POSTER PRESENTATIONS

Mechanism of swertiamarin in oleic acid induced model of hepatic steatosis: *in vitro*

Tushar P. Patel, Komal Rawal, Sanket Soni and Sarita Gupta*

MOLECULAR ENDOCRINOLOGY AND STEM CELL RESEARCH LAB, DEPARTMENT OF BIOCHEMISTRY,

THE MAHARAJA SAYAJIRAO UNIVERSITY OF BARODA, VADODARA, GUJARAT, INDIA. *Corresponding author: sglmescrl@.com

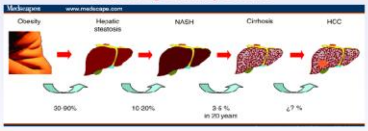
ABSTRACT

Hepatic lipid accumulation and insulin resistance contribute to non-alcoholic fatty liver disease (NAFLD). We hypothesized that hypolipidemic and antioxidant activity of swertiamarin would attenuate events leading to hepatic steatosis and insulin resistance. Steatosis was induced in HepG2 cells by supplementing 1 mM Oleic acid (OA) for 24hrs. OA induced hepatic steatosis in HepG2 cells was marked by significant accumulation of lipid droplets as determined by Oil-Red-O based colorimetric assay, increased triacylglycerol and decreased % LDH release activity. Swertiamarin decreased TAG content by two folds and was effective in reducing LDH release. Swertiamarin reduced insulin resistance and improved sensitivity by restoring the level of insulin receptor, Akt phosphorylation and PI(3)K proteins. In addition, qPCR results confirmed OA up-regulated SREBP-1 and fatty acid synthase, resulting in increased fatty acid synthesis. Swertiamarin effectively increased p-Akt and reduced PPAR level, potential modulators of carbohydrate metabolism which in turn decreased the levels of the gluconeogenic enzyme PEPCK. Hence, swertiamarin effectively reversed NAFLD symptoms by decreasing triglycerides accumulation, fatty acid synthesis, insulin resistance and increasing cellular antioxidants in OA induced hepatic steatosis in HepG2 cells.

INTRODUCTION

➢Defining Nonalcoholic Fatty Liver Disease: A liver biopsy showing moderate to gross macrovesicular fatty change with or without inflammation (lobular or portal), Mallory bodies, fibrosis or cirrhosis.

NAFLD Spectrum of Disease



➢Epidemiology : Prevalence of NAFLD 13-18% and that of NASH specifically 2-3% (1.2-9%). Prevalence of NAFLD in Indian population is 5 – 28% among Asian population.

➢Risk Factors : Classic TRIAD → Obesity, Diabetes and Dyslipidemia

➢Pathogenesis : "2 Hit" Paradigm

➢"First hit" – Excess fat accumulation and insulin resistance

➢"Second hit" – Intrahepatic oxidative stress, Lipid peroxidation, TNF-alpha and cytokine cascade

➢Oleic acid -induced steatosis in HepG2 cells is vitro model of steatosis is critical in understanding the pathogenesis of non-alcoholic fatty liver disease (NAFLD) and searching for effective therapies.

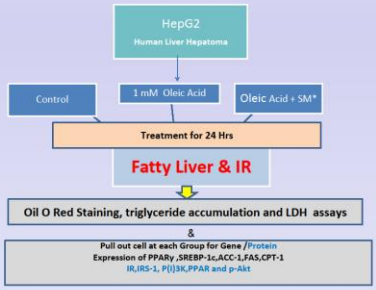
➢Aqueous extract of *Enicostemma littorale* Blume has anti-diabetic activity in the alloxan induced diabetic rats and even in the NIDDM human patients. ***(Maroo J et al., 2002; Vasu V T et al., 2003; Gupta S. et al., 2005., Vasu V T et al., 2005)***

➢Swertiamarin (SM) as a regulator of Hepatic and Adipose tissue gene expression under the control of transcriptional factors like PPARγ, thus confirming that SM improves insulin sensitivity and modulates carbohydrate and fat metabolism. ***(Patel et al. 2013)***

➢Our *in vivo* findings suggest potential role of swertiamarin in regulation of transcription control of fat and carbohydrate metabolism in hepatic tissue.

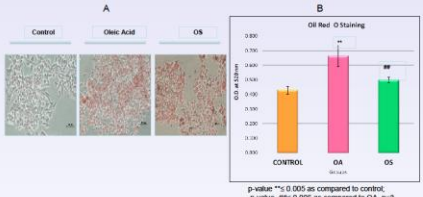
➢Swertiamarin might be effective therapy of non-alcoholic fatty liver disease (NAFLD).

