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Mitochondrial bioenergetics is not impaired in nonobese subjects with type 2 diabetes mellitus

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ABSTRACT

Although mitochondrial dysfunction has been well documented in obese people with type 2 diabetes mellitus, its presence or absence in nonobese subjects with type 2 diabetes mellitus has not been well studied so far. The aim of the present study was to assess the status of mitochondrial oxidative phosphorylation in subcutaneous adipose tissue of nonobese type 2 diabetes mellitus subjects in comparison to control, obese nondiabetic, and obese type 2 diabetes mellitus subjects. Mitochondria were isolated from subcutaneous white adipose tissue obtained from the abdominal region of control, obese nondiabetic, nonobese type 2 diabetes mellitus, and obese type 2 diabetes mellitus subjects. The activities of complex I, I to III, II to III, and IV; transmembrane potential; and inorganic phosphate utilization of mitochondria from different groups were measured. Mitochondrial transmembrane potential, inorganic phosphate utilization, and the activities of respiratory chain complexes were significantly reduced in obese nondiabetic and obese type 2 diabetes mellitus patients compared with those in control subjects. No detectable change in mitochondrial functional parameters was observed in case of nonobese type 2 diabetes mellitus subjects compared with control subjects. Furthermore, a significant difference was noticed in mitochondrial phosphate utilization and activities of respiratory complexes, for example, I, I to III, and II to III, between obese type 2 diabetes mellitus subjects and obese nondiabetic subjects. Obesity modulates mitochondrial dysfunction associated with type 2 diabetes mellitus.

Author contributions: Mrittika Chattopadhyay was responsible for mitochondrial enzyme assays, measurement of transmembrane potential, and updating the literature survey. Ishita GuhaThakurta was responsible for the measurement of mitochondrial inorganic phosphate utilization and all the statistical calculations. Prajna Behera was responsible for the collection of samples and isolation of mitochondria. Kumar Rajeev Ranjan (general surgeon) provided the adipose tissue from control and type 2 diabetes mellitus patients. Manoj Khanna (cosmetic surgeon) provided the adipose tissue from obese nondiabetic subjects and obese type 2 diabetes mellitus subjects. Satinath Mukhopadhyay (endocrinologist) proposed the study and diagnosed and selected the obese and nonobese type 2 diabetes mellitus subjects. Sasanka Chakrabarti (communicating author) designed the experimental protocols, checked and analyzed the data regularly, and wrote the paper.

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1. Introduction

Obesity and type 2 diabetes mellitus are important causes of morbidity and mortality in young and middle-aged people all over the world; and very often, both these conditions coexist in the same subjects. The metabolic dysregulation and complications of obesity and type 2 diabetes mellitus are similar in many respects that include insulin resistance, dyslipidemia, atherosclerosis, hypertension, and cardiovascular and cerebrovascular diseases [1,2]. The most important aspect of metabolic dysregulation in type 2 diabetes mellitus is excess mobilization of fatty acids from adipose tissue; and coupled with impaired oxidation, this results in the storage of triacylglycerol and other lipid moieties in nonadipose tissues like skeletal muscle, liver, heart, and pancreatic β-cells that gives rise to ectopic fat storage syndrome [3-5]. Moreover, excess circulating free fatty acids trigger a chain of metabolic events in liver and also in other peripheral tissues to produce dyslipidemia and impaired glucose utilization [3].

Recently, many studies have indicated several forms of mitochondrial dysfunctions in white adipose tissue in diabetes and obesity both in clinical subjects as well as in experimental models, although it is not clear whether the former is a primary defect or the secondary consequence of the disease process [6-8]. In type 2 diabetes mellitus patients and animal models, downregulation of genes coding for subunits of respiratory complexes, decreased number of mitochondria with diminished mitochondrial DNA (mt DNA) content, and decreased mitochondrial respiration have been reported [9-12]. On the other hand, several forms of mitochondrial dysfunction such as reduction of mitochondrial size and mt DNA content and lower mitochondrial respiration rates in the adipose tissue of nondiabetic obese subjects and animal models of obesity have also been observed [11,13,14]. Most studies regarding mitochondrial impairment in type 2 diabetes mellitus have included only obese diabetic subjects, and it is uncertain to what extent the observations are valid for nonobese type 2 diabetes mellitus subjects [9-11]. Furthermore, the association between mitochondrial dysfunction and type 2 diabetes mellitus has not been so far studied in nonobese individuals. It may be important to note that nonobese type 2 diabetes mellitus constitutes a significant proportion of the type 2 diabetes mellitus population in some countries like India as well as certain developing countries of Asia and Africa [15,16]. A recent epidemiological study has shown a high prevalence of nonalcoholic fatty lever disease and insulin resistance in the nonobese rural population of West Bengal, India [17]. For this purpose, the status of mitochondrial oxidative phosphorylation has been assessed in nonobese type 2 diabetes mellitus patients also in the present study. Secondly, in the present study, more importance has been given to the mitochondrial bioenergetic status instead of the content of mitochondria or mt DNA. The reasons for choosing adipose tissue lie in the fact that the latter plays a crucial role in the metabolic dysregulation associated with type 2 diabetes mellitus and obesity. Adipose tissue, which was earlier portrayed only as a lipid store and an energy reservoir, is now considered as a multifunctional organ playing a central role in lipid metabolism and secreting several bioactive peptides collectively known as *adipokines* with paracrine, autocrine, and endocrine functions [18,19]. Mitochondrial dysfunction in adipose tissue could result in the alteration of pattern of secretion of adipokines that might be causally related to the development of diabetic complications.

