i> : CGGTGGGTCTCCGTAATGG
KEAP-1	<i>f</i> : CAGCAGTTAAGGGCACCAATGC <i>r</i> : CCTGCCTCAGCTTCCCATCA
ADIPONECTIN	<i>f</i> : ACTGCAACTACCCATAGCCCAT <i>r</i> : TGTCGACTGTTCCATGATTCTCC

Figures

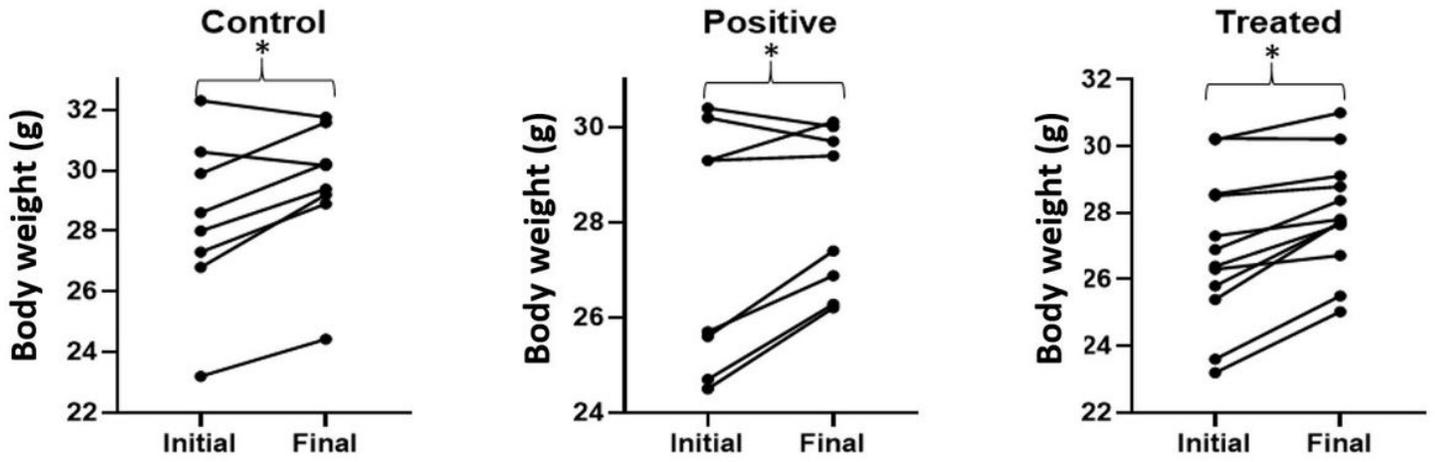


Figure 1

Changes in total animal weight between the beginning and end of the experiment.

Bar graphs show paired data for each mouse for n = 6-12 animals per group. Statistical analysis for was determined by paired t-test (*P < 0.05).

