# COMPARATIVE STUDY OF ADIPOSE TISSUE AND HADSC IN CONTROL AND OBESE INDIAN POPULATION

## CHAPTER 4

## 4. Comparative study of adipose tissue and hADSC in control and obese Indian population

### 4.1. INTRODUCTION

India is a developing country having upto 50% of obese urban population and ranks second for obese children worldwide (Behl & Misra, 2017; Collaborators, 2017). Obesity is causal factor of metabolic and CVD. Increased glycemic intolerance, fat to muscle ratio and cardiovascular diseases are the prime factors attributing metabolic syndrome in Indians even at lower BMI ($Kg/m^2$). Prevention and Management of Obesity and Metabolic Syndrome group has re-categorized Indians as overweight with BMI between 23 and 24.9 and obese with BMI >25(Aziz et al., 2014; Anoop Misra et al., 2009). Increased leptin, resistin, TNFα, CRP and reduced adiponectin levels are associated with obesity in Indian population (Mahadik et al., 2008; Shetty et al., 2004).

Lack of energy expenditure increases fat storage in the adipose tissue through hyperplasia and hypertrophy. Adipose tissue is a dynamic multicellular tissue having an endocrine function as it secrets several growth factors, angiogenic factors, hormones and cytokines (Coelho et al., 2013). It comprises highest number of mature adipocytes, 5% of macrophages, pre-adipocytes and ADSC (1-10%) (B.-S. Kim et al., 2017). Elevated levels of circulating FFA and leptin provoke M1 macrophage class switched from M2, causing inflammation by activation of inflammasome complex through IKKβ, NFκβ, and NLRP3 (Castoldi et al., 2016; Reilly & Saltiel, 2017; Serbulea et al., 2018). Inflammation induces insulin resistance by mitigating insulin signaling through downregulation of insulin signaling proteins IR, IRS, PI3K and AKT. These alterations in the signaling proteins inhibit translocation of Glut4 to the plasma membrane resulting into insulin mediated glucose uptake inhibition in adipocytes of obese humans (Carlson et al., 2003; Kusminski et al., 2007).

Very few studies have been carried out to understand the consequences of obesity on metabolic profile of adipose tissue and ADSC in obese population. Obesity is known to cause insulin resistance, however there are reports which state that obese individuals are insulin sensitive (G. Reaven, 2005; G. M. Reaven, 2004). Microarray analysis of the adipose tissue from obese insulin sensitive Canadian (BMI $\underline{<}$50) population depicted no significant difference in insulin signaling transcripts compared to control subjects (BMI 1$\underline{+}$25.3), however insulin signaling was elevated in adipose tissue of obese insulin resistant subjects (BMI$\underline{\leq}$65). But inflammatory pathways were

activated in both insulin sensitive and insulin resistant obese subjects (MacLaren et al., 2008). In contrast, Indians have been delineated to be metabolically obese due to high truncal fat and visceral adiposity, the major culprits associated with insulin resistance and metabolic syndrome (Indulekha et al., 2015), thus, obesity in India is considered merely at BMI of 25 and above, unlike Caucasian population where it is defined above 30 BMI (Hunma et al., 2016).

Abundance of adipose tissue, profound differentiation, wound healing, angiogenic and migration properties of ADSC with ease of isolation have bestow them chief cells in the field of regenerative medicine. They have been implicated in clinical trials for treatment of diabetes, wound healing, skin grafting and renal assaults.(Jeong, 2008). However, there are very few reports on the deleterious effects of obesity on functions of ADSC. ADSC isolated from obese subjects are unable to differentiate as their stemness and differentiation potentials are mitigated (Oñate et al., 2013; Roldan et al., 2011), also they fail to halt progression of autoimmune encephalitis in animals, (Strong et al., 2016). Inflammatory markers like TNFα, MCP-1, NLRP3 are elevated in ADSC isolated from obese subjects (A Misra et al., 2018; Pérez et al., 2017).

Progression of insulin resistance and metabolic syndrome in Indians with lower BMI is at pace in today's modern era (A Misra et al., 2018), despite of the facts, key findings are yet elusive pertaining to the efficacy of hADSC from obese Indians. The present study demonstrates repercussion of obesity on functionality of adipose tissue and potentials of hADSC isolated from obese subjects (ohADSC) compared to those of control Indian subjects (chADSC).

## 4.2 PLAN OF WORK

This chapter deals with clinical studies with respect to repercussions of obesity on adipose tissue and hADSC of Indian subjects.

**Selection of subjects:**

With written consent surgically excised AT/ lipoaspirates were obtained from 8 healthy control subjects (cAT, BMI $\leq$23) and 8 healthy non-diabetic obese subjects (oAT, BMI $\geq$25) who underwent cosmetic surgery at Nishtha Clinic, Vadodara, India (Table 4.1). The inclusion criteria for recruitment of the subjects under the study were both male and female with age ranging from 19 years to 60 years without any metabolic disease. hADSC were isolated from AT/lipoaspirate of 4 healthy control

(chADSC) and 4 healthy obese subjects (ohADSC) in the current study (Table 4.2). Human ethical approval was obtained from Banker's Heart Institute- Central Drugs Standard Control Organisation (CDSCO) approved institute (ECR/214/Inst/Guj/2013/RR-16) and Institutional ethical committee (IECHR 2016-6), Vadodara, India.

hADSC were isolated from the samples by differential centrifugation method and through sequential passaging. Immuno-characterization was carried out through flow cytometry for purity assessment of hADSC. Comparative assessments were made between chADSC and obese subjects ohADSC. Proliferation of cells of both the groups were assessed by growth kinetic experiment. Stemness profile of hADSC of both groups were monitored by studying pluripotent genes and ATF in undifferentiated hADSC. Then cells of both the groups were subjected to adipogenesis for 22D. Also, RUNX2 was observed in hADSC followed by osteocyte differentiation for 14D. Also expressions of MYF-5 were observed in hADSC of both the groups.

