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F2-isoprostanes and Metabolite, and Breast Cancer Risk

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Free Radicals and F_2-isoprostanes (F_2-IsoPs)

Free radicals or reactive oxygen species (ROS) have been implicated as the causes for many human diseases or conditions, including cancer and many other age-related diseases1–3. However, previous studies have been primarily conducted in animal models 3 and results remain controversial3,4. To develop a reliable non-invasive approach to measure human levels of oxidative stress has long been one of the most critical needs in free radical research5. In 1990, Dr. Jackson Roberts, II and Dr. Jason Morrow, first discovered F_2-isoprostanes (F_2-IsoPs), a unique series of prostaglandin (PG)-like compounds that are formed from the free-radical-catalyzed peroxidation of arachidonic acid *in situ* in phospholipids6. In recent years, F_2-IsoPs have been widely utilized in many epidemiologic and clinical trial studies to evaluate level of lipid peroxidation, one central feature of oxidative stress and free radical damage7. In 2005, a multi-lab validation study, the Biomarkers of Oxidative Stress Study, was organized by the National Institute of Environmental Health Sciences (NIEHS)8. In the study, F_2-IsoPs has been found to be the most accurate oxidative stress biomarker9.

15-F_{2t}-Isoprostane Metabolite (15-F_{2t}-IsopM)

Unmetabolized F_2-IsoPs, however, may be artificially generated *in vitro* in fluids by autoxidation. Furthermore, the level may be significantly affected by the local renal isoprostane production10 as well. After β-oxidation, 15-F_{2t}-Isoprostane(15-F_{2t}-Isop), one major F_2-IsoP, converts to 2,3-dinor-5,6-dihydro-15-F_{2t}-IsoP (15-F_{2t}-IsopM), a metabolite not subject to autoxidation and renal production6. A method with both high sensitivity and accuracy has been developed to measure 15-F_{2t}-IsopM using gas chromatography /negative ion chemical ionization mass spectrometry (GC/NICI MS)10. Nevertheless, unmetabolized F_2-IsoPs, but 15-F_{2t}-IsopM, has been predominantly utilized in previous human or animal studies.

New paradigm for the role of ROS

Overproduction of ROS leads to oxidative stress which may be involved in the etiology and pathogenesis of many diseases. On the other hand, growing evidence from *in vitro* and *in vivo* studies indicates that endogenous basal level of ROS11–13, acting as secondary messengers4, play a key role in the regulation of multiple normal physiologies, including signal transduction, cell proliferation and homeostasis and microorganism defense 5 as well as induction of apoptosis and senescence, two key mechanisms for cancer prevention 5,14. In fact, normal levels of F_2-IsoPs and 15-F_{2t}-IsopM have been defined in healthy

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humans10,15. Therefore, it is possible that the biologic role of ROS is dependent on the endogenous level of ROS.

15-F_{2t}-IsopM, F_2-IsoPs, Obesity, and Breast Cancer Risk

No study has prospectively investigated the etiologic role of F_2-IsoP and its metabolite in the development of breast or other cancers. Using breast cancer as a disease model, we worked with Drs. Milne and Morrow's lab since 2001. We prospectively investigated the associations of urinary F_2-IsoP and 15-F_{2t}-IsopM, as measured using the GC/NICI-MS assay, with breast cancer risk in a nested case-control study within the Shanghai Women's Health Study (SWHS), a population-based cohort study of 74,942 Chinese women between 40 and 70 years of age. We reported the results very recently in the Journal of Clinical Oncology16.

Over the past several years, a number of studies have consistently observed that overweight or obese women had a significantly elevated level of F_2-IsoPs5,17, indicating women with a high level of BMI have an excessive production of ROS and are at high risk of oxidative stress. Therefore, among overweight/obese women, high levels of 15-F_{2t}-IsopM and/or F_2-IsoPs may be related to an increased risk of breast cancer. Conversely, among women with normal BMI, basal levels of ROS18 are necessary to trigger p53 activation, directly mediate apoptosis and induce senescence5. Additionally, F_2-IsoPs was found to increase the glucose-induced synthesis of TGF-β119,20, a critical tumor suppressor at initial stage21. It is, thus, not surprising that several protective factors for breast cancer risk, such as physical activity22,23, parity (normal pregnancy)5,24 and preeclampsia5, were linked to significantly elevated levels of lipid peroxidation25,26. Based on these findings, it is possible that the role of ROS among women with a normal BMI may be different from overweight/obese women. We, therefore, hypothesized that the associations between levels of F_2-IsoPs and 15-F_{2t}-IsopM and breast cancer may vary by BMI status and further evaluated this hypothesis in the SWHS16.

