Journal of Functional Foods 17 (2015) 948–957

Available online at www.sciencedirect.com

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journal homepage: www.elsevier.com/locate/jff

Age-dependency in the metabolism of flaxseed lignans by healthy adults

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ARTICLE INFO

Article history:
Received 11 March 2015
Received in revised form 12 June 2015
Accepted 17 June 2015
Available online 10 July 2015

Keywords:
Enterolignans
Flaxseed
Phytoestrogens
Gut bacteria
Lignans
Cardiovascular disease

ABSTRACT

Flaxseed lignan metabolites, enterodiol (END) and enterolactone (ENL), have biological activities that may have potential therapeutic benefit through their antioxidative and oestrogenic properties. Circulating enterolignan concentrations are influenced by gut bacteria, which itself is subject to change with age. The plasma availability of END, ENL and total enterolignans (END + ENL) after 0 and 4-weeks of dietary milled flaxseed consumption (30 g/d) in healthy, younger (18–29 years) and older (45–69 years) adults was determined by using gas chromatography/mass spectrometry. Plasma total enterolignan concentrations increased in both younger and older adults, however, the distribution of END:ENL was lower in older adults (0.74) compared to younger adults (0.96). Post flaxseed treatment, ENL was detected in the plasma of 100% of the older adults, but only in 78% of the younger ones. The potential therapeutic benefit offered by enterolignans may be particularly important for ageing populations that are prone to chronic diseases.

1. Introduction

Epidemiological studies list cancer and cardiovascular disease as the two main causes of death in North America (Statistics Canada, 2012). Asian populations have traditionally had lower risks of mortality associated with these outlined chronic diseases. This has been in part attributed to a diet high in phytoestrogens (Adlercreutz et al., 1991; Kris-Etherton et al., 2002). Phytoestrogens are plant-based compounds that are classified as isoflavones, coumestans and lignans (Thompson, Boucher, Liu, Cotterchio, & Kreiger, 2006). Isoflavones are found primarily in soy products, coumestans in sprouts and lignans in oil seeds, vegetables, fruits, nuts, whole grains, tea and red

Chemical compounds: Enterodiol (PubChem CID: 123725); Enterolactone (PubChem CID: 114739); Secoisolariciresinol diglucoside (PubChem CID: 9917980).

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Abbreviations: ALA, alpha-linolenic acid; BMI, body mass index; BSTFA, N,O–bis(trimethylsilyl)trifluoroacetamide; CV, coefficient of variation; EDTA, ethylenediaminetetraacetic acid; END, enterodiol; ENL, enterolactone; GC/MS, gas chromatography mass spectrometry; LDL, low-density lipoprotein; SDG, secoisolariciresinol diglucoside; SECO, secoisolariciresinol; μSIS, micro-selected ion storage

http://dx.doi.org/10.1016/j.jff.2015.06.042
1756-4646/

wine (Thompson et al., 2006). Consumption patterns of phytoestrogens in Asian populations are about 20–80 mg/d and are largely isoflavone-based (Adlercreutz et al., 1991; de Kleijn et al., 2001). This is in contrast to Western diets with phytoestrogen intakes typically less than 1 mg/d and this is largely lignan based (80%) with 20% coming from isoflavones and <0.1% coumestans (de Kleijn et al., 2001). Lignans have both weak oestrogenic and antioestrogenic activities, antioxidative potential (Adlercreutz, Heinonen, & Penalvo-Garcia, 2004) and may have a role in disease prevention (Adlercreutz et al., 2004), which warrants further investigation.

Dietary flaxseed has by far the highest content of the lignan secoisolariciresinol diglucoside (SDG) in food (379 mg/100 g) (Thompson et al., 2006). The next highest food sources of SDG include rye (360-fold less) and wheat (870-fold less) (Smeds et al., 2007), asparagus (180-fold less) (Penalvo, Haajanen, Botting, & Adlercreutz, 2005), chestnuts (2170-fold less) (Thompson et al., 2006) and legumes (Mazur, Duke, Wähälä, Rasku, & Adlercreutz, 1998). Other lignans in flaxseed include matairesinol, pinoresinol and lariciresinol, but in much lower quantities, namely 2448, 514 and 134 times lower, respectively (Thompson et al., 2006). In rabbit models, SDG ingestion lowered circulating LDL-cholesterol and attenuated atherosclerosis (Prasad, 1999), reduced glycated haemoglobin and the onset of diabetic symptoms (Prasad, Mantha, Muir, & Westcott, 2000). Mouse models of breast cancer have documented the antitumourigenic effects of dietary flaxseed and pure SDG (Chen, Saggar, Corey, & Thompson, 2009). More recent clinical studies using SDG supplements or a lignan complex report attenuations in glucose concentrations (Zhang et al., 2008), metabolic syndrome composite scores in males (Cornish et al., 2009), diastolic blood pressure (Cornish et al., 2009) and the inflammatory marker, C-reactive protein (Pan et al., 2009). Flaxseed lignans can also reduce the occurrence of breast cancer by 33–70% through the attenuation of angiogenesis, cellular proliferation and apoptosis (Mason & Thompson, 2013). Flaxseed is also a rich source of the omega-3 fatty acid alpha-linolenic acid (ALA) and fibre, both of which have documented benefits in reducing risk factors of CVD (Edel, Rodriguez-Leyva, et al., 2015; Rodriguez-Leyva et al., 2013), however, only lignans can be metabolized to enterolignans and will be the focus of the present discussion.