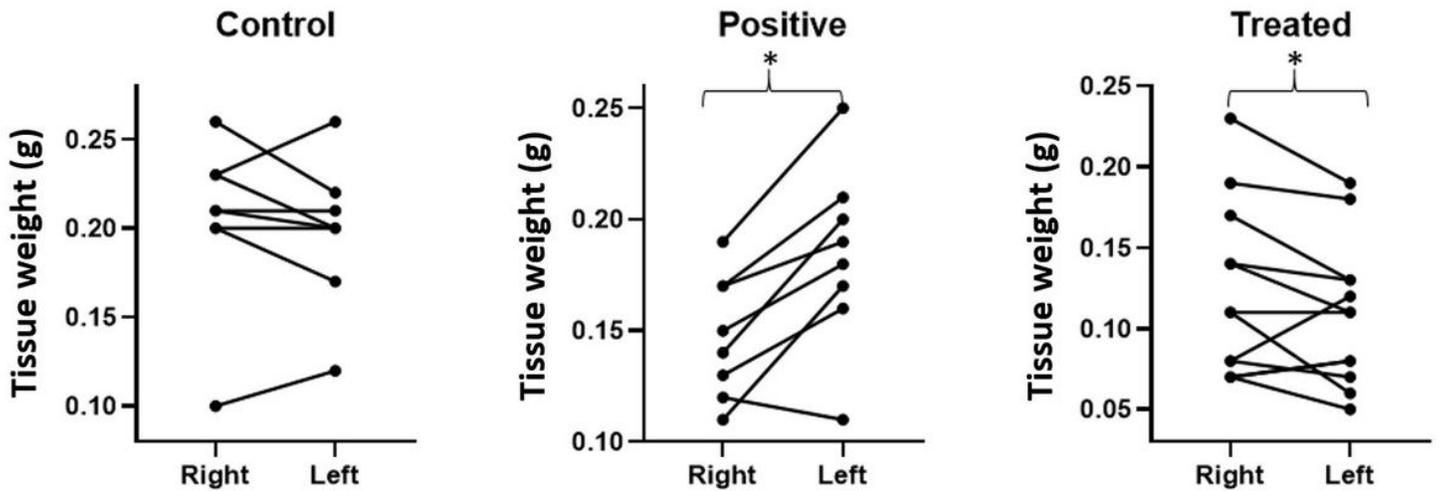


Figure 2

Changes in animal's SAT tissue weight between right (site of injection) and left (control) sites. Data are presented as mean \pm SEM for n = 8-12 animals. Statistical analysis for was determined by paired t-test (*P < 0.05).