We found urinary levels of 15-F_{2t}-IsopM and F_2-IsoPs did not significantly differ by breast cancer status16. Levels of F_2-IsoPs and 15-F_{2t}-IsopM were related to a reduced risk of breast cancer among women with a BMI<2516. Among women with a BMI<23, high F_2-IsoPs was associated with a reduced risk of breast cancer in a dose-response manner (p for trend, 0.006) with an OR of 0.46 (95%CI: 0.26–0.80) for the highest tertile vs. the lowest (p for interaction, 0.006)16. Among women with a low BMI, the reduction in risk appeared in both pre- and post-menopausal women16. In contrast, 15-F_{2t}-IsopM and F_2-IsoPs were associated with an increased risk of breast cancer among women with a BMI≥2516. The associations became stronger with increasing levels of BMI. 15-F_{2t}-IsopM was linked to a 2- to 4-fold elevated risk among women with a BMI≥27.5; the ORs elevated to 10.20 (95% CI: 2.35–44.29) for the middle tertile and 10.27 (2.41–43.80) for the highest tertile vs. the lowest tertile (p for trend, 0.003) (p for interaction with BMI (BMI<29 vs. BMI≥29), 0.0004) among women with a BMI≥2916. The corresponding ORs (95%CIs) further elevated to 13.62 (1.38–134.08) and 23.47 (2.46–223.69) (p for interaction, 0.001) among those with a BMI of 30 or more. Very similar results were obtained in the sensitivity analysis excluding breast cancer patient diagnosed within 3 years from urine collection16. Our novel findings indicate that the role of ROS in the development of breast cancer is different by BMI status and 15-F_{2t}-IsopM is a more sensitive and specific biomarker of oxidative stress than F_2-IsoPs among overweight/obese subjects16

Null Effects of Antioxidant Vitamins in Clinical Trials

Our null overall association and different associations by BMI status may provide a possible explanation for those reported in recent clinical trials that supplementation of α-tocopherol

had no overall benefit for total mortality and for incidence and mortality of major cardiovascular diseases or cancer including breast cancer16,27,28. Very recently, the Physicians' Health Study II randomized controlled trial found individual supplements of 400 IU of vitamin E every other day and/or 500 mg of vitamin C daily provided no overall benefit for total mortality and for incidence or mortality of cancer and major cardiovascular after a mean follow-up of 8 years29.

A previous clinical trial found that supplementation of α-tocopherol at dosages of 200, 400, 800, 1200, or 2000 IU/day did not reduce F_2-IsoPs level after 8 weeks of supplementation among healthy subjects5. In a recent study, a moderate effect was observed solely after 16 weeks of supplementation and when the supplementation dose became high (800 IU/day) and the effect was most apparent when the dose became extremely high (1600 IU/day)30. Thus, no reduction in level of F_2-IsoPs was expected at 400 IU of vitamin E every other day used in the Physicians' Health Study II randomized trial. On the other hand, some previous intervention trials indicated that the antioxidant vitamin E reduces F_2-IsoPs levels in certain disease conditions in which levels of ROS production may be increased5. Very recently, a clinical trial found that high doses of vitamin E (800 IU/day) or Vitamin C (1000 mg/day) reduced the level of F_2-IsoPs after 8 weeks of supplementation only for those with a high basal F_2-IsoPs31. Another recent trial found a high dose of vitamin E (800 IU/day) significantly reduced F_2-IsoPs among overweight subjects32. Taken together, these findings suggest that supplementation use of antioxidants may only be beneficial among overweight/obese subjects or other conditions linked to a high level of associated with a F_2-IsoPs.

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