2. Methods

2.1. Subjects

The subjects of the present study included controls (n = 10), obese subjects (n = 10), obese type 2 diabetes mellitus patients (n = 10), and nonobese type 2 diabetes mellitus subjects (n = 10) in the age group of 30 to 55 years. Controls and nonobese type 2 diabetes mellitus included subjects with body mass index (BMI) of 18.5 to 23 kg/m^2 and waist circumference less than 80 cm for female subjects and less than 90 cm for male subjects (Table 1). These criteria will exclude both obese and truncally obese subjects [20]. Obese subjects and obese type 2 diabetes mellitus patients had BMI of 23 to 27.5 kg/m^2 or higher [20,21].

Table 1 – Clinical characteristics of research subjects

	Control	Nonobese type 2 diabetes mellitus	Obese nondiabetic	Obese type 2 diabetes mellitus
n (F/M)	4/6	3/7	8/2	5/5
Age (y)	36 ± 5	39 ± 3	33 ± 4	35 ± 5
BMI (kg/m^2)	21.2 ± 1.2	22.1 ± 0.8	28 ± 2.8 [*]	29 ± 2.1 [*,†]
Male waist circumference (cm)	81 ± 3.1	80 ± 4.2	112 ± 4.9 [‡]	115 ± 3.3 [‡,§]
Female waist circumference (cm)	68 ± 3.2	70 ± 2.1	114 ± 2.9 [‡]	112 ± 5.2 [‡,§]
Hemoglobin A_{1c}	4.2 ± 0.3	7.2 ± 0.7 [‖]	4.9 ± 0.5	7.4 ± 0.4 [‖]
Fasting plasma glucose (mg/dL)	84 ± 3.9	140 ± 5.3 [‖]	92 ± 3.2	142 ± 5.8 [‖]

Values are means ± SD of 10 observations.
[*] $P < .05$ vs control.
[†] $P < .05$ vs nonobese type 2 diabetes mellitus.
[‡] $P < .005$ vs control.
[§] $P < .005$ vs nonobese type 2 diabetes mellitus.
[‖] $P < .005$ vs control.

Subjects with overt cardiovascular and renal complications, neurodegenerative disorder, or cancer or chronic infective diseases or those taking pioglitazone or rosiglitazone were excluded. Healthy subjects having a family history of diabetes were excluded from the controls. The study was cleared by the Institutional Ethics Committee of Institute of Post Graduate Medical Education and Research. All the departments of the Institute of Post Graduate Medical Education and Research involved in this study, for example, Department of Biochemistry, Department of Surgery, and Department of Endocrinology and Metabolism, are under the control of this ethics committee. The cosmetic surgery clinic that had participated in this study had also agreed to be guided by this ethics committee. A written, informed consent regarding use of his/her adipose tissue for the study was obtained from each subject. The adipose tissue was collected from type 2 diabetes mellitus subjects during planned abdominal surgery for indications unrelated to diabetes. Adipose tissue was collected from control and obese nondiabetic subjects undergoing surgery for various causes. For some subjects (5 obese nondiabetic and 6 obese diabetic), the adipose tissue was collected by liposuction from abdominal region as part of cosmetic surgery. Table 2 presents the different surgical conditions of the subjects under study from whom adipose tissue has been collected.

2.2. Sample collection

Subcutaneous white adipose tissue (5-7 g) from the abdominal region was collected during open surgery from the operation theatre or during liposuction. For all cases of open surgery or liposuction, adipose tissue has been collected from the abdominal region around the umbilicus. The tissue was kept in chilled buffer A (containing 225 mmol/L mannitol, 75 mmol/L sucrose, 5 mmol/L HEPES, 1 mmol/L EGTA, and1 mg/mL bovine serum albumin [pH 7.4]) and immediately brought to the laboratory for further processing.