Further, adipose tissues were also studied for their metabolic profile. Adipose tissue of control subjects (cAT) and adipose tissue of obese subjects (oAT) were examined for lipogenic genes and levels of stored adipokines adiponectin, leptin and levels by adipokine specific ELISA. Gene expressions of inflammatory mediators were observed both in hADSC and adipose tissues of both group of population. Lastly again both the groups' adipose tissues and hADSC were scrutinized for expressions of insulin signaling proteins. The whole plan of work is depicted in Figure 4.1.

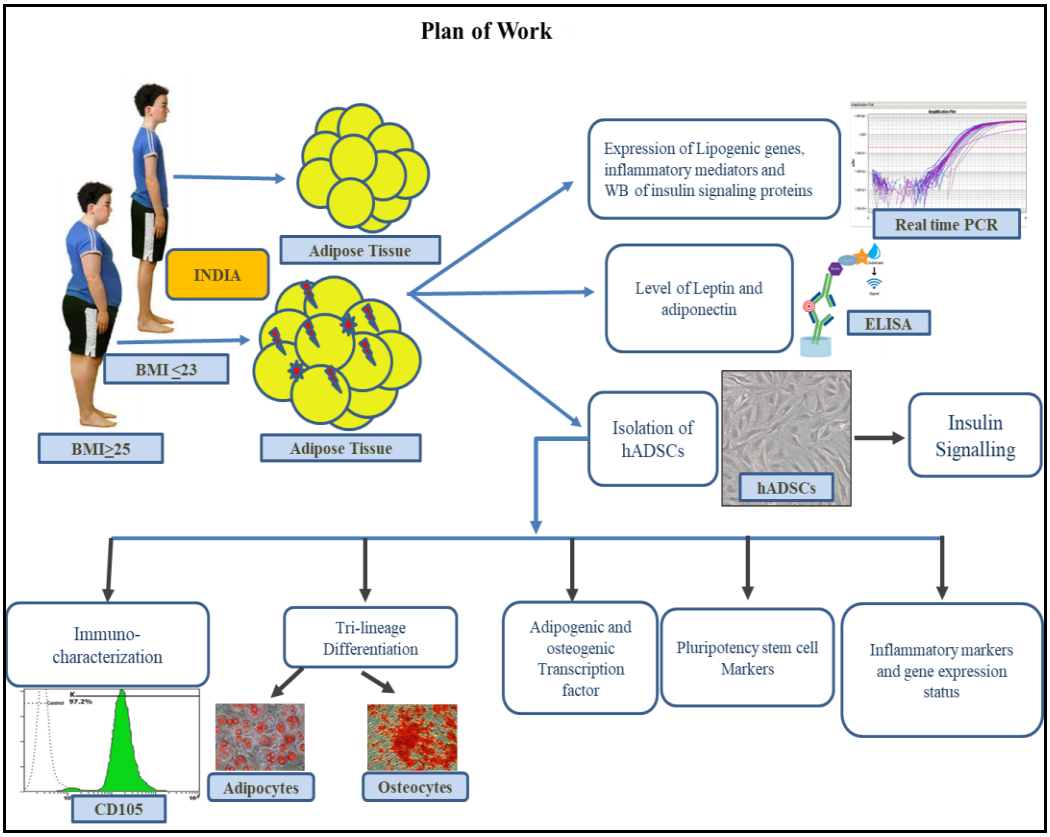


**Figure 4. 1: Plan of work**

## TABLE 4. 1

**Table 4.1. Details of the subjects from whom AT/ lipoaspirates were obtained for the study**

| Sr. No. | Age | Sex | BMI ($Kg/m^2$) | Source of tissue |
|---|---|---|---|---|
| **Control subjects (cAT) (18 to 23 BMI, (average BMI 22.3±0.25)** | | | | |
| 1 | 39 | F | 22.9 | Breast |
| 2 | 40 | M | 23 | Abdomen |
| 3 | 49 | F | 21 | Breast |
| 4 | 48 | F | 22.3 | Breast |
| 5 | 18 | F | 22.4 | Breast |
| 6 | 33 | F | 22.7 | Abdomen |
| 7 | 29 | M | 21.6 | Male breast |
| 8 | 40 | F | 23 | Axillary breast portion |
| **Obese subjects (oAT) (BMI >25 ) (average BMI 31.85+1.65)** | | | | |
| 1 | 24 | F | 26.4 | Breast |
| 2 | 19 | M | 31.3 | Male breast |
| 3 | 39 | F | 33.3 | Abdomen |
| 4 | 40 | F | 41.6 | Abdomen |
| 5 | 41 | F | 28.7 | Breast |
| 6 | 35 | M | 30.8 | Breast |
| 7 | 30 | F | 28.7 | Breast |
| 8 | 48 | F | 34 | Abdomen |

**TABLE 4. 2**

| **Table 4.2. Details of the subjects from whom hADSC were isolated** | | | | |
|---|---|---|---|---|
| **Sr. No.** | **Age** | **Sex** | **BMI** | **Source of tissue** |
| **ControlSubjects (chADSC) (Avg BMI: 22.75± 0.18)** | | | | |
| 1 | 48 | F | 22.23 | Breast |
| 2 | 26 | F | 23 | Thigh |
| 3 | 38 | F | 22.8 | Breast |
| 4 | 25 | M | 23 | Breast |
| **Obese Subjects (ohADSC) (Avg BMI: 33.4± 4.4) (Avg BMI : 33.4±4.4)** | | | | |
| 1 | 24 | F | 26.4 | Breast |
| 2 | 60 | F | 46.3 | Abdomen |
| 3 | 35 | F | 31.6 | Breast |
| 4 | 21 | M | 29.3 | Abdomen |

## 4.3 RESULTS

### 4.3.1 hADSC isolation and immunophenotyping

hADSC isolated from adipose tissue/ lipoaspirate of all the subjects under the study were examined for their purity and stemness at P#3 by flow cytometry. All the cells were positive for mesenchymal stem cell markers $CD44^{+}$ and $CD105^{+}$ and were negative for hematopoetic cell markers $CD31^{-}$ and $CD34^{-}$ . Thus, the results ascribe that the cells isolated were pure ADSC. (Figure.4.2 A-D).

