# Age-related NADH oxidase (arNOX) activity of epidermal punch biopsies correlate with subject age and arNOX activities of serum and saliva

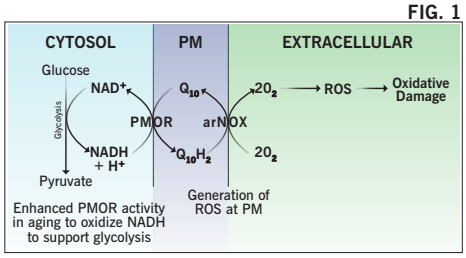
Dale Kern[1], Zoe Draelos[2], Dorothy M. Morré[3] and D. James Morré[3]

[1]NuSkin Enterprises, Provo, Utah, [2]Dermatology Consulting Services, High Point, NC and [3]Purdue University, West Lafayette, IN

## BACKGROUND

The ECTO-NOX (external NADH oxidase) or ENOX proteins are cell-surface located, terminal oxidases involved in the plasma membrane oxido-reductase (PMOR) system. Aging leads to the accumulation of mitochondrial DNA lesions and a shift towards energy production via glycolysis, resulting in a hyperactive PMOR system. ENOX1 (CNOX) and ENOX2 (tNOX) carry out 4 electron transfers to molecular oxygen to form water. However, ENOX3 (arNOX) is unique in that it generates superoxide at the cell surface (Fig. 1) and its activity is elevated in individuals of 50-70 years of age compared to those of 20-40 years of age (1,2) Generated superoxide can then form $H_2O_2$ and other reactive oxygen species (ROS) capable of damaging adjacent cells, circulating lipoproteins (3) and components of the skin's extracellular matrix (ECM).

FIG. 1




*Modified from Morr é, Lenaz & Morr é, 2000, J. Expt. Biol. 201:1512*

## OBJECTIVE

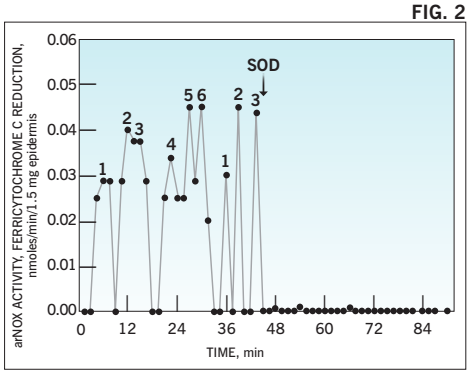
To demonstrate the presence of arNOX (ENOX3) in human epidermis and dermis and examine possible correlations with age and sun exposure.

## METHODS & MATERIALS

This was a single center study designed to obtain human skin, serum, and saliva samples from a variety of age groups for arNOX level determination and further laboratory study. From both sun-exposed and non sun-exposed sites, three mm full-thickness skin punch biopsies were taken from sixteen healthy women age 25-73 of Fitzpatrick skin type I & II. The epidermis and dermis of each biopsy were carefully separated and frozen in PBS. Serum and saliva samples were also collected from each of the 16 subjects. All epidermal, dermal, serum and saliva samples were sent to Purdue University for arNOX activity measurement.

arNOX activity was measured as the production of superoxide based on the standard method where reduction of ferricytochrome c by superoxide was monitored from the increase in absorbance at 550 nm with reference at 540 nm (4). The oscillatory pattern of arNOX activity with a 26 min period and inhibition by superoxide dismutase (Fig. 2) served as the basis for the activity assay. Rates were determined using a SLM Aminco DW-2000 spectrophotometer in the dual wavelength mode with continuous measurements (over 1 min every 1.5 min). After 45 min, 60 $\mu$l (containing 60 units) SOD were added and the assay was continued for an additional 45 min as a further check for the specificity of the arNOX activity.

FIG. 2




## RESULTS

For all six tissue samples arNOX activity and subject age were positively correlated, with arNOX activity exceeding background (blank) rates beginning at about age 30 (extrapolation) and reaching a maximum between ages 55 and 65 (Fig. 3-8). For sun-exposed epidermis and both sun-exposed dermis and sun-protected dermis, arNOX activity values reached a plateau or declined between ages 55 and 72. However, for serum and saliva, activity increased with increasing age beginning at about age 30.

