Effect Of Obesity And Vitamin E On Mitochondrial Function In Human Chondrocytes

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Introduction: The pathomechanics and metabolic activity associated with articular cartilage degeneration in knee osteoarthritis (OA) has not been well elucidated. Obesity, however, is a well known risk factor for knee OA that is associated with increases in regular physiologic loading on to the affected cartilage. Obesity is also linked to mitochondrial dysfunction across a variety of tissues (e.g., skeletal muscle and liver), often characterized by increased production of reactive oxygen species (ROS). This dysfunction has previously been reported in OA chondrocytes; however, the specific mechanisms of ROS mediated losses in function remain unclear. Previous studies have demonstrated a role for ROS in mechanotransductive pathways that should be more active in obese patients. The purpose of the present study was to compare differences in mitochondrial function between cartilage from obese patients and normal BMI patients with OA of the knee and to determine any effect of vitamin E (tocopherol, an antioxidant which combats lipid peroxidation) on chondrocyte mitochondrial respiration. Vitamin E was investigated to delineate the role of lipid peroxidation in obesity-associated pathogenesis of OA.

Methods: Proximal tibial plateau bone specimens were collected during total knee arthroplasty. Five specimens from obesity patients (BMI > 30) and 4 specimens from normal BMI patients (BMI < 25) were analyzed. We included only varus OA knee with Outerbridge 0-1 on lateral tibial plateau (to serve as healthy control cartilage) and Outerbridge 2-4 on medial tibial plateau (to serve as OA cartilage). The chondrocytes from lateral and medial tibial plateau of each specimen were culture separately. Cellular bioenergetics were measured using a Seahorse Bioscience XF96. Oxygen consumption rate (OCR) and extracellular acidification rate (ECAR), a measure of lactate excretion following glycolysis, were measured. OCR analysis was conducted as described by Seahorse Bioscience. Briefly, basal respiration was calculated as the difference between untreated OCR and final electron transport blockade (2 μM rotenone and 5 μM antimycin A -inhibited) OCR. Maximum respiration was calculated as the difference between uncoupled respiration in the presence of 0.25 μM carbonyl cyanide-4-(trifluoromethoxy) phenylhydrazone (FCCP) and rotenone/antimycin A-inhibited OCR. Spare respiratory capacity, a measure of the mitochondrial ability to respond to challenge, was taken as the difference between the basal and maximum respiration. Proton leakage, often associated with membrane peroxidation, was calculated as the difference between 2 μM oligomycin-inhibited OCR and rotenone/antimycin A-inhibited OCR. ECAR rates were only utilized from the untreated portion of the assay. Data are expressed as the mean rate of oxygen consumption in amol/min/cell based on no less than 4 wells per sample.

Results: The average age was 63 ± 8 years old with no significance differences in age between groups. The average BMI in obese group was 34.7 ± 6.4 and normal BMI group was 24.2 ± 1.5. The chondrocytes harvested as controls from the obese group showed higher maximum respiration (p = 0.08) and spare
capacity \( (p = 0.04) \) when compared with healthy chondrocytes from normal BMI group (Figure 1). Lesion chondrocytes did not show any significant difference between obese and normal BMI group under control medium. However, after incubation with alpha-tocopherol diacetate (to facilitate uptake) for 4 days at a concentration of 200 \( \mu \text{M} \), all OCR activities trended towards increases in both groups however only maximum respiration in the obese group showed significance difference \( (p 0.04) \) (Figure 2, 3). No differences were found in comparing ECAR between all chondrocytes.

**Discussion:** Healthy chondrocytes in the obese group demonstrated significantly more maximum respiration and spare capacity, potentially indicating an increased capacity for mitochondrial output in response to the obese condition. We postulate that chondrocytes in the obese group have adapted themselves to increased repetitive physiologic loading by increasing their respiratory capacity. This increase in oxygen consumption associated with increased loading is in accordance with previous studies showing load-responsive oxygen consumption in chondrocytes. Unfortunately, the arthritic tissue known to harbor mitochondrial and oxidative defects appears to lose this adaptation, likely because the arthritic condition swamps normal tissue function. The application of vitamin E to lesion chondrocytes of the obese group appeared to restore respiratory capacity to healthy obese cartilage. This suggests that in the knee OA pathogenesis of obese patients there may be a lipid peroxidation event that disrupts chondrocyte mechanoresponses. Patients with OA of the knee might benefit from vitamin E supplementation. Prospective randomized studies are being performed to evaluate this hypothesis.

**Significance:** Chondrocytes in normal obese cartilage have increased respiratory capacity. That capacity is lost in arthritic obese cartilage, but may be restored with vitamin E supplementation.
Mitochondrial respiration analyses in healthy cartilage

**Graph:**
- **Y-axis:** Respiratory rate (pmol O2/h/nl)
- **Categories:**
  - Basal respiration
  - Maximum respiration rate
  - Super-maximal respiration capacity
  - Protein levels

**Legend:**
- **Stripes** = Normal BMI
- **Black** = Obese

**Statistical Significance:**
- P < 0.05
- P < 0.04
Mitochondrial respiration analysis in Lesion cartilage of Normal BMI group (Control vs Vit E)
Mitochondrial respiration analysis in Lesion cartilage of Obese group (Control vs Vit E)

Respiration (pmol O2/min)

- Basal Respiration control
- Basal Respiration vit E
- Maximum Respiration control
- Maximum Respiration vit E
- Spore Respiratory control
- Spore Respiratory vit E
- Proton Leakage control
- Proton Leakage vit E

P = 0.04
P = 0.15

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