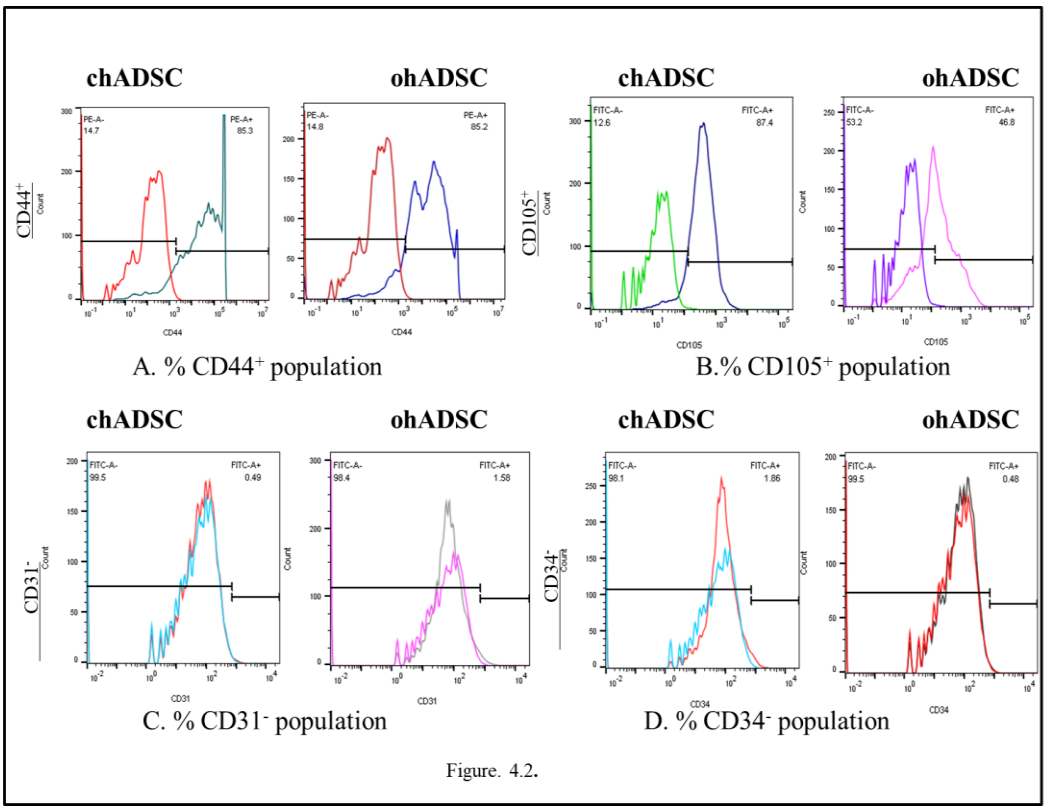


**Figure 4. 2:** hADSC isolation and immunophenotyping: hADSC from both the groups at P#3 were characterized for surface markers through flow cytometry A. Expression of $CD44^{+}$ B. Expression of $CD105^{+}$ C. Expression of $CD31^{-}$ D. Expression of $CD34^{-}$. n=4, chADSC and n=4, ohADSC. Percent positive population is shown in the graph.

### 4.3.2. Obesity restricts growth of hADSC

Obesity refrain hADSC to grow and proliferate. Thus, to observe the effects of obesity on proliferation of hADSC, growth curve experiment was performed to elucidate the doubling time of hADSC and their kinetics.

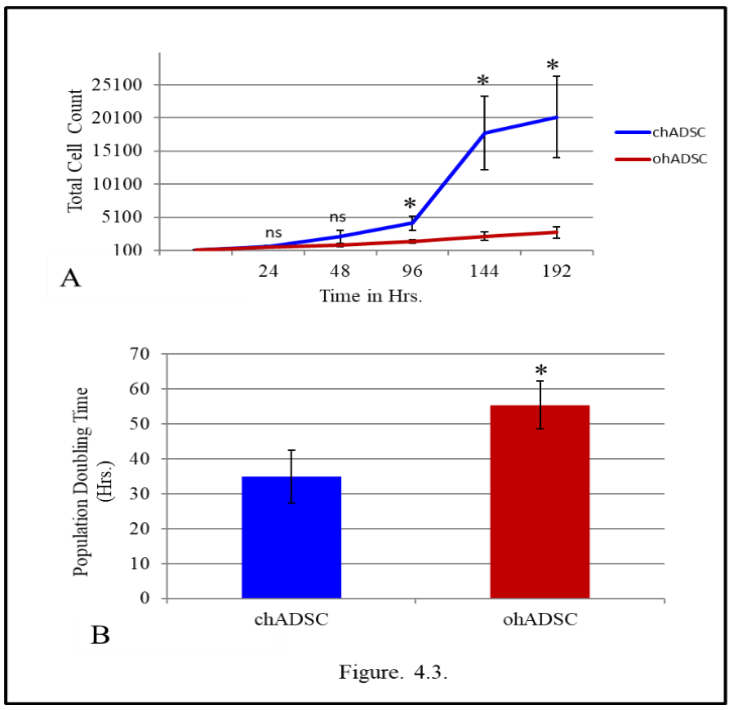


**Figure 4. 3:** Obesity restricts growth of hADSC: A. Growth curve of the hADSC at P#3 of chADSC and ohADSC were calculated and plotted. B. PDT was calculated for both chADSC and ohADSC. n=4, chADSC and n=4, ohADSC. Significance is expressed as p value* <0.05. Data is represented as Mean ± S.E.M.