FIG. 3

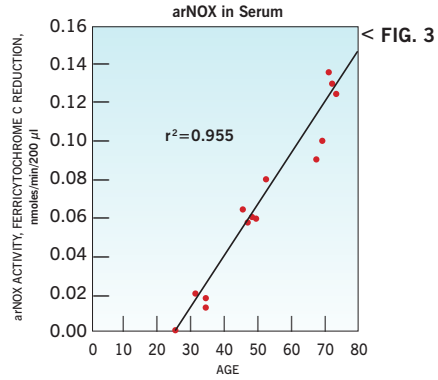



FIG. 4

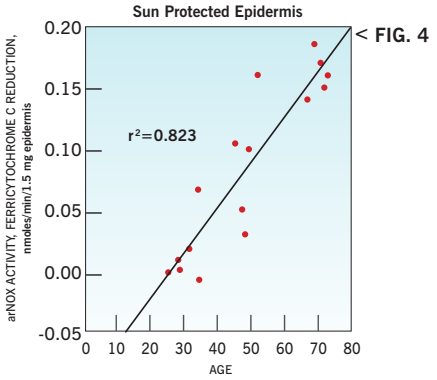



FIG. 5

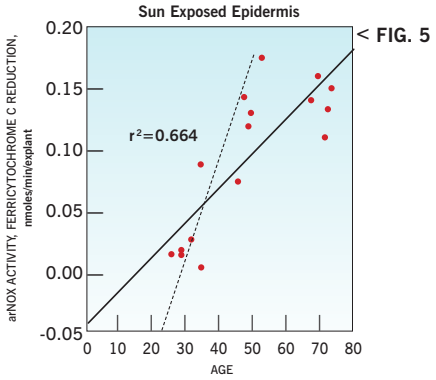



FIG. 6

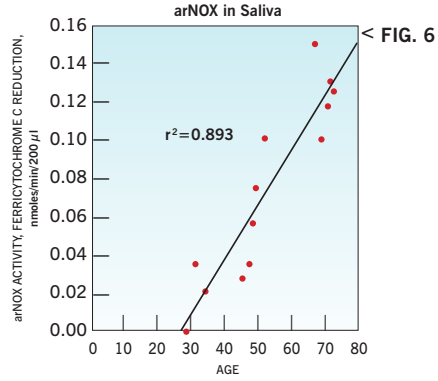



FIG. 7

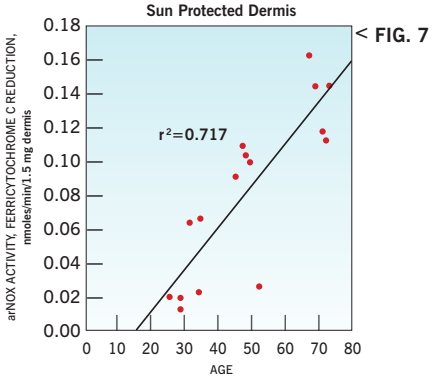
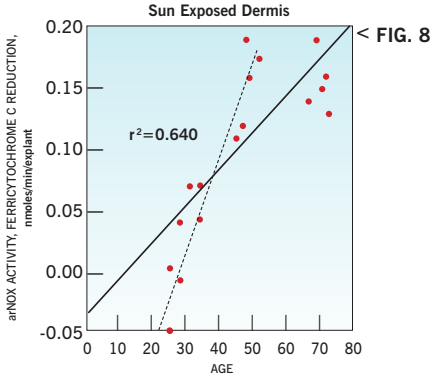



FIG. 8




## CONCLUSION

We have demonstrated that arNOX (ENOX3) is found in both the epidermis and dermis at both sun-exposed and non-sun exposed sites. arNOX levels correlate with chronological age. Because of decreasing arNOX levels in the oldest subjects, the data suggest that arNOX inhibitors may be of cutaneous value in persons between ages 45 and 65.

## REFERENCES

1. Morré DM, Guo F, Morré DJ. An aging-related cell surface NADH oxidase (arNOX) generates superoxide and is inhibited by coenzyme Q. Mol. Cell. Biochem. 2003;254:101-109.
2. Morré DM and Morré DJ. Specificity of Coenzyme Q inhibition of an aging-related cell surface NADH oxidase (ECTO-NOX) that generates superoxide. BioFactors 2003;18:33-43.
3. Morré DJ and Morré DM. Aging-related cell surface ECTO-NOX protein, arNOX, a preventative target to reduce atherogenic risk in the elderly. Rejuvenation Res. 2006;9(2):231-236.
4. Butler J, Koppenol WH, Margoliash E. Kinetics and mechanism of the reduction of ferricytochrome c by the superoxide anion. J. Biol. Chem. 1982;257:10747-10750.