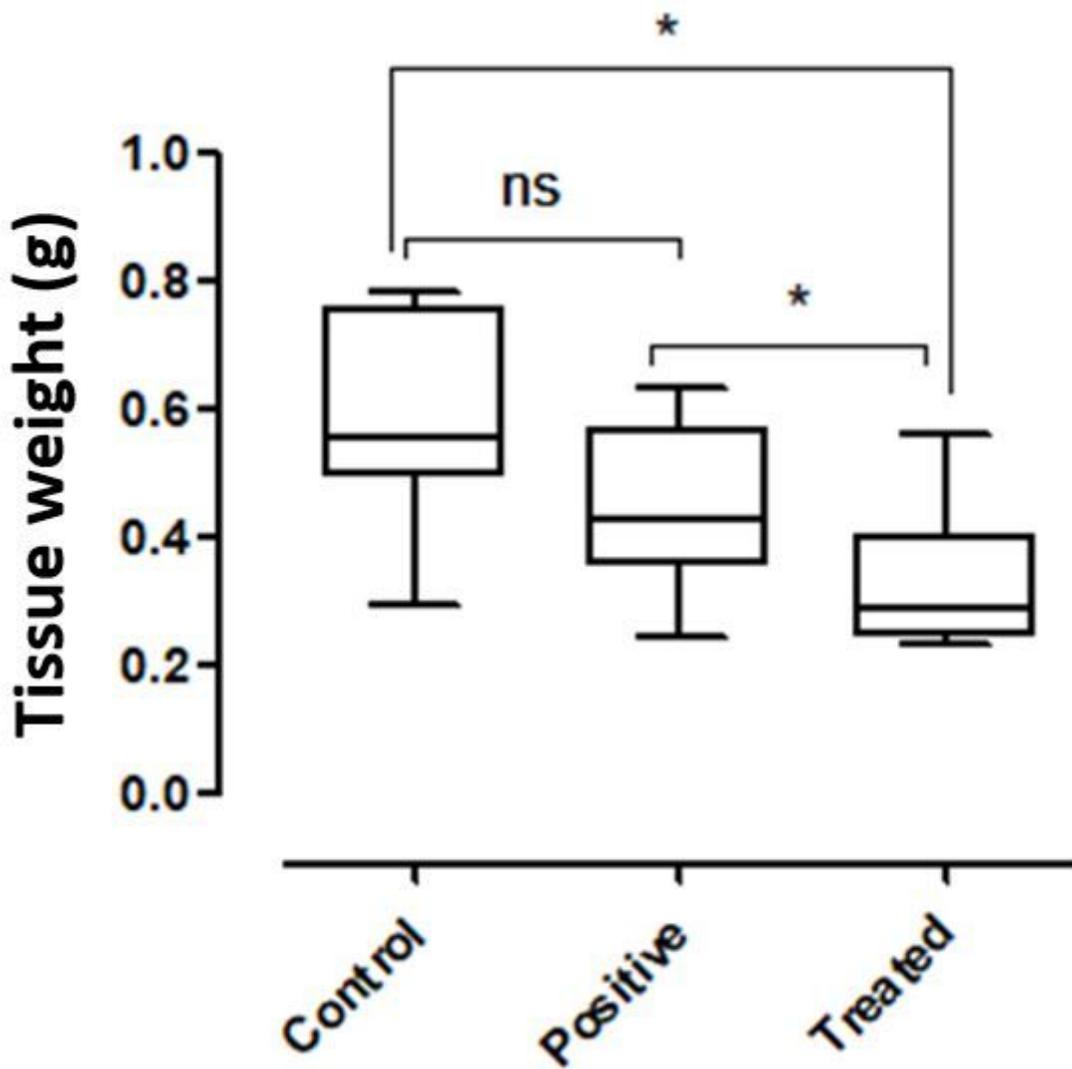


Figure 3

Changes in the animal's omental tissue weight with GATA-3 inhibitor treatment. Data are presented as median \pm IQR for n = 8-12 animals. Statistical analysis for was determined by un-aired t-test (**P < 0.02, *** P < 0.01).

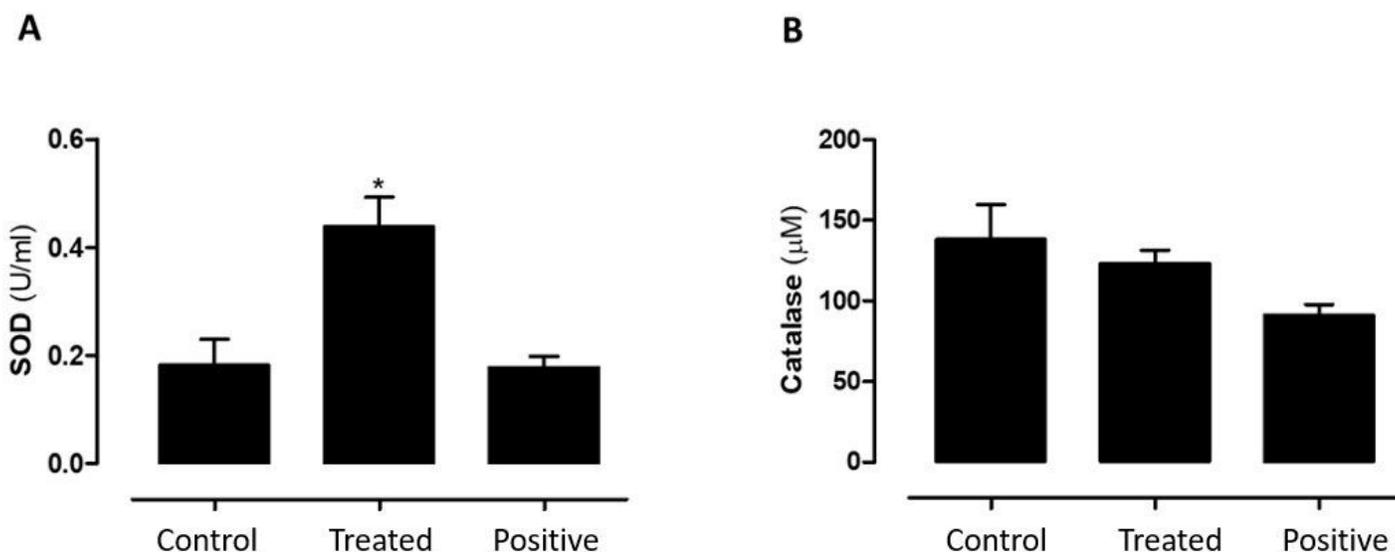


Figure 4

(A) SOD and (B) Catalase levels detected from mice serum. Data are presented as mean \pm SEM for n = 4-12 animals. Statistical analysis for was determined by One-way ANOVA (*P < 0.05).