The growth curve graph deduced that chADSC showed persistent increase in the number of cells with time however, ohADSC were found to be significantly non-proliferative during growth curve experiment (Figure. 4.3.A). The PDT calculated for ADSC for both the group of cells depicted a wide difference in their doubling time which was found to be 34.9H and 55.4H for chADSC and ohADSC respectively (Figure. 4.3.B.).

### 4.3.3 Obesity is detrimental to stemness of hADSC in Indians

hADSC from obese Caucasians have diminished stemness at higher BMI. To elucidate repercussions of obesity on metabolic profile of hADSC in Indian subjects, stemness of hADSC of both the groups were observed both at cellular and molecular levels.

### 4.3.3.1 Obesity alters pluripotency of hADSC

To check the pluripotent markers in hADSC under current study, expression of key pluripotent transcriptional factors OCT4, SOX2 AND NANOG were evaluated from both chADSC and ohADSC. Gene expression studies showed that

the expression levels of OCT4 and SOX2 were found to be upregulated in ohADSC, whereas there was reduction in the expression level of NANOG (Figure. 4.4.A).

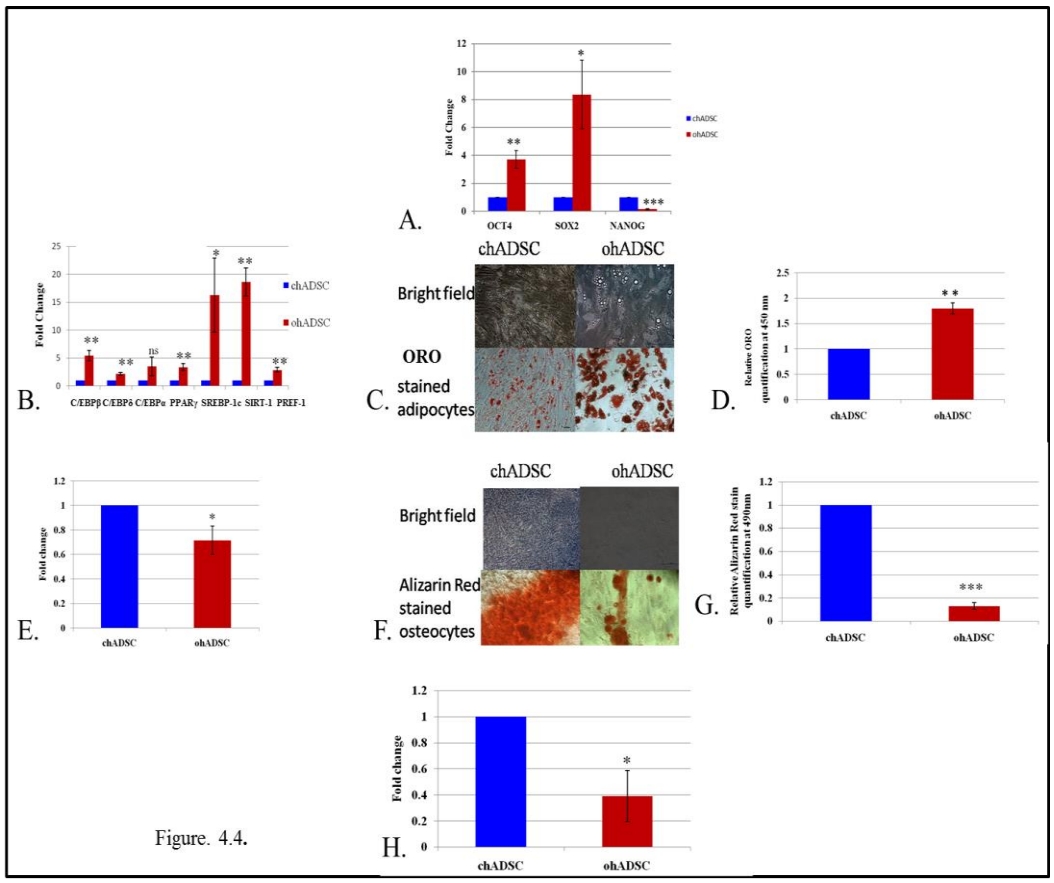


Figure. 4.4.

**Figure 4. 4: Obesity is detrimental to stemness of hADSC in Indians: A comparative analysis governing stemness of chADSC and ohADSC were performed.** A. Gene expressions of pluripotent stem cell markers. B. Gene expression of ATF. C. ORO stained adipocytes after differentiation: Bright field image of adipocytes on 22$^{nd}$D observed under Phase Contrast Inverted Microscope under 20X objective. D. Quantification of ORO stain: The graph of ORO is plotted as relative values of O.D. where control is considered as 1. E. Gene expression of OTF, RUNX2. F. Alizarin Red stained osteocytes after differentiation: Bright field image of osteocytes on 14$^{th}$D observed under Phase Contrast Inverted Microscope under 20X objective. G. Quantification of Alizarin Red stain: The graph is plotted as relative values of O.D. where control is considered as 1 H. Gene Expression of MYF5: n=4, chADSC and n=4, ohADSC for all the experiments. Results are expressed as fold change $\pm$ S.E.M for gene expression studies with β Actin as internal control. Significance is expressed as p value *<0.05, **<0.01, ***<0.0001, ns- non-significant.