PLAN OF WORK



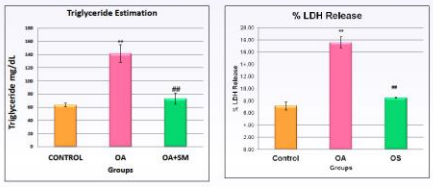
RESULTS

Figure 1: A) OA-induced steatosis in HepG2 cells determined by ORO staining. B) ORO-based colorimetric assay. Quantification of Oil O Red stain after extraction procedure.



p-value **≤ 0.005 as compared to control; p-value ##≤ 0.005 as compared to OA. n=3

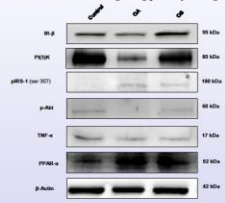
Figure 2A : Effect of swertiamarin on A) Triglyceride accumulation. B) % LDH release.



p-value **≤ 0.005 as compared to control; p-value ##≤ 0.005 as compared to OA. n=3

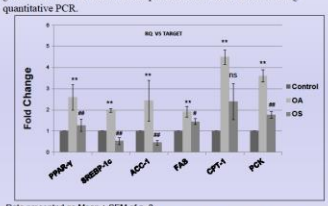
p-value **≤ 0.005 as compared to control; p-value ##≤ 0.005 as compared to OA. n=3

Figure 3 : Effect of Swertiamarin on Signaling pathways using fatty liver in HepG2 cells .



Western blot study showing the effect of SM treatments on the expression of PPAR and Insulin signaling proteins: IR,IRS-1,Akt and PI(3)K in the Hepg2 as compared to OA treated group . β-actin was taken as an internal control.(20 ng protein)

Figure 4 : Effect of swertiamarin treatments on the expression of metabolic genes in the liver steatosis. The expression levels were checked using quantitative PCR.



Data presented as Mean ± SEM of n=3.
p-value **≤ 0.005 as compared to control; p-value ##≤ 0.005 as compared to OA. n=3.
p-value ; ns > 0.05 as compared to control & OA

DISCUSSION

❖Hepatic steatosis results from increased fatty acid influx to hepatoctyes, reduced lipid oxidation and decreased VLDL excretion .(Cui et al., 2010)

❖HepG2 cells were supplemented with pathophysiologic levels of oleic acid to mimic the influx of excess FFAs into hepatocytes, giving rise to hepatic steatosis. (Barve et al., 2007)

❖Insulin mediated glucose uptake and proliferation of HepG2 cells were hampered in steatosis due to excess fat accumulation which suggest a link between antioxidant imbalance, insulin resistance and obesity-related complications.

❖The results demonstrated that cells incubated with swertiamarin remarkably decreased the ORO staining, TAG accumulation and the % LDH release.

❖Insulin resistance is a major factor responsible for NAFLD and management of this condition reduces its risk.

❖Swertiamarin improved insulin sensitivity by restoring the level of insulin receptor, Akt phosphorylation, PPAR-α and PI(3)K proteins as previously proved by Patel et al.,2013 in an *in vivo* model.

❖Swertiamarin controls fatty acid synthesis by down-regulation of SREBP1c and ACC-1.Mitochondrial fatty acid oxidation was reduced by CPT-1 gene expression control.

❖PPARs, one of the major players for triglyceride biosynthesis, gluconeogenesis and fat metabolism was also shown to be controlled by swertiamarin (Patel et al.,2013).

CONCLUSION

Swertiamarin effectively reversed NAFLD symptoms by decreasing triglyceride accumulation, fatty acid synthesis, insulin resistance and increasing cellular antioxidants in OA induced hepatic steatosis in HepG2 cells. Hence swertiamarin is promising to carry out more experimental and clinical studies to understand the molecular mechanisms to overcome NAFLD symptoms.

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MESCRL
Hypothesis-experiments-innovations

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Metabolic assessment of adipose tissue from control and obese human subjects.