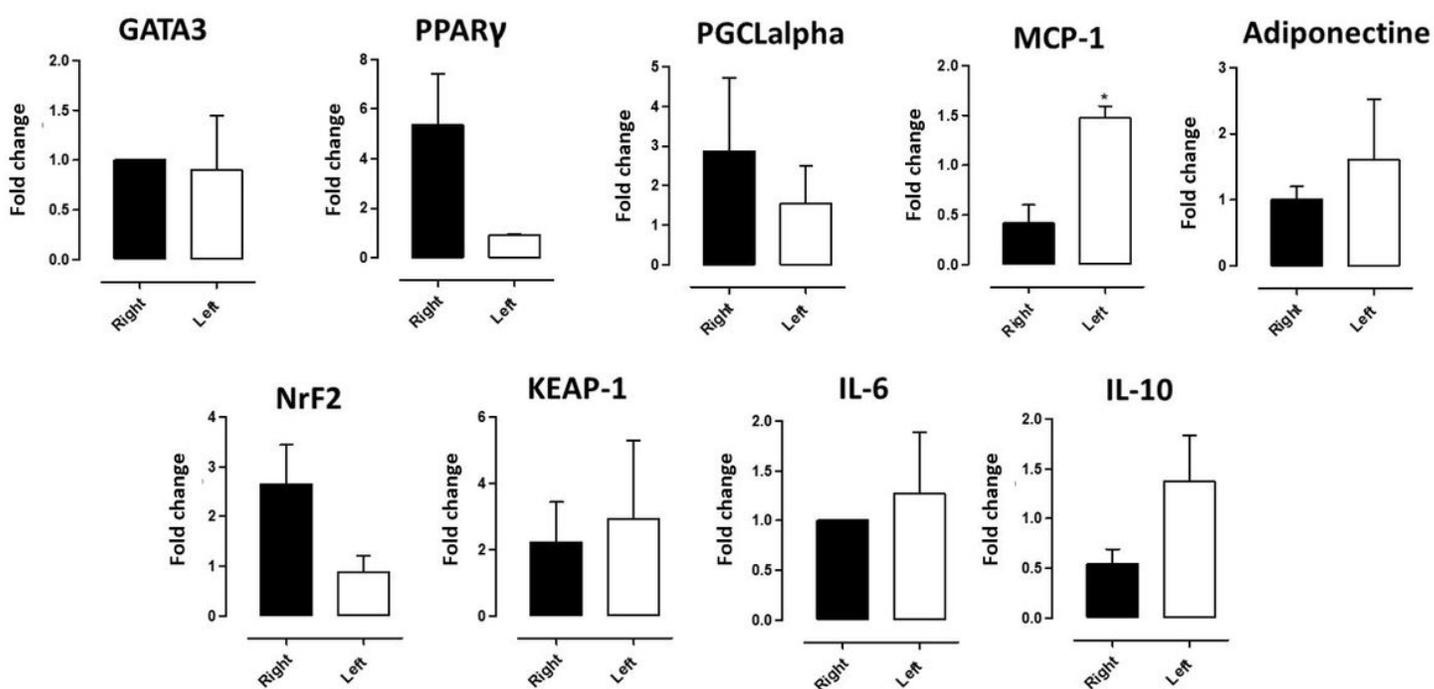


Figure 5

Gene expression in the stromal vascular fraction derived from GATA-3 inhibitor treated animal's right (treatment site) and left (opposite control side). Differences in gene expression levels were compared for GATA3, PPAR γ , PGC1alpha, MCP-1, Adiponectin, NrF2, KEAP-1, IL-6 and IL-10. Data are presented as

mean \pm SEM for n = 6 replicates from 3 animals each. Statistical analysis for was determined by paired t-test (*P < 0.05).