#### 4.3.3.2 hADSC of Indian obese are pre-committed to adipogenesis

hADSC of both the groups were analysed for adipogenic and osteogenic differentiation potentials. Expression levels of early transcriptional factors C/EBPβ and C/EBPδ were significantly upregulated in ohADSC. The main regulator of adipocyte differentiation is PPARγ which was found to be significantly high along with C/EBPα. Moreover, the transcriptional factor SREBP-1c that regulates PPARγ induction and lipid synthesis in adipocyte was also found to be upregulated in ohADSC compared to chADSC. Moreover, to maintain the stemness and to inhibit the adipogenic induction in undifferentiated hADSC, transcription factors that inhibit adipocyte differentiation SIRT-1 and PREF-1, were upregulated in ohADSC compared to chADSC (Figure.4.4. B).

As observed through gene expression study, upregulation of adipogenic genes in undifferentiated hADSC destines their fate into adipocyte formation. hADSC of both the groups were differentiated into adipocytes by inducing them with adipogenic cocktail which changed the morphology of the cells from fibroblastic to oval or round shape. Small oil droplets were observed after 3$^{rd}$D of adipogenic induction in ohADSC whereas, in chADSC the droplet formation started around 5$^{th}$D of the induction. The oil drops in adipocytes of ohADSC were huge in size and contained more lipid content when compared to those of chADSC. Subsequently at 22$^{nd}$D, adipocytes containing large lipid droplets were observed. Quantification of ORO stain at 450nm substantiated elevated adipogenesis with hypertrophic adipocytes derived from ohADSC group compared to those of chADSC (Figure. 4.4.C and D).

#### 4.3.3.3 Indian obese have reduced osteogenic potentials of hADSC

It has been observed that obesity hampers osteocyte formation from hADSC of obese subjects. Gene expression study of RUNX2 gene, an early transcriptional factor for osteogenesis was found to be significantly reduced in ohADSC compared to chADSC (Figure. 4.4. E). Further, hADSC of both the groups were subjected to osteocyte differentiation. Calcium formation and deposition was observed phenotypically by granularity in the differentiated cells. ohADSC derived ostoecytes showed very less granules or aggregates. The intracellular and extracellular calcium produced by osteocytes were stained with Alizarin Red (Figure. 4.4. F) stain at 14$^{th}$D of differentiation subsequently followed by quantification at 490nm (Figure. 4.4. G). The

results were similar to the gene expression of RUNX2 which was suppressed in ohADSC, exemplifying loss of stemness in ohADSC.

#### 4.3.3.4 Obesity ablates B-adipose tissue progenitors

B-adipose tissue has higher mitochondrial activity with expenditure of energy. hADSCs have been ascribed to differentiate into brown adipose tissue or the beige cells those derived from white adipocytes. Beige cells are known to be produced from a specific transcriptional factor MYF-5. To elucidate the effects of obesity on MYF-5 progenitors, gene expression study was conducted. ohADSC had significantly reduced expression of MYF-5 gene compared to those of chADSC (Figure. 4.4. H).

### 4.3.4 hADSC and adipose tissue of Indian obese have high inflammatory markers

Positive energy balance in obesity triggers immune system that induces macrophage infiltration which alter lipid metabolism. Gene expression studies were done to elucidate the expressions of inflammatory genes in both the groups of hADSC and adipose tissue in the present study.

The gene expression studies revealed that MCP-1, was found to be upregulated in ohADSC and oAT compared to chADSC and cAT respectively, which denote infiltration of macrophages in adipose tissue. A state of systemic inflammation is established in obesity through NLRP3, an inflammasome complex protein. Expression of this gene was upregulated in ohADSC and oAT compared to those of controls. IKKβ stimulates the activity of inflammasome complex thus, the expression of IKKβ was found to be significantly upregulated in ohADSC. As, there is a direct link between inflammation and hypoxia, HIF1A gene was examined which was significantly elevated in ohADSC and oAT compared to those of controls (Figure. 4.5.A and B.).