Komal Rawal, Vyakhaya Kataria, Tushar Patel and Sarita Gupta*.
Molecular Endocrinology and Stem Cell Research Laboratory,
Department of Biochemistry, Faculty of Science, The M.S.University of Baroda.
***Corresponding author: saritagupta9@gmail.com, sglmescrl@gmail.com**

ABSTRACT

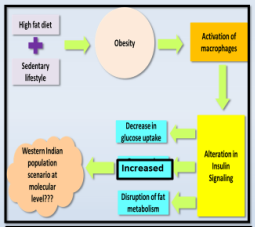
Sedentary lifestyle accompanied with high calorific diet alters energy homeostasis thus, causing a clinical manifestation called as obesity. More than 30% obese are diagnosed diabetic across the world. Amongst all, Indian obese has high glucose intolerance and cardiovascular disease incidences. High levels of free fatty acids activate adipose tissue resident cytotoxic macrophages that enhance inflammation thus, causing metabolic disorders. Increased feeding and cytokine levels leads to hyperleptinemia and leptin resistance, thus the control over food intake is lost, resulting into metabolic disorders. Inspite of Indians being metabolically obese, no study has been performed to scrutinize adipose tissue metabolism and insulin signaling in obese Indian subjects. Thus, the aim of the study was to evaluate molecular aspects of lipid metabolism and insulin signaling in adipose tissue of obese and control subjects. Subjects with BMI (Kg/m^2) >25 were considered to be obese. Adipose tissue from non-diabetic control and obese subjects were studied for major lipid metabolic gene expressions and insulin signaling proteins. Also the protein expression of inflammatory mediators like TNFα and Erk1/2 were checked. Gene expression studies depicted that PPARγ, major transcriptional factor of adipogenesis, was found to be significantly increased in obese subjects along with elevated levels of leptin , highlighting the dysregulated lipid metabolism in obese subjects under study. Insulin receptor and pAKT, key proteins of insulin signaling were found to be elevated significantly, indicating expansion of adipose tissue in obesity. Increased expression of TNFα (hallmark of inflammation) in obese subjects provides evidence for commencement of metabolic disorders like diabetes, CVDs and cancer. Thus, the study with a larger subject number would signify the metabolic status of obesity prevailing in India.

Keywords: Human adipose tissue, PPARγ, Leptin, Insulin signalining, TNFα.

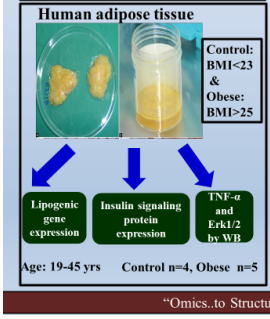
INTRODUCTION

- There has been a continuous increase in population of obese people with India being one of the top most countries having most obese population with high association of diabetes and high cardiovascular diseases incidences.
- Adipose tissue primarily works as energy stores of the cell, but excess energy intake and little or no energy expenditure leads to hypertrophy of the adipose tissue resulting into obesity (Choi et al, 2014).
- High calorific diet increases PPARγ expression and their dependent lipogenic genes like FAS, ACC-1, leptin (Tanti et al, 2013).
- Leptin is a major hormone secreted by adipose tissue that regulates food intake. But during obesity, leptin levels increase signifiantly thus causing leptin resistance (Clauda et al;2009).
- Prolonged hyperleptinimia and chronic inflammation in obese with progression in age alters insulin signaling causing insulin resistance and CVDs.

HYPOTHESIS



PLAN OF WORK



RESULTS

EXPRESSION PROFILE OF MAJOR FAT METABOLIC GENE

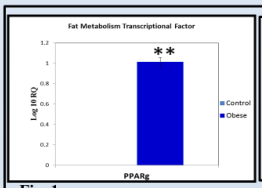


Fig. 1a

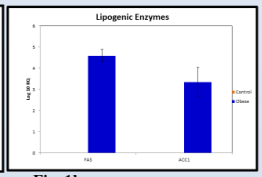


Fig. 1b

. Fig 1a. Gene expression of PPARγ, Fig 1b. Gene expression of Lipogenic enzymes. Data is presented as mean ± SEM in log scale where control is considered 0 (n=3 control, n=3 obese), *P<0.01 as compared to C)