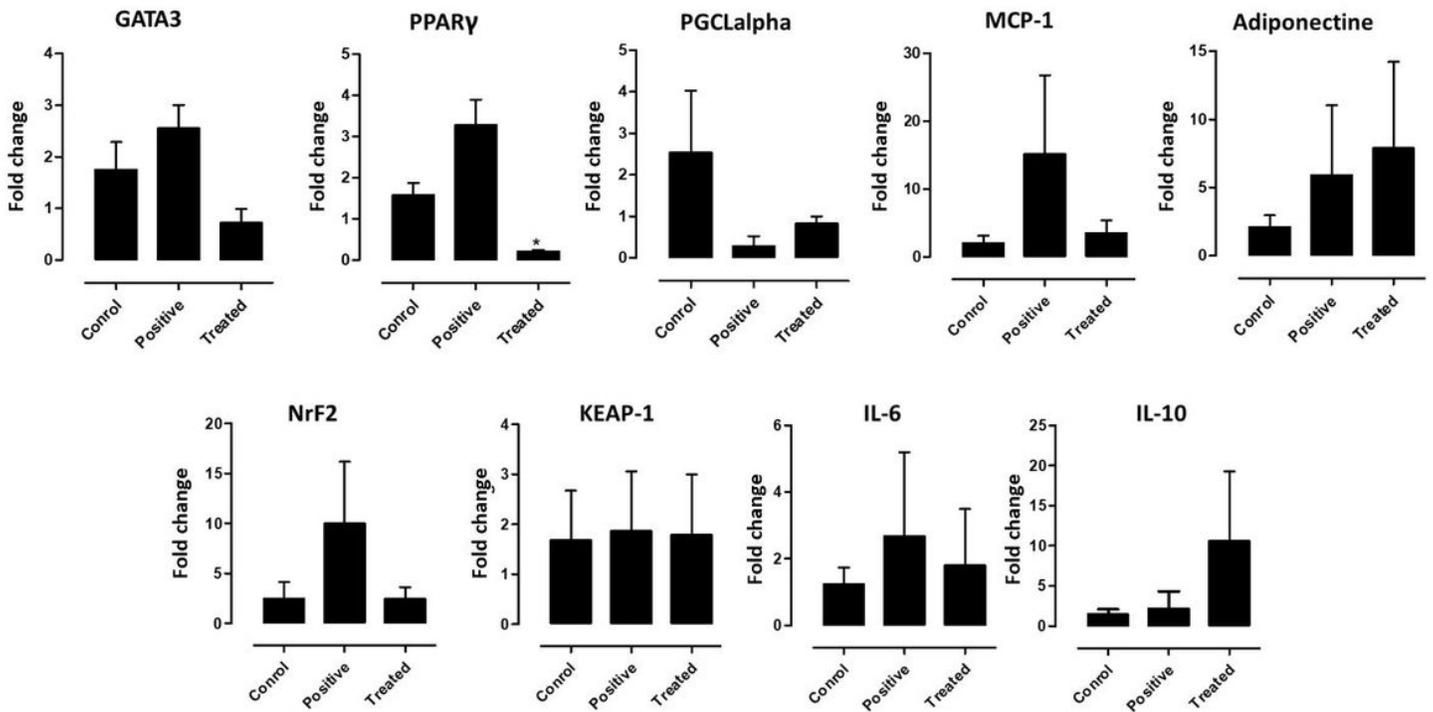
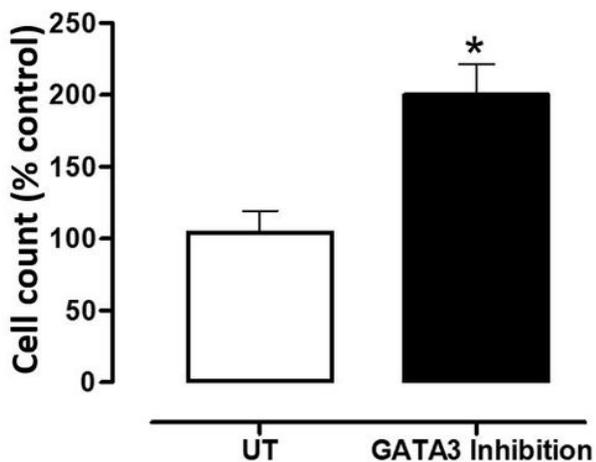


Figure 6

Gene expression in the stromal vascular fraction derived from the omental adipose tissue in vehicle control, positive control and GATA-3 inhibitor-treated animals. Differences in gene expression levels were compared for GATA3, PPAR γ , PGC1 α , MCP-1, Adiponectin, Nrf2, KEAP-1, IL-6 and IL-10. Data are presented as mean \pm SEM for n = 6 replicates from 3 animals each. Statistical analysis for was determined by ANOVA (*P < 0.05).