Table 2 – Types of subjects and surgery

Groups	No. of patients taken from different types of surgery
Control (n = 10)	4 Gallbladder stone (cholecystectomy) 3 Hernia 2 Appendicitis 1 Gastrectomy (peptic ulcer)
Nonobese type 2 diabetes mellitus (n = 10)	5 Gallbladder stone (cholecystectomy) 2 Gastrectomy (peptic ulcer) 2 Appendicitis 1 Cholecystectomy (chronic cholecystitis)
Obese nondiabetic (n = 10)	5 Liposuction 4 Gallbladder stone (cholecystectomy) 1 Hernia
Obese type 2 diabetes mellitus (n = 10)	6 Liposuction 1 Hernia 2 Appendicitis 1 Gallbladder stone (cholecystectomy)

2.3. Isolation of adipose tissue mitochondria

Mitochondria were isolated from tissue sample by differential centrifugation as adopted from earlier articles [22,23]. Briefly, the tissue sample (5-7 g) was kept over a chilled Petri dish and minced, followed by homogenization in 30 mL of buffer A. After initial centrifugation at 2000*g* for 5 minutes at 4°C, the bulk of the fat was found floating on the top, whereas the nuclei, whole cells, and debris were sedimented in the form of pellet. The supernatant layer between the pellet and the floating fat layer was aspirated out carefully and centrifuged at 12 000*g* for 15 minutes at 4°C. The brown mitochondria pellet thus obtained was resuspended in 10 mL of washing buffer (containing 225 mmol/L mannitol, 75 mmol/L sucrose, and 5 mmol/L HEPES [pH 7.4]) and centrifuged at 12 000*g* for 10 minutes at 4°C. The pellet was washed twice in the same way and finally resuspended in 50 mmol/L potassium phosphate buffer and stored at –20°C for subsequent enzyme assays. The enzyme activities in mitochondrial samples were measured within 48 hours. For carrying out measurement of mitochondrial membrane potential or phosphate utilization, the freshly obtained mitochondrial pellet was resuspended in isotonic buffer B containing 145 mmol/L KCl, 50 mmol/L sucrose, 1 mmol/L EGTA, 1 mmol/L magnesium chloride, and 10 mmol/L phosphate buffer (pH 7.4). For some preliminary experiments (5 observations of each group), mitochondrial marker enzyme citrate synthase was measured in adipose tissue in all 4 groups of subjects to examine any variability of mitochondrial purification among the different groups.

2.4. Citrate synthase assay

Citrate synthase activity was measured following an earlier published method based on the spectrophotometric measurement of released coenzyme A using 5,5′-dithiobis-2-nitrobenzoic acid (Ellman reagent) [24]. The mitochondrial suspension (20-50 μg) was added to an assay mixture containing 100 μmol/L acetyl CoA, 10 μmol/L 5,5′-dithiobis-2-nitrobenzoic acid, and 0.1% (vol/vol) Triton X-100 in 100 mmol/L Tris buffer (pH 8) in a final volume of 1 mL. The reaction was initiated by the addition of 20 μL of 10 mmol/L potassium oxaloacetate, and the rate of change of absorbance was recorded at 412 nm. The enzyme activity in nanomoles of coenzyme A released per minute per milligram of protein was calculated using molar extinction coefficient of 5-thio-2-nitrobenzoic acid at 412 nm (13.6 L/[mmol cm]).

2.5. NADH-ferricyanide reductase (partial complex I assay) assay

The complex I activity of adipose tissue mitochondria was estimated by using ferricyanide instead of ubiquinone as the electron acceptor in an assay mixture containing 0.17 mmol/L NADH, 0.6 mmol/L ferricyanide, and Triton X-100 (0.1% vol/vol) in 50 mmol/L phosphate buffer (pH 7.4) at 30°C as published earlier [22]. The reaction was initiated by the

addition of mitochondrial suspension (10-30 μg protein) to the sample cuvette, and the rate of oxidation of NADH was measured by the decrease in absorbance at 340 nm. Complex I (NADH-ferricyanide reductase) assayed by this method is not rotenone-sensitive because the latter compound acts at the O_2-side and not the substrate side of the flavoprotein from where ferricyanide accepts electrons [25].

2.6. NADH-cytochrome c reductase (complex I-III activity) assay

The activity of NADH-cytochrome c reductase was assayed spectrophotometrically by following the NADH-supported reduction of ferricytochrome c to ferrocytochrome c at 550 nm [24]. The reaction was initiated by the addition of mitochondrial suspension (10-30 μg protein) to the assay mixture containing 100 mmol/L phosphate buffer (pH 7.4), 0.2 mmol/L NADH, 0.1 mmol/L cytochrome c, and 1 mmol/L KCN in 1-mL cuvette. The increase in absorbance at 550nm was monitored for a period of 2 minutes. The same assay was repeated with rotenone (1 μmol/L) to determine the inhibitor sensitive rate. Rotenone sensitive enzyme activity in nanomoles cytochrome c reduced per minute per milligram of protein was calculated using molar extinction coefficient of reduced cytochrome c (19 200 L/[mol cm]).