GENE EXPRESSION AND SERUM LEVELS OF LEPTIN

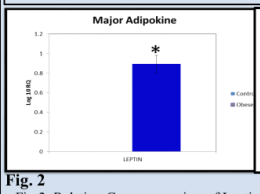


Fig. 2

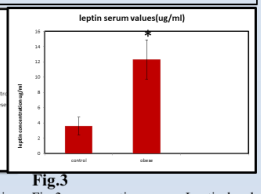
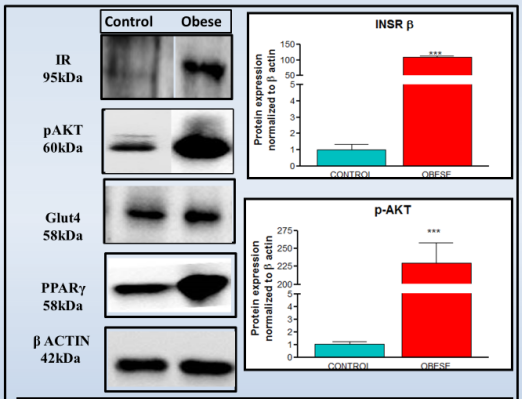


Fig.3

Fig 2: Relative Gene expression of Leptin Fig 3: comparative serum Leptin levels Data is presented as mean ± SEM in log scale where control is considered as 0. (n=3 control, n=3 obese),*P<0.05 as compared to C).

EXPRESSION OF INSULIN SIGNALING PROTEINS



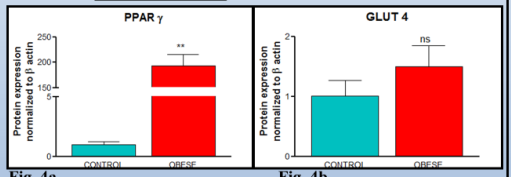


Fig. 4a **Fig. 4b**

Fig4a. &4b. Immunoblotting of major insulin signaling proteins IR, pAkt and PPARγ Data plotted as mean ± SEM. IR (n= 3 control, n=5 obese), pAkt (n=3 control n=6 obese),Glut-4 (n= 4 control,n=5, obese), PPARγ (n=4 control, n=3 obese).(***P<0.001, **P<0.01).

EXPRESSION OF INFLAMMATORY MEDIATOR

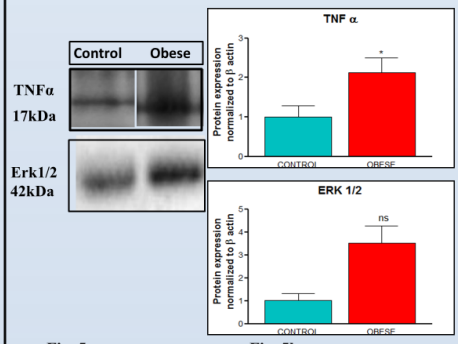


Fig. 5a **Fig. 5b.**

Fig5a. &5b. Immunoblotting of TNFα and Erk1/2, (n= 3 control, n=4 obese) Data plotted as mean ± SEM*P<0.05).

DISCUSSION

- FFA induces high expressions of PPARγ, thus increasing lipogenic gene expression causing hypertrophy and hyperplasia of adipose tissue in obese.
- The endocrine function of adipose tissue is altered, thus causing dysregulation in leptin leading to hyperphagia which disturbs energy homeostasis in obesity.
- Hyperactivation of insulin signaling in insulin sensitive obese stimulates adipogenesis, through PPARγ activation.
- TNF-α and FFA's are secreted at high amounts in adiposity and play an important role in the development of insulin resistance and Type II Diabetes Mellitus.

CONCLUSION

First study initiated to assess metabolic functions and insulin signaling on Indian Obese subjects. Obeese have dysregulated lipid metabolism along with elevated inflammation which could be worsened with progression in age. A study with large number of subjects would delineate the relation of obesity and insulin resistance with increased age subjects.

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Human adipose derived stem cells plasticity in adipose tissue of obese Indians

Komal Rawal[1], Kashish Israni[1], Vyakhaya Kataria[1], Kishan Purohit[1], Hiren Bhatt[2], Tushar Patel[1] and Sarita Gupta[1]

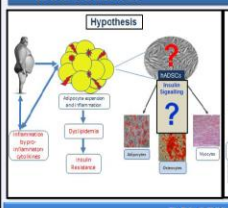
[1]Department of Biochemistry, Faculty of Science, M.S. University of Baroda, Vadodara, Gujarat, India.
[2]Nishtha Hospital, Vadodara, Gujarat, India.