A



B

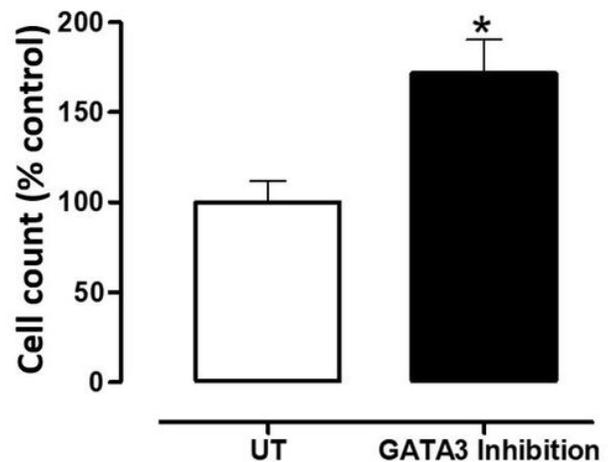


Figure 7

Effect of GATA3 inhibition on (A) Primary adipocytes proliferation and (B) Adipogenic Capacity. Data are presented as mean \pm SEM for n = 15 replicates from 5 donors. Statistical analysis for effect of GATA3 inhibition was determined by Mann–Whitney U test (*P < 0.05).

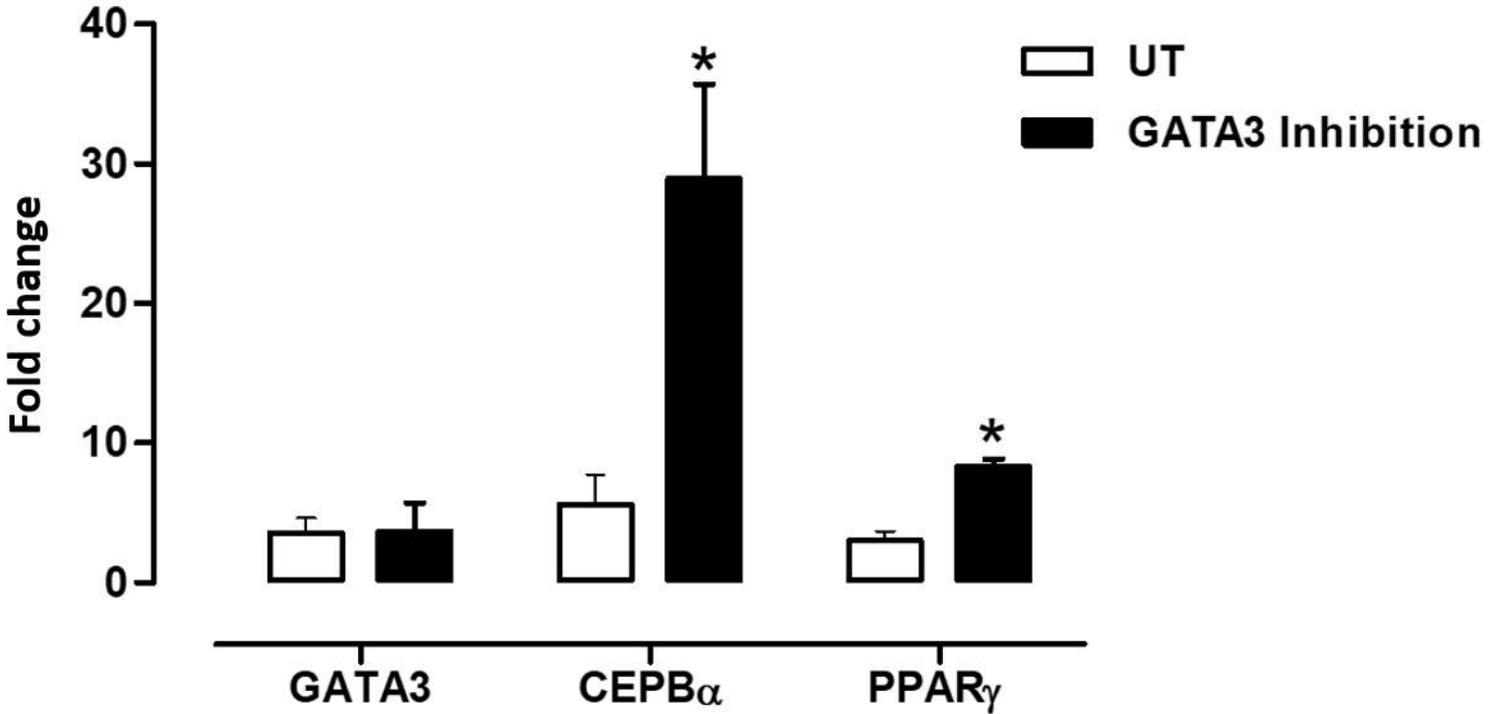


Figure 8

Effect of GATA3 inhibition on gene expression from primary adipocyte cells after differentiation. Data