2.7. Succinate cytochrome c reductase (complex II-III) assay

The activity of succinate cytochrome c reductase (complex II-III) was assayed by following the succinate-supported reduction of ferricytochrome c to ferrocytochrome c at 550 nm [24]. The sample cuvette contained 100 mmol/L phosphate buffer, 2 mmol/L succinate, 1 mmol/L KCN, 0.3 mmol/L EDTA, and 1.2 mg/mL cytochrome c in a total volume of 1 mL. The reaction was initiated by adding mitochondrial suspension (10-30 μg) in the sample cuvette. The increase in absorbance at 550 nm was monitored for a period of 3 minutes. The same assay was repeated with antimycin (10 μmol/L) to determine the inhibitor sensitive rate. The enzyme activity was calculated from antimycin A sensitive rate taking into consideration the mitochondrial protein in the cuvette and the molar extinction coefficient of reduced cytochrome c at 550 nm (19 200 L/[moles cm]) [26].

2.8. Cytochrome oxidase (complex IV) assay

The activity of complex IV was assayed by following the oxidation of reduced cytochrome c (ferrocytochrome c) at 550 nm [27]. Reduced cytochrome c (50 μmol/L) in 10 mmol/L phosphate buffer (pH 7.4) was added in each of two 1-mL cuvettes. In the blank cuvette, ferricyanide (1 mmol/L) was added to oxidize ferrocytochrome c; and the reaction was initiated in the sample cuvette by the addition of mitochondrial suspension (protein content10-30 μg). The rate of decrease of absorbance at 550 nm was measured at room temperature, and the activity of the enzyme was calculated from the first-order rate constant taking into account the concentration of reduced cytochrome c in the cuvette and the amount of mitochondrial protein added [22,27].

2.9. Measurement of transmembrane potential of adipose tissue mitochondria

Mitochondrial transmembrane potential was measured by using a mitochondrial membrane potential sensitive carbocyanine dye, 5,5',6,6'-tetrachloro-1,1',3,3'-tetraethyl-benzamidazolocarbocyanine iodide (JC-1). The monomeric and aggregated forms of JC-1 show emission maxima at 527 nm and 590 nm, respectively, when excited at 490 nm [28]. When isolated mitochondria are incubated with JC-1, the dye accumulates within mitochondria driven by the negative transmembrane potential and forms concentration-dependent aggregates (J-aggregates). The excess monomeric dye in the medium could be removed by several washings, and the red fluorescence of intramitochondrial J-aggregates measured at 590 nm is indicative of mitochondrial transmembrane potential. Briefly, an aliquot of mitochondria was incubated at 37°C for 30 minutes in isotonic buffer B containing 10 mmol/L pyruvate, 10 mmol/L succinate, and 1 mmol/L adenosine diphosphate (ADP) in the presence of 5 μmol/L JC-1. At the end of the incubation, the dye loaded mitochondria were collected by centrifugation, washed extensively with isotonic buffer A to remove the excess dye, and then resuspended in the same buffer in appropriate dilution, followed by the measurement of fluorescence intensity (λex 490 nm, λem 590 nm) in a JASCO (Tokyo, Japan) FP 6500 spectrofluorometer [26]. Arbitrary fluorescence values were transformed to relative fluorescence unit in percentage using quinine sulphate (0.1 μg/mL in 0.1 N H_2SO_4; λex 360 nm and λem 457 nm) as the reference compound.

2.10. Measurement of phosphate utilization of adipose tissue mitochondria

Mitochondrial phosphate utilization was estimated following earlier published procedures [26,29]. Briefly, in a total volume of 250 μL, an aliquot of 25 μL mitochondrial suspension was added to a medium containing 125 mmol/L KCl, 75 mmol/L sucrose, 0.1 mmol/L EGTA, 1 mmol/L $MgCl_2$, 10 mmol/L HEPES, 2 mmol/L phosphate, 0.3% bovine serum albumin, 0.5 mmol/L ADP, 10 mmol/L pyruvate, 10 mmol/L succinate, and 10 mmol/L glucose; and a further 5 U of hexokinase was added immediately to the incubation medium. The mixture was incubated at 37°C for 30 minutes. The incubation was terminated by the addition of 5% ice-cold trichloroacetic acid, and the amount of inorganic phosphate left in the incubation mixture was measured spectrophotometrically. A 0-minute sample was also assayed for inorganic phosphate content where hexokinase addition was immediately followed by the treatment with 5% ice-cold trichloroacetic acid. Glucose and hexokinase in the reaction mixture converted adenosine triphosphate (ATP) to glucose-6-phosphate and ADP, helped to maintain the level of ADP in the system, and also prevented the liberation of free inorganic phosphate from ATP by the action of phosphatases.