ABSTRACT: Indians are delineated to be metabolically obese at lower BMI compared to western population due to high glucose intolerance, fat/muscle ratio and cardiovascular diseases. It is plethora of positive energy, free fatty acids and inflammation that remodel adipose tissue (AT) by altering adipokines, cell types and reduced stemness of human adipose derived stem cells (hADSC). Thus, the present study was commenced to unravel repercussions of obesity in Indian subjects by assessment of hADSC from control (BMI<23) and obese (ohADSC, BMI>25) along with metabolic alterations in AT. hADSC isolated from surgically excised AT were characterized by flow cytometry of CD44+, CD105+, CD31-, CD34-, growth curve and tri-lineage differentiation (adipogenesis, osteogenesis and myogenesis). Further, gene expression analysis of key adipogenic transcription factors [ATF (CEBPB, CEBPD, PPARG, SREBF1, SIRT1, DLK1)], inflammatory mediators [IM (CCL2, IKBKB, NLRP3, HIF1A)] and pluripotent stem cell markers [POU5F1 (OCT3/4), SOX2, NANOG] along with protein expression of candidate insulin signaling proteins (pIR, PI3K, pAKT, Glut4) were performed in hADSC. mRNA of CEBPB, PPARG, FAS, ACACA, LEP and ADIPOQ(adiponectin) from AT were analysed. Our results demonstrated that, ohADSC had reduced CD44+ population and growth rate, hampered differentiation potentials where adipogenic fate was preferred over osteogenesis. Elevation in IM, ATFs, OCT3/4 with reduced SOX2, NANOG and insulin signaling in ohADSCs, exemplify regulatory role of inflammation and insulin resistance on growth and stemness of ohADSCs. Upregulation of lipogenic genes with high leptin levels and low adiponectin levels were evident in obese AT. Thus, concluding that ADSCs being the self-resilient are compromised and might be one of the factors contributing to metabolic deregulation in obese AT culminating in NAFLD and diabesity. This is the first study to the best of our knowledge on comparative assessment in Indian subjects demonstrating the dynamic plasticity of AT, highlighting involvement of ADSCs thus, introducing a new avenue for treatment of diabesity.

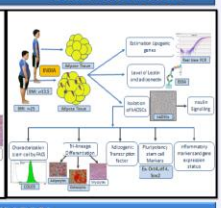
INTRODUCTION

- India is the third largest country suffering from obesity due to high fat diet and sedentary life. More than 30% obese Indians along with obese worldwide are found to have hyperglycemia, hyperinsulinemia, dyslipidemia with high levels of circulating cytokines,thus, diagnosing them clinically diabetic (Mahadik et al, 2008)
- Circulating free fatty acids aggravate infiltrating adipose resident macrophages, leading to hyperplasic and hypertrophic adipocytes.
- Dyregulated lipid metabolism and inflammation alters insulin signaling resulting into insulin resistance like condition, leading to ectopic fat deposition culminating into metabolic disorder.
- Enhanced inflammation in obesity is one of the causal factor in reduction of multi lineage differentiation potentials of ADSCs isolated from massively obese patients compared to healthy subjects , thus altering plasticity of ADSC (Roldan et al, 2011) and functionality of adipose tissue.
- Inspite of Indians being metabolically compromised there is a black hole on the understanding of repercurrsion of obesity on hADSC of obese Indians.

HYPOTHESIS



PLAN OF WORK



DISCUSSION

- High serum leptin levels with low adiponectin levels are found in obesity. (Mahadik et al, 2008)
- The population doubling time, calculated from growth curves, was greater in human obese ADSCs compared with nonobese ADSCs.(Perez et al, 2015)
- Obesity reduces the differentiation capacity (stemness) of the adipose ASCs . (Onate et al. 2013 bio med, Roldan et al. 2011).
- Adipocyte dysfunction makes an important contribution to metabolic disease Obese subjects showed downregulation of stemness and upregulation of adipogenic genes with respect to control .(Onate et al, 2013).
- IKK- links inflammation to obesity-induced insulin resistance. (Perez et al, 2015)

CONCLUSION

Insulin signaling is hamper in ohADSC with concomitant elevation in inflammatory mediators suggesting link of inflammation and obesity mediated insulin resistance.

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RESULTS

TRILINEAGE DIFFERENTIATION

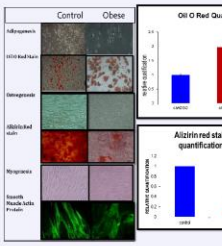


Fig1a. chADSC and ohADSC were differentiated into adipocytes, osteocytes and myocytes. Adipogensis, was carried out and quantified by Oil Red O Staining, n, chADSC=3,ohADSC=4. Osteogenesis was carried out and quantified by Alizirin Red staining, n, chADSC=4,ohADSC=4. , p value*<0.05,**<0.01. Data is represented as +SEM.

iPHENOTYPING

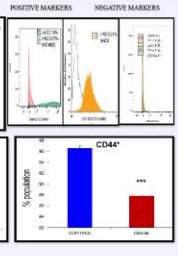


Fig1b. hADSC were characterized through flow cytometry for +&-CD cell surface markers. chADSC =4,ohADSC=4, , p value*<0.05,**<0.01. Data is represented as ±SEM.

