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Comparison of growth, serum biochemistries and *n*−6 fatty acid metabolism in rats fed diets supplemented with high-gamma-linolenic acid safflower oil or borage oil for 90 days

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ABSTRACT

Recently, steps have been taken to further developments toward increasing gamma-linolenic acid (GLA) concentration and lowering costs in plant seed oils using transgenic technology. Through identification and expression of a fungal delta-6 desaturase gene in the high linoleic acid safflower plant, the seeds from this genetic transformation produce oil with >40% GLA (high GLA safflower oil (HGSO)). The aim of the study was to compare the effects of feeding HGSO to a generally recognized as safe source of GLA, borage oil, in a 90 day safety study in rats. Weanling male and female Sprague–Dawley rats were fed a semi-synthetic, fat free, pelleted diet (AIN93G) supplemented with a 10% (wt/wt) oil blend containing HGSO or borage oil, with equivalent GLA levels. Results demonstrated that feeding diets containing HGSO or borage oil for 90 days had similar biologic effects with regard to growth characteristics, body composition, behavior, organ weight and histology, and parameters of hematology and serum biochemistries in both sexes. Metabolism of the primary *n*−6 fatty acids in plasma and organ phospholipids was similar, despite minor changes in females. We conclude that HGSO is biologically equivalent to borage oil and provides a safe alternative source of GLA in the diet.

1. Introduction

One of the fastest growing segments of nutritional products in the United States are those enriched with essential fatty acids, in particular the omega-3 fatty acids (eicosapentaenoic acid (EPA), docosahexaenoic acid) and gamma-linolenic acid (GLA; 18:3*n*−6). The use of GLA in functional food, dietary supplements, personal care products, and animal health products has significantly increased recognition of its nutritional and health benefits.

Research on GLA has centered on its therapeutic role in alleviating chronic disease states such as inflammatory disease (Rheumatoid arthritis, critically ill patients and dermatitis), diabetic neuropathy, premenstrual syndrome, and cardiovascular disease risk factors (Fan and Chapkin, 1998). One of the proposed contributing factors in the etiology of these disease states is a chronic imbalance between GLA and its metabolites, dihomo-GLA (DGLA; 20:3*n*−6) and arachidonic acid (20:4*n*−6). Conversion of the essential fatty acid, linoleic acid (18:2*n*6) to GLA is rate limited by delta-6 desaturase activity (Fig. 1). GLA may become essential under certain pathological conditions (as noted above) that depress delta-6 desaturase activity and thus reduce the production of the elongase product of GLA, DGLA (Fan and Chapkin, 1998). This is important because DGLA can be converted by inflammatory cells to 15-hydroxy-eicosatrienoic acid and prostaglandin E_1 which possess anti-inflammatory and antiproliferative properties (Horrobin, 1992; Ziboh and Fletcher 1992). Numerous preclinical (Mancuso et al., 1997a; Mancuso et al., 1997b; Murray et al., 1995, 2000; Palombo et al., 1997, 1999) and clinical (Gadek et al., 1999; Pacht et al., 2003; Singer et al., 2006; Pontes-Arruda et al., 2006) studies have shown that nutritional formulas enriched with a combination of EPA (20:5*n*3) and GLA can favorably reduce an inflammatory response while promoting vasodilation and oxygen delivery in patients with acute lung injury. Recently Covar and colleagues (Covar et al., 2010) evaluated the safety and efficacy

Abbreviations: AAALAC, Association for assessment and accreditation of laboratory animal care; DGLA, dihomo-gamma-linolenic acid; EPA, eicosapentaenoic acid; FID, flame ionization detector; GLA, gamma-linolenic acid; GLC, gas–liquid chromatography; HGCO, high gamma-linolenic acid canola oil; HGSO, high gamma-linolenic acid safflower oil; QMR, quantitative magnetic resonance; TLC, thin-layer chromatography.

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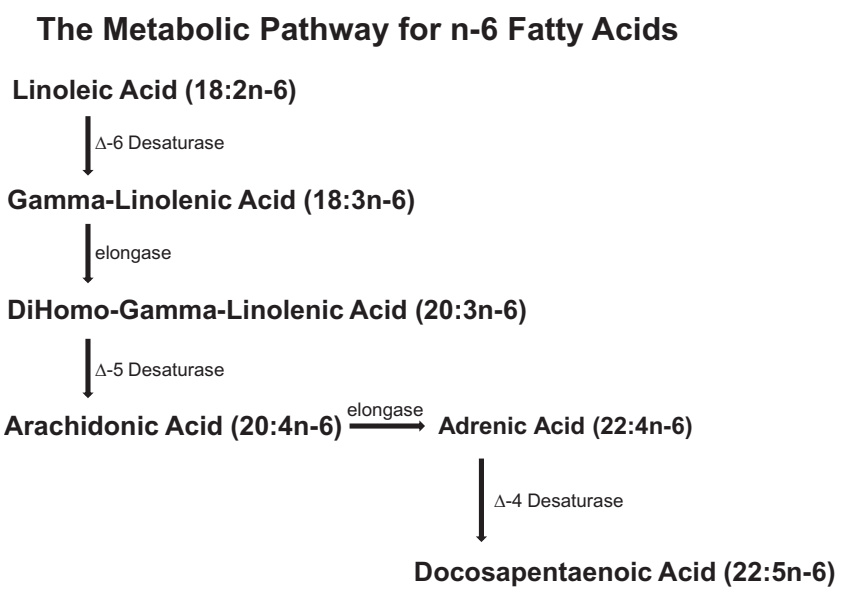

Fig. 1. The metabolic pathway for *N*−6 fatty acids.

of a novel nutritional formula enriched in EPA and GLA and antioxidants in children 6–14 years of age with mild persistent asthma. Daily consumption of this nutritional formula for 12 weeks prevented the deterioration in bronchial hyper-responsiveness, while showing patterns of improvement in well established biomarkers of inflammation and pulmonary function.