2.11. Protein estimation

The protein content of the samples was estimated after solubilization of the sample in 1% sodium dodecyl sulfate by the method of Lowry et al [30].

Table 3 – Comparison of mitochondrial complex activities and membrane potential between samples obtained from open surgery and liposuction

Adipose tissue isolation procedure	Complex I activity (nmol of NADH oxidized/[min mg protein])	Complex I-III activity (nmol of cytochrome c reduced/[min mg protein])	Complex II-III activity (nmol of cytochrome c reduced/[min mg protein])	Complex IV activity (nmol of reduced cytochrome c oxidized/[min mg protein])	Membrane potential (relative fluorescence unit [%])
General surgery	1728 ± 131.6	277 ± 17.24	232.2 ± 8.4	248.8 ± 9.3	57.78 ± 1.7
Liposuction	1685 ± 57.48	268 ± 12.24	234.5 ± 8.2	245.6 ± 6.9	57.8 ± 1.2

Values are the means ± SE of 5 observations.

2.12. *Statistical analysis*

Statistical analysis was done by Student *t* test, unpaired. Each experiment was performed in duplicate with at least 10 different samples, and the values were expressed as the means ± SEM. For results presented in Tables 3 and 4, the number of observations was 5 for each group. A *P* value less than .05 was considered to be statistically significant.

3. Results

3.1. *Measurement of mitochondrial bioenergetic parameters: effect of adipose tissue isolation procedure*

Because the adipose tissue was collected from the operation theatre during open surgery for most of the patients and by liposuction in few cases (5 cases of obese nondiabetic and 6 cases of obese diabetic), we did some preliminary experiments to evaluate if adipose tissue collection procedure had any influence on mitochondrial functional status as measured in our study. Our results clearly demonstrate (Table 3) that the procedure of adipose tissue collection (liposuction or open surgery) did not affect mitochondrial functional parameters such as complex activities and membrane potentials. These preliminary experiments were carried out with healthy controls (5 observations) undergoing open surgery and similar healthy controls (5 observations) undergoing liposuction purely for cosmetic purposes. These values are not included in our data presented in Fig. 1.

3.2. *Measurement of mitochondrial marker enzyme citrate synthase in different groups of subjects*

The activity of mitochondrial marker enzyme citrate synthase was determined in adipose tissue obtained from all 4 groups of subjects. The results presented in Table 4 show that no significant difference exist in mitochondrial citrate synthase activity in adipose tissue from the different groups, indicating a similar degree of purification of mitochondrial samples. Furthermore, because no significant difference existed in the citrate synthase activity among the different groups, no normalization of complex activities was performed with respect to citrate synthase activity.

Table 4 – Citrate synthase activity of adipose tissue mitochondria obtained from 4 different groups

	Control	Obese nondiabetic	Nonobese diabetic	Obese diabetic
Citrate synthase activity (nmol of coenzyme A released/[min mg of protein])	655 ± 20.55	646 ± 23.71	659 ± 22.14	660 ± 23.08

Values are the means ± SD of 5 observations.

3.3. *Mitochondrial electron transport chain activity*

No statistically significant difference was observed between controls and nonobese type 2 diabetes mellitus subjects with respect to complex I, I to III, II to III, and IV activities (Fig. 1A). However, there were statistically significant differences between control and obese nondiabetic subjects ($P < .005$) and also between control and obese type 2 diabetes mellitus subjects in mitochondrial complex I activity in adipose tissue ($P < .001$). Complex I activity in adipose tissue was reduced by approximately 19% in obese nondiabetic subjects and about 28% in obese type 2 diabetes mellitus subjects. Furthermore, a noticeable difference was also seen between the obese nondiabetic and obese type 2 diabetes mellitus subjects ($P < .05$) in mitochondrial complex I activity. Similarly, complex I to III (Fig. 1B), complex II to III (Fig. 1C), and complex IV (Fig. 1D) activities of adipose tissue mitochondria were significantly reduced in nondiabetic obese and obese type 2 diabetes mellitus subjects compared with controls. Furthermore, the decrease in complex I, I to III, or II to III activity in obese type 2 diabetes mellitus subjects was more pronounced than that in obese nondiabetic subjects. However, complex IV activity was decreased to nearly the same extent in adipose tissue of obese and obese type 2 diabetes mellitus subjects.

3.4. *Mitochondrial membrane potential*

There was no significant difference in membrane potential between control and nonobese type 2 diabetes mellitus subjects. However, a significant loss in the mitochondrial membrane potential as indicated by a decrease in fluorescence of J-aggregates was found in adipose tissue of obese and obese type 2 diabetes mellitus subjects compared with that in controls ($P < .005$ and $P < .001$, respectively) (Fig. 2).