Ingestion of SDG does not result in its entry into the circulation (Setchell et al., 2014). SDG is metabolized by microflora in the gut to the mammalian enterolignans, enterodiol (END) and enterolactone (ENL), which are considered to be the two primary bioactive forms of SDG (Setchell et al., 2014). The less abundant flaxseed lignans are also metabolized to one or both of these enterolignans (Clavel, Borrmann, Braune, Dore, & Blaut, 2006). SDG metabolism involves bacterial hydrolysis to the aglycone secoisolariciresinol (SECO), subsequent demethylation and dehydroxylation to yield END, which may then undergo dehydrogenation within the intestinal tract to ENL (Axelson, Sjovall, Gustafsson, & Setchell, 1982; Setchell et al., 1981). Clavel has demonstrated in anaerobic models of the gut that specific strains of bacteria promote the formation of END and ENL (Clavel et al., 2006). Once enterolignans are formed, they permeate the intestinal mucosa and are repackaged into sulphate- or glucuronide-conjugates in the liver (Axelson & Setchell, 1981). Following conjugation, they enter the enterohepatic circulation and may be eliminated in urine and bile, or they may circulate systemically (Axelson & Setchell, 1981) and/or be stored in a variety of tissues (Saarinen & Thompson, 2010). A portion may also be deconjugated in the large intestine and eliminated in the faeces. Other factors that may influence enterolignan formation include intestinal pH and oxygen gradients (Flint, Scott, Louis, & Duncan, 2012), bile acids, transit time (Rowland, Wiseman, Sanders, Adlercreutz, & Bowey, 2000), diet (Zimmer et al., 2012), flaxseed cultivar (Spence, Thornton, Muir, & Westcott, 2003), flaxseed form (Kuijsten, Arts, van't Veer, & Hollman, 2005) and antibiotic usage (Kilkkinen et al., 2002). An outcome of this is that there can be large inter-individual variations in enterolignan bioavailability in human plasma samples (Kuijsten, Arts, van't Veer, et al., 2005; Kuijsten, Arts, Vree, & Hollman, 2005).

Subject age may also influence lignan metabolism and enterolignan absorption. It is well established that gut microbiota composition changes throughout a person's lifespan (Flint et al., 2012). The three phases of change occur from birth to weaning, from weaning to a habitual diet and throughout old age. Generally, a decline in microbial variety has been observed in older populations (O'Toole & Claesson, 2010), with increases in enterobacteriaceae, declines in bacteroides (Enck et al., 2009; Woodmansey, 2007), and reports of reduced (Woodmansey, 2007) or stable numbers of bifidobacteria and lactobacilli (Enck et al., 2009). Individuals older than 65 years of age had greater bacteroidetes and less firmicutes compared to their younger counterparts (28–46 years) (Claesson et al., 2011). The health effects of the microbial environment on lignan metabolism and enterolignan absorption are still largely unknown. Gender may also play a role in gut microbiota composition as significantly higher enterolignan producing organisms are present in females compared to males (Clavel et al., 2005).

Chronic diseases are also influenced by increased age (Centers for Disease Control and Prevention, Chronic Disease Prevention and Health Promotion, National Vital Statistics System, 2007) and oxidative stress (Stocker & Keaney, 2004; Wiseman & Halliwell, 1996). It is well established that blood antioxidative potential improves with consumption of foods rich in antioxidants, namely lycopene enriched tomato sauce (Abete et al., 2013) or golden flaxseed (Pilar et al., 2014) as examples. Enterolignans exhibit free-radical scavenging capabilities *in vitro* (Kitts, Yuan, Wijewickreme, & Thompson, 1999) and *in vivo* (Vanharanta et al., 2002). Therefore, flaxseed lignan therapy may be a potential solution for Western populations whose incidence of chronic disease in elderly subjects continues to rise. However, prior to using dietary flaxseed in a diseased population, lignan metabolism and enterolignan absorption needs to be studied in a healthy population to assess circulating concentrations. Therefore, the aim of this study was to examine lignan metabolism through enterolignan plasma availability in healthy, younger and older populations consuming dietary milled flaxseed fortified in muffins as a source of lignans.

2. Materials and methods

2.1. *Ethics and study design*

This study design was approved by the University of Manitoba Research Ethics Board and the St. Boniface Hospital