COMPARATIVE GENE EXPRESSION PROFILE

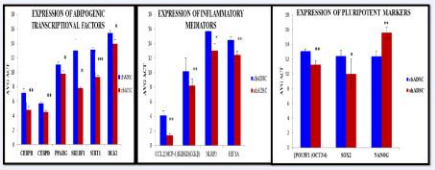


Fig4a: Adipogenic transcriptional factors gene expression , Fig4b, Inflammatory Mediators Fig 4c.Pluripotent Markers chADSC=4,ohADSC=4p value*<0.05,**<0.01, ***<0.0001. Data is represented as ±SEM.

ADIPOKINE LEVELS

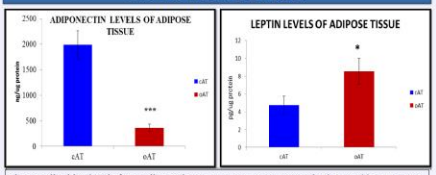


Fig. 5. Adipokine levels from adipose tissue, n, cAT=8,oAT=8, p value*<0.05,**<0.01. Data is represented as ±SEM

GROWTH CURVE OF hADSCs

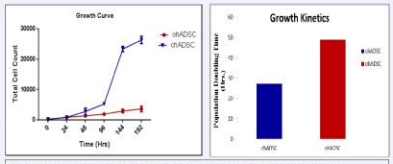


Fig.2. Growth curve experiment was done to evaluate the growth pattern of chADSC and ohADSC. N,chADSC=3, ohADSC=6,**- p value,0.01

INSULIN SIGNALING PROTEIN EXPRESSION

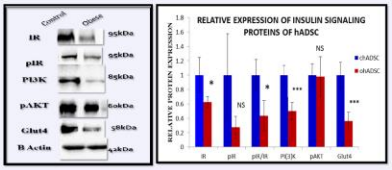


Fig 3. Insulin signaling cascade in hADSC was studied by western blotting. N,C=4,O=4(IR), C=4,O=5(PI3K), C=3,O=3(pIR), pAKT (c=4,O=3, n,.C=3,O=3(pIR), Glut4(C=4,O=3), *- pvalue<0.05, **- pvalue<0.01, ±SEM

LIPOGENIC GENE EXPRESSION

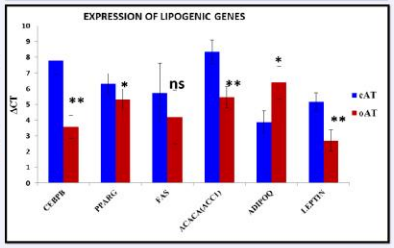


Fig.6. Expression profile of lipogenic genes from adipose tissue, n, cAT=8,oAT=8, p value*<0.05,**<0.01. Data is represented as ±SEM

Resistin alters human adipose derived stem cells through insulin resistance

Komal Rawal[1], Kishan Purohit[1] and Sarita Gupta[1]

[1]Department of Biochemistry, Faculty of Science, The M. S. University of Baroda.

Vadodara, Gujarat, 390021, India.

ABSTRACT

Background: Obesity mediated metabolic disorders like diabetes, cardiovascular diseases, NAFLD are most prevalent globally. Developing countries like India have plethora of obese subjects and associated clinical manifestation. Imbalance in energy homeostasis remodels adipose tissue which polarizes macrophages that enhance secretion of pro-inflammatory adipokines like TNFα, resistin, leptin with reduced adiponectin and IL-10 levels. Moreover, resistin profoundly increases obesity, mitigates lipid metabolism and is directly associated with peripheral insulin resistance. It has been reported that resistin induces insulin resistance in human adipocytes but its effects on human adipose derived stem cells (hADSCs) is sparsely explored. Therefore, the present study was designed to unravel the role of resistin on stemness and insulin sensitivity of hADSCs.