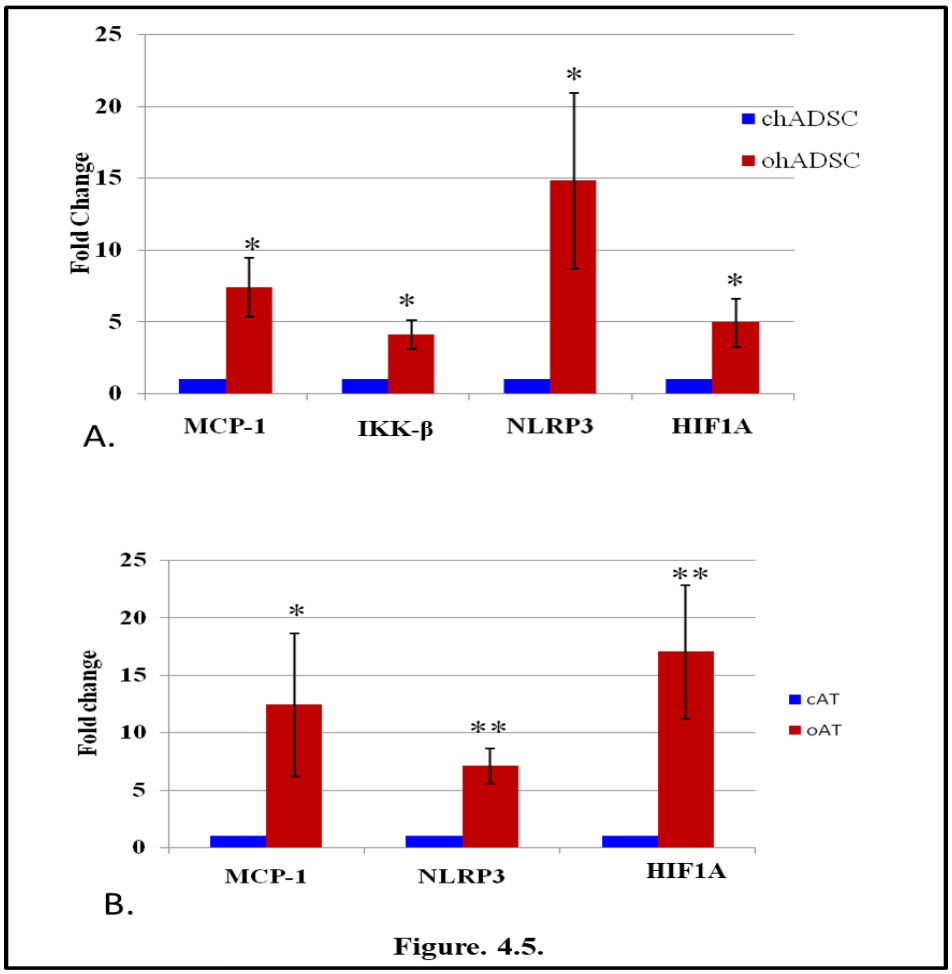


**Figure 4. 5:** hADSC and adipose tissue of Indian obese have high inflammatory markers: A. Gene expressions of inflammatory mediators in chADSC and ohADSC at P#3. n=4, chADSC and n=4, ohADSC. B. Gene expressions of inflammatory mediators in cAT and oAT cAT, n=8 and oAT, n=8. Results are expressed as fold change ± S.E.M for gene expression studies with β Actin as internal control. Significance is expressed as p value* <0.05, **<0.01, ***<0.0001.

### 4.3.5 Adipose tissues of Indian obese have high lipogenesity

Adipose tissue has versatile functions; among which endocrine is one of the prime function. During adipogenesis adipose tissue expands due to hypertrophy and hyperplasia. In the present study lipogenic profile of adipose tissue from both the groups was evaluated. Real time PCR of lipogenic genes was performed from cAT and oAT. C/EBPβ and PPARγ2 were found to be elevated in oAT, however there was reduction in C/EBPα expression. Further, lipogenic genes like FAS and ACC-1 were also upregulated in oAT. Obese subjects had reduced expression of adiponectin with higher expressions of leptin and resistin (Figure. 4.6.A.). The stored levels of adipokines were in compliance with their gene expressions as adiponectin levels were

reduced with elevated leptin and resistin levels in oAT (Figure. 4.6.B-D), thus causing alterations in endocrine function of adipose tissue.

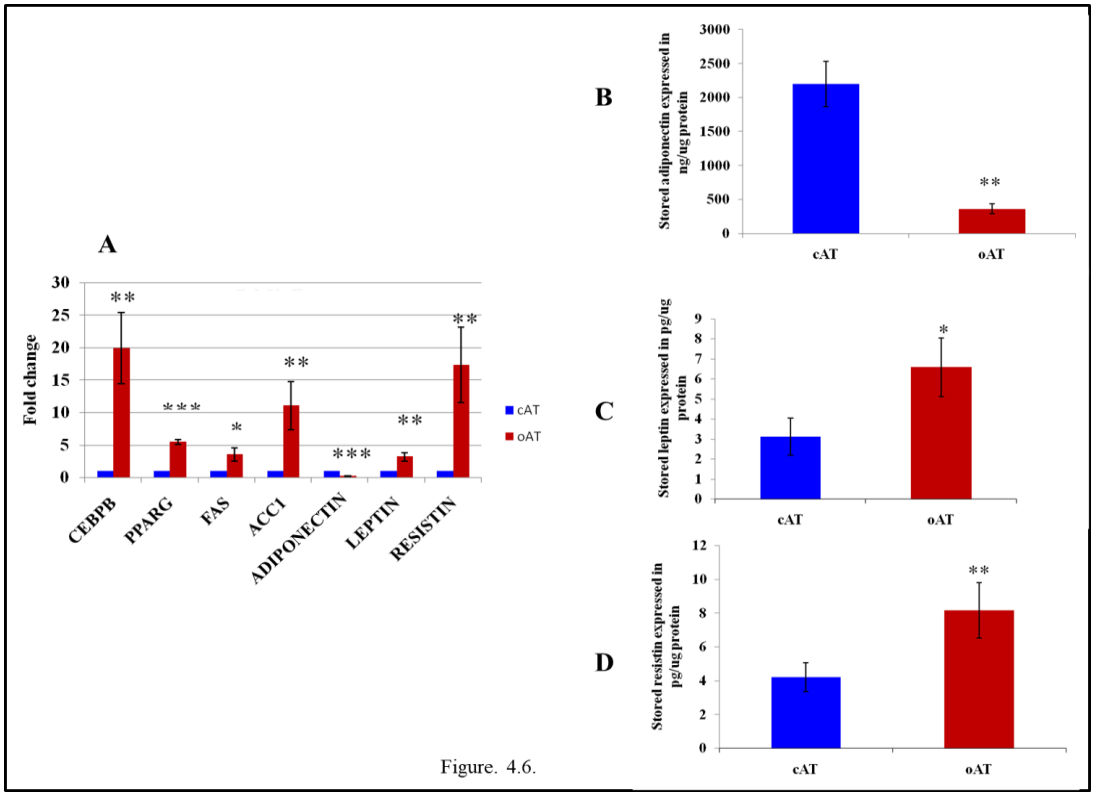


**Figure 4. 6:** Adipose tissue of Indian obese have high lipogenesity: A. Gene expression of lipogenic genes of cAT and oAT. Results are expressed as Fold change± S.E.M. with β Actin as internal control. Stored adipokine levels from cAT and oAT were checked by adipokine specific ELISA. B. Adiponectin levels were quantified and expressed in ng/ug protein. C. Leptin levels were quantified and expressed in pg/ug protein. D. Resistin levels were quantified and expressed in pg/ug protein. cAT, n=8, oAT, n=8. Significance is expressed as p value* <0.05, **<0.01, ***<0.0001. Data is represented as Mean ± S.E.M.