3.5. *Mitochondrial inorganic phosphate utilization capacity*

Nonobese type 2 diabetes mellitus patients showed no significant changes compared with controls with regard to mitochondrial phosphorylation capacity. However, the

capacity of mitochondria to use inorganic phosphate (indicative of ATP synthesis capacity) was found to be significantly reduced (about 36%) in nondiabetic obese subjects compared with that in control subjects; and the former was further reduced in obese type 2 diabetes mellitus subjects ($P < .001$ vs control) (Fig. 3). The difference in mitochondrial phosphorylation capacity between obese nondiabetic and obese type 2 diabetes mellitus subjects was also statistically significant.

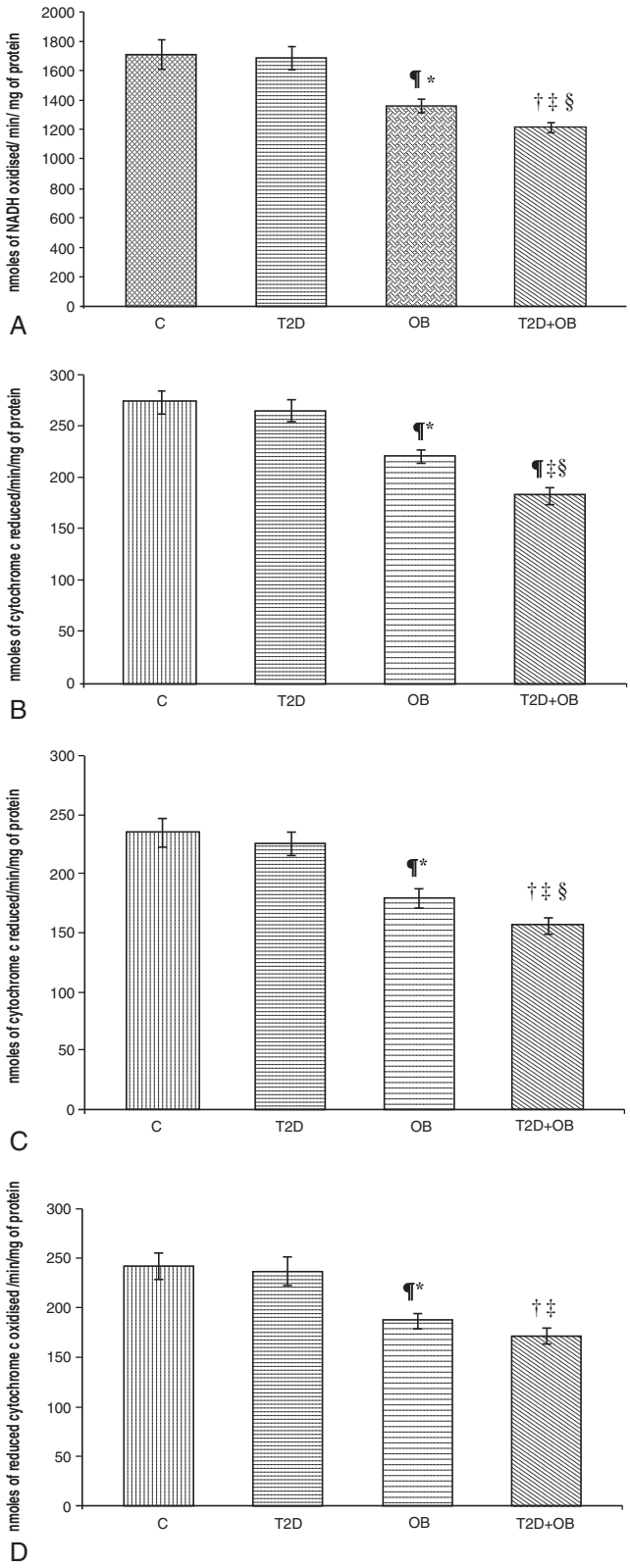

Fig. 1 – Respiratory chain activities of adipose tissue mitochondria. Mitochondrial complex activities in the adipose tissue of control (C), obese nondiabetic (OB), obese type2 diabetes mellitus (T2D + OB), and nonobese type2 diabetes mellitus (T2D) subjects. The mitochondria were isolated from subcutaneous adipose tissue by differential centrifugation, and the activities of respiratory complexes were measured as described in "Methods." A, Complex I. B, Complex I to III. C, Complex II to III. D, Complex IV. The values are the means ± SEM of 10 observations. Statistical significance was calculated by Student *t* test, unpaired. A, ¶$P < .01$ vs C; †$P < .001$ vs C; *$P < .005$ vs T2D; ‡$P < .001$ vs T2D; §$P < .05$ vs OB. B, ¶$P < .001$ vs C; *$P < .005$ vs T2D; ‡$P < .001$ vs T2D; §$P < .005$ vs OB. C, ¶$P < .005$ vs C; †$P < .001$ vs C; *$P < .005$ vs T2D; ‡$P < .001$ vs T2D; §$P < .05$ vs OB. D, ¶$P < 0.005$ vs C; †$P < .001$ vs C; *$P < .01$ vs T2D; ‡$P < .005$ vs T2D.