are presented as mean \pm SEM for n = 6 donors. Statistical analysis for effect of GATA3 inhibition was determined by Mann–Whitney U test (*P < 0.05).

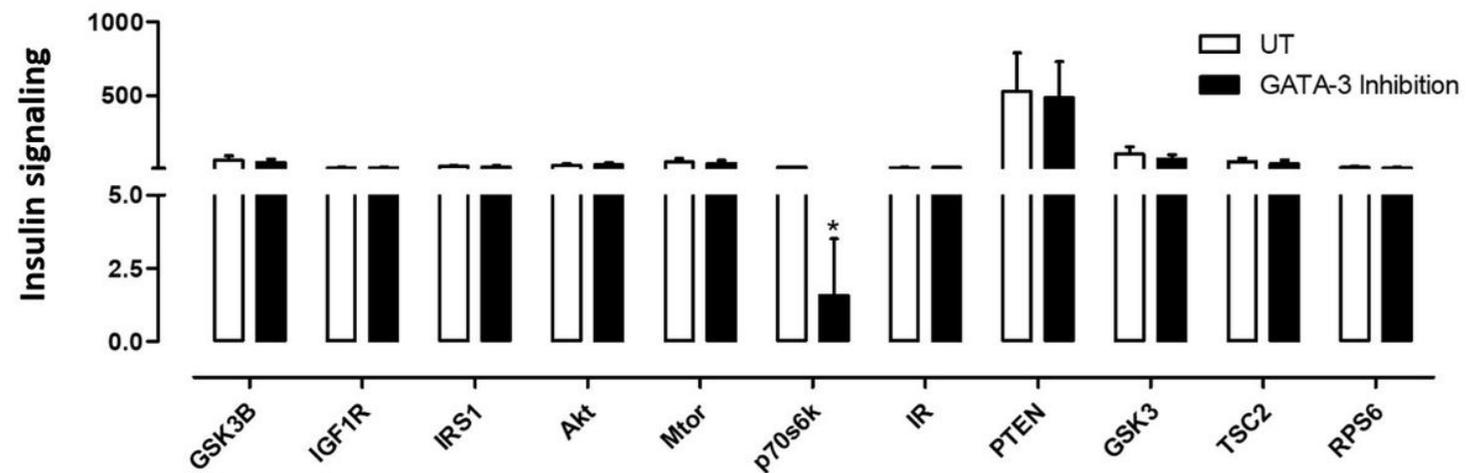


Figure 9

Effect of GATA3 inhibition on insulin signaling from primary adipocyte cells. Data are presented as mean \pm SEM for n = 4 samples. Statistical analysis for effect of GATA3 inhibition was determined using two-way ANOVA followed by Bonferroni Posttests ($*P < 0.05$).