### 4.3.6 Obesity causes insulin resistance in hADSC but provokes insulin signaling in adipose tissue of Indians

Elevated inflammation in obesity alters insulin signaling of adipose tissue causing dyslipidemia. hADSC have not been examined for their insulin signaling function in any of the population. Western blot was performed to scrutinize expression of insulin signaling proteins from chADSC and ohADSC.

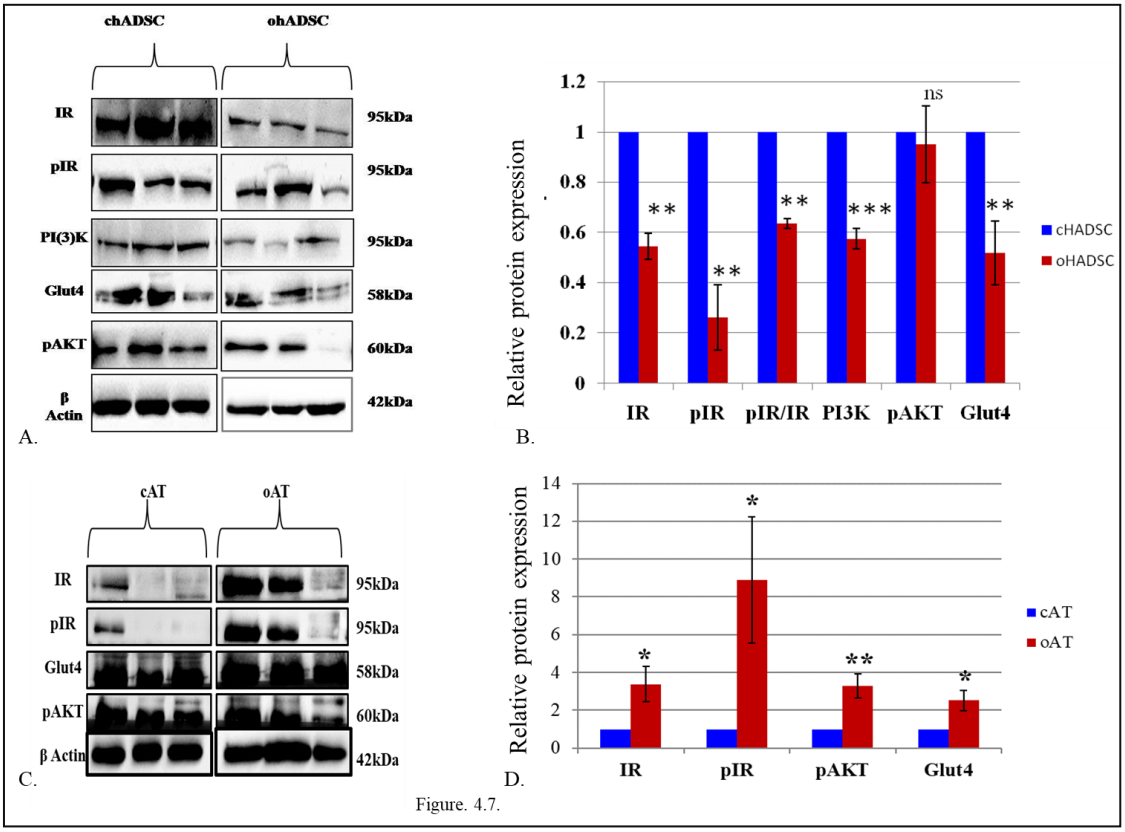


Figure. 4.7.

**Figure 4. 7:** Obesity causes insulin resistance in hADSC but provokes insulin signaling in adipose tissue of Indians: A. Protein samples of chADSC and ohADSC at P#3 were run in two different gels and blots are representative of 3 different subjects' chADSC and ohADSC respectively for insulin signaling proteins. B. Densitometric analysis of western blot of hADSC of both the groups represented as relative protein expression. n=4 for chADSC, n=4 for ohADSC. C. Protein samples of cAT and oAT were run in two different gels and blots are representative of 3 different subjects' cAT and oAT respectively for insulin signaling proteins. D. Densitometric analysis. cAT, n=8, oAT, n=8. Significance is expressed as p value * <0.05, **<0.01, ***<0.0001, ns-non significant. Data is represented as Mean ± S.E.M.

Western blot analysis revealed that the protein expression of key insulin signaling proteins IR, pIR, pIR/IR (activated IR), PI3K and Glut4 were significantly downregulated with no significant difference in expression of pAKT in ohADSC compared to those of chADSC, thus a state of insulin resistance was observed in ohADSC of Indians (Figure 4.7. A and B). Similarly, insulin signaling protein expressions were observed in cAT and oAT which was scrutinized for first time in Indian population. Western blot analysis revealed that key insulin signaling proteins IR, pIR, pAKT along with Glut4 expressions were upregulated significantly in oAT when compared to those of cAT. (Figure. 4.7.C. and D.).