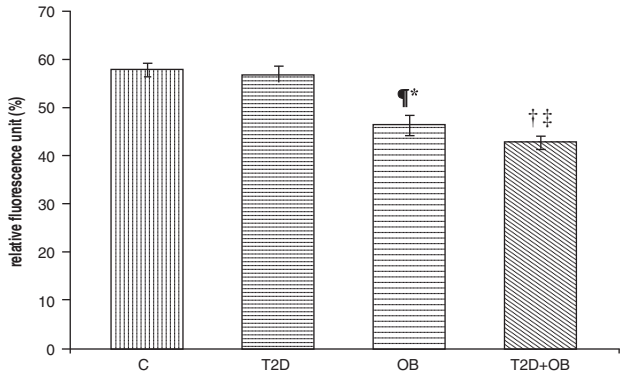

Fig. 2 – Mitochondrial membrane potential in adipose tissue. Mitochondrial membrane potential was measured spectrofluorometrically in freshly isolated adipose tissue mitochondria of control (C), obese nondiabetic (OB), obese type2 diabetes mellitus (T2D + OB), and nonobese type2 diabetes mellitus (T2D) subjects using the cationic dye JC-1 as described in "Methods." The values (relative fluorescence unit as percentage) are the means ± SEM of 10 observations. Statistical significance was calculated by Student *t* test, unpaired. ¶$P < .005$ vs C; †$P < .001$ vs C; *$P < .005$ vs T2D; ‡$P < .001$ vs T2D.

4. Discussion

Although several forms of mitochondrial impairments such as decreased mitochondrial content and mt DNA copy number have been reported in adipose tissue of type 2 diabetes mellitus and obesity, a detailed mitochondrial bioenergetic status in adipose tissue in these conditions has not been clearly worked out in human beings [9-14]. Furthermore, the mitochondrial status in nonobese type 2 diabetes mellitus subjects is practically unknown. Our study has shown that in obese nondiabetic as well as obese type 2 diabetes mellitus

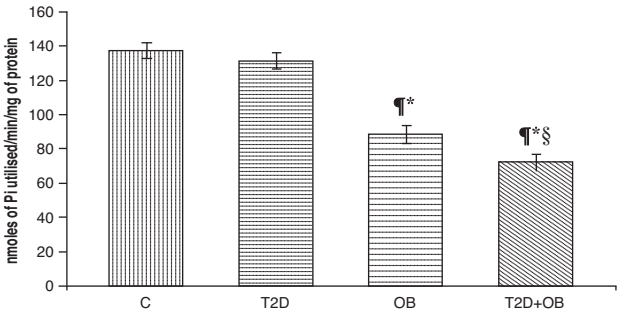

Fig. 3 – Mitochondrial phosphorylation capacity in adipose tissue. Isolation of mitochondria and measurement of phosphorylation capacity were performed in control (C), obese nondiabetic (OB), obese type2 diabetes mellitus (T2D + OB), and nonobese type2 diabetes mellitus (T2D) subjects as described in "Methods." The values are the means ± SEM of 10 observations. Statistical significance was calculated by Student t test, unpaired. ¶P < .001 vs C; *P < .001 vs T2D; §P < .05 vs OB.

subjects, there occurs a significant impairment of activities of mitochondrial respiratory chain complexes. Direct measurement of activities of respiratory chain complexes in adipose tissue in obesity and type 2 diabetes mellitus with obesity have not been reported much, although an impairment of mitochondrial electron transport chain activity has been observed in skeletal muscles in these conditions [31,32]. However, the downregulation of mitochondrial genes encoding respiratory chain subunits has been demonstrated in visceral adipose tissue of type 2 diabetes mellitus subjects; but similar changes are not observed in subcutaneous adipose tissue [9]. The inhibition of respiratory chain complex activities could result in mitochondrial membrane depolarization with associated decrease in phosphate utilization or ATP synthesis that is also clearly noticed in our study in obese nondiabetic as well as obese type 2 diabetes mellitus subjects. It is, however, also possible that the mitochondrial depolarization noticed in this study occurs as a result of uncoupling induced by excess free fatty acids that accumulate in adipose tissue in obesity and obese type 2 diabetes mellitus. The decrease in mitochondrial phosphorylation capacity in obese nondiabetic and obese type 2 diabetes mellitus subjects could be explained from the loss of the mitochondrial membrane potential and respiratory chain complex activity, although a direct inhibition of F_0-F_1 ATP synthase activity or adenine nucleotide translocase activity could not be ruled out. Mitochondrial membrane depolarization in cultured 3T3-L1 adipocyte cell line has been shown to occur following high free fatty acids and high glucose stress [33]. In mouse models of obesity and type 2 diabetes mellitus, a decreased synthesis of ATP in adipose tissue has been reported; and furthermore, the downregulation of several messenger RNA transcripts including that of ATP synthase has also been demonstrated in other experimental model of obesity and type 2 diabetes mellitus [11,34]. However, a loss of mitochondrial membrane potential or a decreased phosphorylation capacity has not been shown earlier in adipose tissue of obese nondiabetic or obese type 2 diabetes mellitus subjects.

