Available online at www.sciencedirect.com
SCIENCE DIRECT
Biochimica et Biophysica Acta 1737 (2005) 52 – 60
BIOCHIMICA ET BIOPHYSICA ACTA
BBA
http://www.elsevier.com/locate/bba

Dietary coconut oil increases conjugated linoleic acid-induced body fat loss in mice independent of essential fatty acid deficiency ☆

Kimberly M. Hargrave [a], Michael J. Azain [b], Jess L. Miner [a,*]

[a] Department of Animal Science, C220 Animal Science, University of Nebraska, Lincoln, NE 68583, USA
[b] Animal and Dairy Science Department, University of Georgia, Athens, GA 30602, USA

Received 15 June 2005; received in revised form 15 August 2005; accepted 24 August 2005
Available online 13 September 2005

Abstract

Conjugated linoleic acid (CLA) induces a body fat loss that is enhanced in mice fed coconut oil (CO), which lacks essential fatty acids (EFA). Our objective was to determine if CO enhancement of CLA-induced body fat loss is due to the lack of EFA. The CLA–EFA interaction was tested by feeding CO and fat free (FF) diets for varying times with and without replenishment of individual EFA. Mice fed CO during only the 2-week CLA-feeding period did not differ from control mice in their adipose EFA content but still tended ($P=0.06$) to be leaner than mice fed soy oil (SO). Mice raised on CO or FF diets and fed CLA were leaner than the SO+CLA-fed mice ($P<0.01$). Mice raised on CO and then replenished with linoleic, linolenic, or arachidonic acid were leaner when fed CLA than mice raised on SO ($P<0.001$). Body fat of CO+CLA-fed mice was not affected by EFA addition. In summary, CO-fed mice not lacking in tissue EFA responded more to CLA than SO-fed mice. Also, EFA addition to CO diets did not alter the enhanced response to CLA. Therefore, the increased response to CLA in mice raised on CO or FF diets appears to be independent of a dietary EFA deficiency.

Keywords: Conjugated linoleic acid; Coconut oil; Essential fatty acid; Body fat; Mouse

1. Introduction

Conjugated linoleic acid (CLA) refers to a group of isomers of linoleic acid. Dietary CLA reportedly has anti-carcinogenic [1,2], anti-atherogenic [3–5], and anti-obesity [6,7] effects. Many of the effects can be attributed to specific CLA isomers. The isomer responsible for the induction of body fat loss is *trans*-10,*cis*-12 [6,7].

Linoleic and linolenic acids are dietary essential fatty acids. Linoleic acid (*cis*-9,*cis*-12) is metabolized to arachidonic acid by Δ6-desaturase, elongase, and Δ5-desaturase. Therefore arachidonic acid is conditionally essential, as it is not required in the diet if linoleic acid consumption is sufficient [8]. Linolenic acid is metabolized in the same manner to eicosapentaenoic acid, and then further elongated and desaturated to docosahexaenoic acid. CLA isomers are also metabolized via these enzymes to conjugated isomers of arachidonic acid [9–11]. The metabolites of CLA may contribute to the biological effects observed when CLA isomers are fed. Supporting this, CLA metabolites conjugated eicosadienoic (C20:2 *trans*-12,*cis*-14) and eicosatrienoic (C20:3 *cis*-8,*trans*-12,*cis*-14) acids have been shown to inhibit lipoprotein lipase activity and stimulate glycerol release in 3T3-L1 adipocytes similar to CLA [12]. Therefore, competition for the desaturase and elongase enzymes likely exists between these 18-carbon fatty acids and may impact the ability of CLA to induce a loss of body fat by altering the amount of CLA metabolized.

If this interaction between essential fatty acids and CLA exists, then diets with reduced essential fatty acid (linoleic, linolenic, and arachidonic acids) concentrations may allow for a greater effect of CLA. We previously determined that mice raised from weaning on 7% coconut oil diets (nearly devoid of essential fatty acids) for 6 weeks prior to the addition of CLA were leaner than mice raised on soy oil diets and supplemented with CLA [13]. Coconut oil-fed mice with heavier average weaning weights appeared to have lower coconut oil enhance-

☆ This manuscript has been assigned Journal Series No. 14930, Agricultural Research Division, University of Nebraska.

* Corresponding author. Tel.: +1 402 472 0518; fax: +1 402 472 6363.
E-mail address: jminer1@unl.edu (J.L. Miner).

1388-1981/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbalip.2005.08.016

ment of CLA-induced leanness (unpublished observations). This may indicate that heavier mice at weaning have more fat and therefore greater initial stores of essential fatty acids. These stores may be maintained to a greater degree and counteract the coconut oil effect. However, in addition to the difference in essential fatty acid concentration, coconut oil is more saturated than soy oil and has a higher concentration of medium chain fatty acids (8–12 carbons). Therefore, the difference in the response to CLA in mice fed coconut oil versus soy oil may be independent of the lack of dietary essential fatty acids.

The current studies were designed to determine if coconut oil enhancement of CLA-induced leanness is due to a dietary essential fatty acid deficiency. We used three approaches: (1) mice raised on a standard chow diet were fed coconut oil only during the 2-week CLA-feeding period; (2) mice raised on coconut oil were compared to mice raised on a fat free diet; and (3) individual essential fatty acids were replenished to mice raised on coconut oil.

2. Materials and methods

2.1. Animals and diets

Mice were obtained from the University of Nebraska Animal Science colony, housed individually, and maintained in a controlled environment at 22 °C under a photoperiod of 12 h light, 12 h dark. Mice used in all studies were from a control line four-way composite developed at the University of Nebraska [14,15] which was maintained without genetic selection.

