THE EFFECT OF ARACHIDONIC ACID AND EICOSAPENTAENOIC ACID SUPPLEMENTATION ON PROSTACYCLIN PRODUCTION IN RATS FED A BUTTER-ENRICHED DIET

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Previous studies have shown that dietary saturated fat in the form of butter (relatively low n-6:n-3 fatty acid ratio) decreased arterial prostacyclin production dose dependently in rats (O'Dea et al. 1988). This decrease was paralleled by a decrease in arachidonic acid (AA) and an increase in eicosapentaenoic acid (EPA) in phospholipids of plasma, aorta and liver. Oral supplementation of butter-fed rats with AA reversed these changes completely (Steel et al. 1990).

Hence prostacyclin production seems to be regulated either directly by tissue levels of its precursor AA or indirectly by levels of EPA which competitively inhibit AA metabolism by the cyclooxygenase pathway, or a combination of both. Dietary administration of EPA to rats has previously shown varied results, including increased in vitro PGI_2 but no detectable PGI_3 formation when administering the ethyl ester of EPA (Hamazaki et al. 1982) and a decrease in PGI_2 with no detectable PGI_3 formation (Morita et al. 1983).

In our study, four groups of male Sprague-Dawley rats were fed a high-fat butter-enriched diet (50% energy as fat) for two weeks. Three of the groups were then supplemented orally with either 90mg/day of linoleic acid (LA) (free acid), AA or EPA (as ethyl esters) for a further two weeks while remaining on the high-fat diet. Forty eight-hour urine samples were collected at the end of the second and fourth weeks in metabolic cages. The rats were sacrificed and tissue collected for fatty acid analysis (plasma, platelets and aorta). Prostacyclin production was determined by arterial incubation followed by RIA. Urinary prostacyclin determination was performed by extraction of the urinary metabolite 2,3 dinor-6-keto-$PGF_{1\alpha}$ and its Δ^{17} analog followed by combined Gas Chromatography-Mass Spectrometry (GC-MS) analysis in NICI mode using a deuterated internal standard and Selected Ion Monitoring (SIM).

Rats on the high-fat diet showed a significant decrease in plasma phospholipid AA and an increase in EPA, with a parallel significant decrease in aortic prostacyclin production ($p<0.05$ for all significant results) compared with a low-fat control group. Oral supplementation with AA resulted in a rise in plasma AA level and in vitro prostacyclin production, coupled with a fall in plasma EPA in comparison with the high-fat control group (all significant). EPA supplementation resulted in the opposite trend, with a rise in plasma EPA and fall in plasma AA. EPA supplementation also showed a significant increase for in vitro prostacyclin in comparison with the high-fat control group, but was also significantly less than the level reached by AA supplementation.

GC-MS analysis of urines showed a significant increase in the PGI_2-M in the AA supplemented rats but a decrease in the EPA supplemented rats. The PGI_3-M level increased in the EPA supplemented rats as shown in an early study (Knapp and Salem 1989), and decreased in the AA supplemented group (all relative to the high-fat control diet).

In conclusion this study shows that AA administration in rats leads to increased in vitro prostacyclin production by aortic strips accompanied by an increase in whole body PGI_2 production as indicated by urinary metabolite levels, measured by combined GC-MS. EPA administration leads to increased in vitro prostacyclin production, but a significant decrease in whole body production of PGI_2, with a small increase in PGI_3 production (GC-MS urinary analysis).

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