The other most significant aspect of this study, however, is the inclusion of nonobese type 2 diabetes mellitus patients. Nonobese type 2 diabetes mellitus constitutes a significant population of type 2 diabetes mellitus patients in India [15,16]. Most of the earlier studies related to mitochondrial dysfunction have included only obese type 2 diabetes mellitus subjects. Because both obese nondiabetic and obese type 2 diabetes mellitus subjects present with mitochondrial dysfunctions in adipose tissue, it is interesting to assess the contribution of obesity per se to mitochondrial dysfunction associated with obese type 2 diabetes mellitus. The results presented here bring out a rather unexpected finding in the sense that no detectable mitochondrial functional alteration takes place in adipose tissue of nonobese type 2 diabetes mellitus. Mitochondrial dysfunction is a very important component of a complex array of metabolic dysregulations in adipose tissue of type 2 diabetes mellitus and obesity where altered fatty acid oxidation and lipid storage, impaired insulin signaling, and insulin-stimulated glucose uptake take place [12,35,36]. These results indicate, however, that the metabolic dysregulation in obese type 2 diabetes mellitus patients may be somewhat different from nonobese type 2 diabetes mellitus patients in the context of mitochondrial functions. In agreement with our finding, it has been shown recently that several other metabolic parameters such as plasma free fatty acids and triglyceride levels in nonobese type 2 diabetes mellitus subjects do not differ in a statistically significant way from those present in healthy controls, whereas these parameters are significantly elevated in obese diabetes subjects [37]. However, a significantly increased mitochondrial impairment in adipose tissue of obese type 2 diabetes mellitus patients compared with that from only obese nondiabetic subjects as observed in this study clearly implies that coexistence of type 2 diabetes mellitus can aggravate mitochondrial injury associated with obesity.

Although this study has provided some useful information, several limitations may also be mentioned. Firstly, the results should be reconfirmed with a larger sample size; and a longitudinal study should be conducted to know the relationship between mitochondrial functional impairment and disease progression. Furthermore, the present study has used subcutaneous adipose tissue for assessment of mitochondrial functional status; and it is therefore not established if the results will apply to visceral adipose tissue also. Thirdly, adipose tissue may contain other cell types like preadipocytes, stem cells, vascular endothelial cells, and smooth muscle cells; and mitochondria from such cells may contaminate adipocyte mitochondria during purification procedure. Although these contaminations are probably quantitatively, very small, and distributed in all the different samples, it can still be argued that mitochondrial metabolic alterations as observed here in obese and obese type 2 diabetes mellitus subjects using whole adipose tissue do not truly reflect the scenario in adipocytes. It will be thus important to confirm the findings by undertaking a similar study with mitochondria isolated from pure adipocytes instead of whole adipose tissue. The fact that mitochondrial functional impairment is not seen in adipose tissue of nonbese type 2 diabetes mellitus needs to

be explored further in other tissues, for example, skeletal muscles; and this could provide mechanistic insights to the genesis of mitochondrial dysfunction in obese nondiabetic or obese type 2 diabetes mellitus subjects. The compromised bioenergetics of mitochondria in adipose tissue from obese nondiabetic and obese type 2 diabetes mellitus patients as shown here can lead to altered pattern of release of adipokines, for example, adiponectin, leptin, and resistin [38,39]. These adipokines have an important role in the development of insulin resistance and also have anti-inflammatory and proinflammatory functions and thus may modify the metabolic and other complications of obesity and obese type 2 diabetes mellitus [38,40]. In particular, adiponectin has been related to vascular dysfunctions in several studies [41]. Based on these results, it will be tempting to estimate and compare the levels of different adipokines in the sera of subjects of obese nondiabetic, nonobese type 2 diabetes mellitus, and obese type 2 diabetes mellitus patients and to relate these parameters to development of different complications associated with each condition. Furthermore, mitochondrial stabilizing/protective agents like α-lipoic acid, creatine, and coenzyme Q can open pathways for new therapeutic approaches in type 2 diabetes mellitus.

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Conflict of Interest

The authors declare no conflict of interest.

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