## 4.4. DISCUSSION

Excess visceral fat, reduced primary fat storage compartment and leaky tertiary adipose compartments have delineated Indians to be metabolically obese and vulnerable to metabolic disorders at very low BMI compared to Caucasian population (Indulekha et al., 2015; Sniderman et al., 2007). hADSC in India, have gained fame in the field of regenerative medicine as they have been transplanted for treating problems like diabetes and renal clinical manifestations (Dave et al., 2018; Vanikar, Trivedi, Gopal, et al., 2014). hADSC from obese Caucasian population have been described to have diminished stemness, metabolism and regenerative properties (Pérez et al., 2015; Roldan et al., 2011; Serena et al., 2016). Approaches towards scrutinizing metabolic profiles of hADSC and adipose tissue of Indian obese are at underpinning levels. Therefore, we made an attempt to scrutinize the influence of obesity on metabolic profile of hADSC and adipose tissue of Indian subjects.

In the present study growth of ohADSC was found to be restricted which comply with that of hADSC derived from obese Caucasian population (Pérez et al., 2015). Pluripotent markers showed reduced expression of NANOG with upregulation of OCT4 and SOX2 in ohADSC compared to chADSC. Perez et al., had found reduced expression of NANOG with elevated levels of SOX2 in hADSC of obese subjects at 20 # (Pérez et al., 2015). hADSC isolated from S-adipose tissue of obese subjects had increased expression of OCT4 with reduced differentiation markers which substantiated the undifferentiated state of hADSC in Caucasian population (Petrangeli et al., 2016). PPARγ2 and C/EBPα, important ATF were upregulated in hADSC of obese Caucasians (Baptista et al., 2009; Roldan et al., 2011). All the ATF were found to be upregulated in ohADSC which emphasizes these cells to be pre-committed to adipogenesis compared to chADSC. Also, hypertrophic adipocytes were derived from ohADSC as evidenced by ORO staining in ohADSC. Further, scrutiny of osteogenic potentials revealed that expression of RUNX2 was downregulated in ohADSC along with reduced osteocyte formation as evidenced by Alizarin Red staining. Obese Caucasian population have been reported to have similar osteogenic profile of hADSC with a very large BMI (Baptista et al., 2009; Roldan et al., 2011).

ADSC have been described to have pool of MYF-5 progenitors which can predominantly differentiate into brown adipocytes and myocytes (A. Park et al., 2014). Assault of obesity on gene expression of MYF-5 in hADSC of any human population

has been studied for the first time in the present study. Gene expression study revealed that ohADSC has reduced expression of MYF-5 compared to those of chADSC which indicates reduced energy expenditure compared to that of chADSC.

Interaction between obesity and inflammation obscure the metabolic profile of hADSC. In silico and gene expression studies have shown upregulation of inflammatory genes in hADSC of obese subjects (Esser et al., 2013; Pérez et al., 2015; Serena et al., 2016). Expressions of all key inflammatory mediators were upregulated both in ohADSC and oAT compared to chADSC and cAT respectively, thus exemplifying the pivotal association of inflammation with obesity in Indians.

Metabolic profile of oAT in the current study was found to be highly lipogenic as expressions of lipogenic genes and stored levels of adipokines like leptin and resistin were upregulated with concomitant reduction in both gene expression and stored levels of adiponectin. In Caucasian population, there was no difference between expression of ATF from adipose tissue of control and insulin sensitive obese population(MacLaren et al., 2008). However, few have reported that lipogenic genes were upregulated in adipose tissue of obese Caucasians compared to their lean counterparts (Berndt et al., 2007; Giusti et al., 2004; Ruschke et al., 2010).

To further investigate the effects of obesity on insulin signaling, hADSC of both the groups were compared and key insulin signaling proteins IR, pIR, pAKT and Glut4 were significantly downregulated in ohADSC compared to those of chADSC, thus ohADSC were found to be insulin resistant. This is the first population study which states that obesity mitigates insulin signaling of hADSC and renders insulin resistance in these cells. However, oAT had elevated levels of insulin signaling proteins compared to those of cAT, which might have triggered adipogenesis in hADSC of the obese subjects under the study. Moreover, there are no studies on insulin signaling in adipose tissue of obese subjects from India. Microarray of insulin signaling transcripts in adipose tissue did not show any difference in their expressions in control and obese Caucasians but there were differences in gene expressions in V- and S- adipose tissue (MacLaren et al., 2008).

Thus, the current study for the first time, to the best of our knowledge, highlight that self-resilent hADSC become insulin resistant, despite of the subjects being non-diabetic, highlighting their propensity to be susceptible to diabetes and metabolic

syndrome. ohADSC of Indians are pre-committed to adipogenesis and fail to differentiate into osteogenesis. Plausible role of inflammation in abrogation of stemness of ohADSC in Indian population is highlighted here. Thus, the study has delineated ohADSC to be highly compromised with reduced stemness and physiological functions in metabolically obese Indians.

## 4.5 CONCLUSION

The present study dissects the metabolic profile of ADSC and adipose tissue in Indian obese subjects. The results of the present study emphasize the involvement of inflammation in downregulation of insulin signaling that governs the metabolic profile of hADSC of obese Indian population. hADSC of the obese subjects are found to be precarious and alarms the Indian population from onset of metabolic syndrome. Altered adipokines, inflammatory markers and increased insulin signaling in oAT highlight probability of insulin resistance and metabolic disorders in the subjects under the study with progression in obesity. India is diverged in genetic makeup thus; a need has aroused to conduct a cohort study to scrutinize the potentials of the most robust hADSC of Indians with their diverse clinical status. This study also provides the direction for improvising the medications and novel therapies to treat hADSC targeting the intervening signaling molecules that govern obesity mediated alterations and alarm the cautious use of hADSC in regenerative medicine.

## SUMMARY OF CHAPTER-4 :