These beneficial effects have increased the awareness and demand for GLA enriched oils. Presently, commercial sources of GLA are borage oil, evening primrose oil, and black current seed oil. The increased demand for GLA in functional foods will require a more concentrated, lower cost, and improved availability of oils containing GLA. Therefore, development of oil seed crops which produce substantial quantities of GLA using transgenic technology has been a major goal of plant biotechnology.

Through identification and expression of a fungal delta-6 desaturase gene in the canola plant, the developed seeds from this genetic transformation produced oil with >30% GLA (high GLA canola oil (HGCO) (Huang et al., 1999). The chemical and physical characteristics of this novel oil have been evaluated (Liu et al., 2001) as well as a series of animal studies conducted showing the bioequivalence and safety of HGCO to borage oil which is normally consumed by humans (Tso et al., 2002; Palombo et al., 2000; Liu et al., 2004; Wainwright et al., 2003). Recently, Arcadia Biosciences, Inc. (Davis, CA) has taken the steps to further development toward increasing GLA concentration and lowered costs in plant seed oils by producing a high GLA safflower oil (HGSO) comprising >40% GLA with a significantly reduced level of linoleic acid. This level of GLA was achieved through genetic transformation of a high linoleic acid safflower variety with a delta-6 desaturase gene that codes for the enzyme which converts linoleic acid (18:2*n*−6) to GLA (18:3*n*−6). Arcadia Biosciences currently markets HGSO as a dietary supplement under the name SONOVA 400.

Though HGSO is considered a new plant seed oil containing GLA, it has similarities to borage oil and HGCO, possessing triglycerides with elevated levels of GLA as well as monounsaturated and *n*−6 fatty acids. Based upon the knowledge gained from the results of the absorption, growth and feeding studies in rats with diets containing HGCO, we chose to compare the effects of feeding HGSO to a generally recognized as safe source of GLA, borage oil, for a period of 90 days on growth characteristics, diet consumption, behavior, appearance, and organ morphology (relative organ size and histology) in male and female rats. In addition, the phospholipid fatty acid composition of plasma and various organs was determined to compare the metabolism of GLA given as either HGSO or borage oil.

2. Materials and methods

The in vivo portion of this study was conducted at the University of Cincinnati-Metabolic Diseases Institute (Cincinnati, Ohio), an Association for Assessment and Accreditation of Laboratory Animal Care, International (AAALAC) accredited facility, in accordance with the animal use protocol approved by the Institutional Animal Care and Use Committee (IACUC), using guidelines set forth by the National Institute of Health's Guide for the Care and Use of Laboratory Animals.

2.1. Animals

Pathogen-free male (159–179 g; $n = 25$) and female (128–157 g; $n = 25$) Sprague–Dawley®™ rats were purchased from Harlan (Indianapolis, IN). The animals were housed in their own room, within a barrier animal facility, in conventional, plastic shoebox cages (Alternative Design, Siloam Springs, AK), one rat per cage, with corn cob bedding (Bed-o'cobs®, The Andersons, Inc.) under light (12 h light–dark cycle), temperature (72 ± 1 °F) and humidity (30–70%) controlled conditions for the duration of the study. All animals had ad libitum access to food and water.

2.2. Test diets

Each diet was a semi-synthetic, fat free, pelleted diet fortified with vitamins and minerals (AIN93G; Harlan Teklad, Madison, WI) (Table 1) and supplemented with 10% (wt/wt) of an oil blend containing either HGSO or borage oil, with the resulting levels of GLA being equivalent between both diets. The fatty acid compositions of the original HGSO and borage oils, along with the two oil blends in the experimental diets, are presented (Table 2). All test diets were packed in batches of 0.5 kg in vacuum-sealed freezer bags and stored at approximately −20 °C. Fresh aliquots of diet were provided to the animals every other day.

2.3. Experimental design

After acclimation for 1 week, the male and female rats were placed in one of two weight matched groups for each sex, and assigned to receive one of the two experimental diets for the entire ninety day study period. Clinical appearance, body weight, and food intake were monitored weekly. At the end of twelve weeks, the rats were anesthetized with sodium pentobarbital (Fatal Plus, Vortech Pharmaceuticals, Ltd.) and euthanized by exsanguination. Blood from each rat was collected from the abdominal aorta and immediately placed into three different 4.0 ml sterile blood collection tubes (BD Vacutainer®); two tubes contained the anti-coagulant EDTA and the other tube contained Lithium Heparin. The blood in the EDTA tubes was stored in wet ice and covered with aluminum foil until centrifuged for fifteen minutes at 750 g and 4 °C. The plasma was carefully extracted and placed into an additive free tube. The plasma was stored at −20 °C until analyzed that evening for hematological studies and analysis of cholesterol and fatty acid composition.