Mice were fed a purified base diet (Modified AIN-93G) containing (g/kg): isolated soy protein 200, cornstarch 395.42, dextronized cornstarch 132, sucrose 100, cellulose 50, oil source 70, AIN-93G mineral mix 35, AIN-93G vitamin mix 10, L-cystine 2.54, L-methionine 2.54, and choline bitartrate 2.5 (Dyets, Inc., Bethlehem, PA). A modified AIN-93G with soy protein replacing casein was used in order to avoid the possibility of consumption of essential fatty acids or CLA from casein. Oil sources consisted of soy oil and hydrogenated coconut oil (Dyets, Inc.). Cornstarch replaced the oil source in the fat free diet (w/w). The CLA mixture contained approximately equal concentrations of the *cis*-9,*trans*-11 and *trans*-10,*cis*-12 isomers and was provided by BASF (Offenbach/Quiech, Germany). CLA was included in the diets to provide 0.5% of the diet as CLA isomers and replaced the oil source or cornstarch (w/w; 0.6% CLA-containing ingredient).

2.2. Animal protocol

The University of Nebraska Institutional Animal Care and Use Committee approved all animal procedures. Study 1 was designed to determine the extent of a CO–CLA interaction in mice with unaltered tissue EFA. Male mice ($n=24$, 15-week-old and $n=56$, 17-week-old) were allowed 1 week of adaptation to individual cages and purified diets. The mice were blocked by initial body weight (within age group) and assigned to one of four experimental diets: SO (7% soy oil); SO+CLA (soy oil+0.5% CLA isomers); CO (7% coconut oil); or CO+CLA (coconut oil+0.5% CLA isomers). Body weight and feed intake were measured weekly. Feed intake was measured as disappearance from glass jars equipped with a cylindrical stainless steel screen attached to a small-holed lid. No feed spillage was observed. Following 14 days of consumption of experimental diets the mice were killed and body fat and lean mass were determined by dual X-ray densitometry (PIXImus, Madison, WI). Densitometry analysis was conducted as described [16] except that mice were fasted 5 h and the measurements were made immediately following death by carbon dioxide asphyxia. Retroperitoneal and epididymal fat pads and livers were collected, weighed, flash frozen in liquid nitrogen, and stored at −80 °C.

Study 2 was designed to determine whether a fat free diet would interact with CLA in a similar manner to CO. Study 2 was also designed to determine if weaning age, and ultimately the amount of tissue EFA, would exaggerate the CO–CLA interaction. Male mice ($n=120$) were blocked by litter and assigned to one of two weaning dates. An earlier than average age of 18 days (compared to 21 days) was chosen to minimize the development of essential fatty acid stores in the early-weaned (EW) mice. Late-weaned (LW) mice were weaned 1 week later at an age of 25 days. Ninety mice were weaned at 18 days of age and allotted to one of three base diets: SO (7% soy oil); CO (7% coconut oil); or FF (fat free). The additional 30 mice were weaned at 25 days of age and fed the CO diet. After 6 weeks consuming the base diets, half of each group was randomly selected to receive 0.5% CLA isomers (in place of the base oil or cornstarch) for an additional 2 weeks. Body weight, feed intake, body fat, lean mass, and tissue weights were collected as in study 1.

Study 3 was designed to determine the extent to which specific EFA could neutralize the CO–CLA interaction. Three experiments of 80 weanling (3 weeks of age) male mice ($n=240$ total) were blocked by initial body weight and assigned to one of two base diets: SO (7% soy oil, $n=16$ mice) or CO (7% coconut oil, $n=64$ mice). Starting on day 40, the CO diet was replenished with no fatty acid, 1% linoleic acid, 0.1% linolenic acid, or 0.05% arachidonic acid (replacing coconut oil w/w). The individual essential fatty acids were fed to meet the dietary requirement of the mouse according to the Nutrient Requirements of Laboratory Animals, NRC 1995. On day 42 of the study, half of each group was randomly selected to receive 0.5% CLA isomers (in place of the base oil) for an additional 2 weeks. EFA replenishment was continued throughout the CLA-feeding period. Body weight, feed intake, body fat, lean mass, and tissue weights were collected as in studies 1 and 2, with the additional collection of subcutaneous fat pads.

2.3. Fatty acid analysis

Total fatty acids from retroperitoneal fat pads were extracted and methylated according to the method of Park and Goins [17], and fatty acid methyl esters were analyzed by gas chromatography as previously described [18]. Liver lipids were additionally analyzed in mice from study 3 as this is the primary site of fatty acid desaturation and elongation in rodents.

2.4. Statistical analysis

All data were analyzed using a fixed model testing the main effects and interaction of fat source (coconut oil vs. soy oil in study 1; coconut oil vs. fat free vs. soy oil in study 2; and all 5 oil/fatty acid combinations in study 3), and CLA vs. no CLA. In study 2, mice weaned at 25 days were excluded from the initial analysis. The data from mice fed coconut oil in study 2 were similarly analyzed testing the main effects and interaction of weaning age (18 vs. 25 days) and CLA vs. no CLA. F tests, least-squares means, and standard errors of the means were calculated using the Mixed procedures of SAS (SAS Inst. Inc., Cary, NC). Means were separated by least significant difference test in cases of significant fat source by CLA interaction. For all tests, $P<0.05$ was considered significant.

3. Results

3.1. Body weight and feed intake

In study 1, mice consuming coconut oil, regardless of CLA addition, weighed less ($P<0.05$) on the final day of the study than mice consuming soy oil (Table 1). There was a fat source by CLA interaction ($P<0.05$) on feed intake during both weeks of experimental diet feeding (Table 1). Mice fed the CO diet consumed more than those fed SO. The presence of CLA in the diet caused mice consuming coconut oil to eat less, but did not affect intake by mice consuming soy oil.