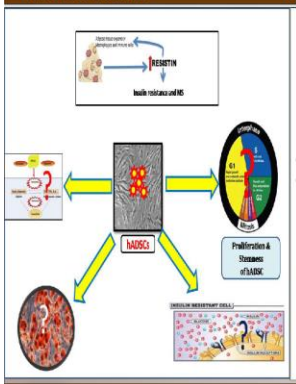
Methods: Healthy subject's ADSC were isolated, immune-characterized were treated with 50ng/ml resistin for 48 hours. Glucose uptake, insulin signaling and expressions of pluripotent and inflammatory markers were observed. Effects of resistin on proliferation of hADSCs were studied by MTT assay and cell cycle analysis. hADSCs were explored for their adipogenic potentials in presence of resistin. **Results:** Western blot analysis and insulin mediated 2NBDG glucose uptake revealed that resistin induces insulin resistance in hADSCs by downregulating insulin signaling and insulin mediated glucose uptake. Gene expressions of key inflammatory markers *MCP-1, IKBKB and NLRP3* were found to be significantly elevated in resistin treated hADSC. Expressions of pluripotent markers were altered on treatment of resistin. Moreover, resistin restricted growth of hADSC by cell cycle alteration and cause enhanced adipogenesis compared to control cells.

Discussion and Conclusion: This is the first study which highlights that resistin, a key pro-inflammatory adipokine causes insulin resistance in hADSC. Hence, resistin could be one of the potential target candidates for treating metabolic diseases.

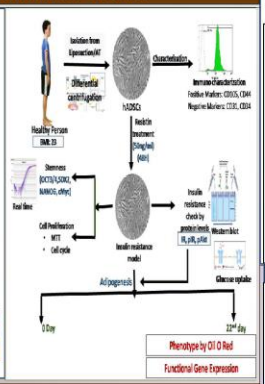
INTRODUCTION

- Adipose tissue is an endocrine organ which secrets several adipokines like leptin, adiponectin, resistin, visfatin, etc. (Indulekha et al, 2015).
- Resistin is predominantly secreted by adipocytes in mice and macrophages in humans. (Park et al, 2013)
- It elevates obesity and is associated with several metabolic alterations leading to diabetes and cardiovascular diseases (Ikeda et al, 2013).
- TNFα inhibits adipogenesis whereas resistin increases adipogenesis in 3T3L1 cells and mice (Hammarstedt et al, 2007).
- Resistin was found to be elevated in metabolically obese Indians and metabolically healthy obese compared to TNFα levels in both the groups of Indians (Indulekha et al, 2015).
- Resistin alters metabolism of human mature adipocytes.
- Elevated serum and gene expressions of resistin in obese Indians, post-menopausal Indian women were in concordance with insulin resistance (Sadashiv et al, 2012).

HYPOTHESIS



PLAN OF WORK



DISCUSSION

- 50ng/ml of resistin causes insulin resistance in mature adipocytes by activating IKKβ and JNK pathways (Park et al, 2013).
- Resistin inhibits lipid metabolism and hampers maturation of adipocytes from 3T3-L1 cells.
- Resistin knock down mice were protected from hyperlipidemia and obesity (Ikeda et al, 2013)
- There was a direct correlation between resistin gene expression, serum resistin and insulin resistance in post menopausal women in India.(Sadashiv et al, 2012).
- There is an association of RETN -420C/G polymorphism with T2D risk, FBG,BMI and total cholesterol (Rathwa et al, 2018).

CONCLUSION

- This is the first report as per our knowledge which states that proinflammatory adipokine **resistin** alters stemness and metbolism of hADSC derived adipocytes through insulin resistance.
- This could be a potential therapeutic target for amelioration of obesity mediated metabolic alterations.

RESULTS

Figure 1. Immunophenotyping of hADSC

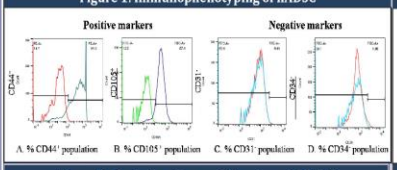


Figure 2. Resistin alters pluripotent markers in hADSC

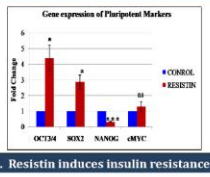


Figure 3. Resistin alters proliferation of hADSC

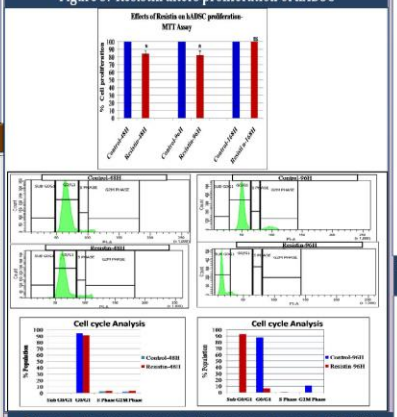


Figure 4. Resistin activates inflammatory markers in hADSC

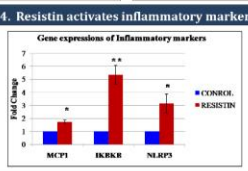


Figure 5. Resistin induces insulin resistance in hADSC

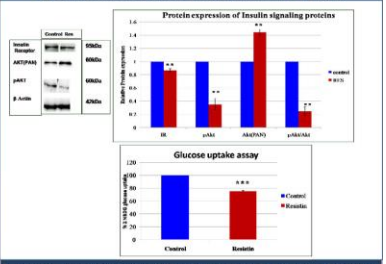
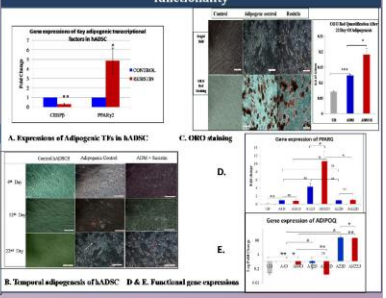


Figure 6. Resistin aggravates adipogenesis and alters adipocyte functionality



All the experiments were performed in triplicates. n=3, p Value*<0.05, **0.01, *<0.0001, ns- non-significant**

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"8th Annual Meeting of Indian Academy of Biomedical Sciences and Conference on Deliberation on Translation of Basic Scientific Insights into Affordable Healthcare Products". CSIR - NIIST, Thiruvananthapuram, Kerala, India." 25th To 27th February, 2019.

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Publications

1. Komal Rawal, Tushar Patel, Kishan Purohit, Kashish Israni, Vyakhaya Kataria, Hiren Bhatt and Sarita Gupta, Influence of obese phenotype on metabolic profile, inflammatory mediators and stemness of hADSC and adipose tissue in Indians. Clinical Nutrition- Under Revision.

2. Tushar P. Patel, Komal Rawal, Sanket Soni, Sarita Gupta. Swertiamarin ameliorates oleic acid induced lipid accumulation and oxidative stress by attenuating gluconeogenesis and lipogenesis in hepatic steatosis, Biomedicine & Pharmacotherapy 83 (2016) 785–791.

3. Tushar P. Patel, Komal Rawal, Ashim, K. Bagchi, Gauri Akolkar, Nathalia Bernardes, Danielle da Silva Dias, Sarita Gupta. Insulin resistance: an additional risk factor in the pathogenesis of cardiovascular disease in type 2 diabetes. Heart Failure Reviews, 2015, 20: 633-754.

Awards

1.Travel Award: M.S. University of Baroda, 2019

2.International Travel Grant Award sanctioned and availed by DST-SERB but also DBT-CTEP and CSIR sanctioned my international Travel Grant Award.

3.CSIR-SRF. File No. 09/114/0204/2016-EMR-I. date of Implementation: 2nd April, 2017.

4. Prof. U.M. Rawal Memorial award for Best Poster presentation,Gujarat Science Congress. 2015.

Poster Presentations and Conferences

1. 8th Annual Meeting of Indian Academy of Biomedical Sciences and Conference on Deliberation on Translation of Basic Scientific Insights into Affordable Healthcare Products". CSIR -NIIST, Thiruvananthapuram, Kerala, India." 25th To 27th February, 2019.

2. Poster Presentation "Resistin alters Human Adipose Derived Stem Cells through insulin resistance".

3.Poster Presentation "Human Adipose Derived Stem Cells plasticity in adipose tissue of obese Indians" in Keystone Symposia conference "Organ Crosstalk in Obesity and NAFLD", Keystone, Colorado, USA. 21st to 25th January, 2018.

- Poster Presentation entitled "Metabolic assessment of adipose tissue from control and obese human subjects in Symposium "Omics to Structural Basis of Diseases", M.S.University of Baroda, 2016.
- Poster Presentation for "Mechanism of Swertiamarin in oleic acid induced model of hepatic steatosis: In vitro" .XXIX Gujarat Science 2015, Science city, Ahmadabad, Gujarat..
- National Symposium on "Emerging Trends in Biochemical Sciences", 2014, M.S.University of Baroda, 2016.