ELSEVIER

Nutrition Research 23 (2003) 651–658

NUTRITION RESEARCH

www.elsevier.com/locate/nutres

Apparent skeletal muscle loss related to dietary trans fatty acids in a mixed group of omnivores and vegetarians

Richard Hubbard[a,*], James Westerdahl[a], Albert Sanchez[b], Merritt Horning[c], Jacques Barth[d]

[a]*Departments of Nutrition and Pathology, Schools of Medicine and Public Health, Loma Linda Univ, Loma Linda, CA 92350, USA*
[b]*Pacific Health Education Center, Bakersfield, CA, USA*
[c]*Lassen Foundation, Chico, CA, USA*
[d]*Prevention Concepts, 1150 W. Olympic, Suite 10, Los Angeles, CA, USA*

Received 17 October 2002; received in revised form 12 February 2003; accepted 13 February 2003

Abstract

The well-fed free-living adult subjects in this study show evidence of skeletal muscle loss, evaluated from the increased levels of plasma alanine ($p < 0.0001$) and decreased urinary levels of isoleucine, leucine, lysine, glycine and alanine (all p values <0.005). Plasma fatty acid analysis showed low percentages of cis-linoleic acid associated with high percentages of both trans-linoleic acid and Mead's acid. Combining the fatty acid findings with the amino acid results in a multiple regression analysis revealed that the low levels of cis-linoleic acid are independently associated with high levels of both trans-linoleic acid ($p = 0.049$) and Mead's acid ($p = 0.0001$) and with low levels of both urinary alanine ($p = 0.047$) and plasma glycine ($p = 0.001$). These results suggest an interactive relationship between cis and trans linoleic acid that could easily disrupt prostaglandin control of absorption/utilization of the amino acids from dietary protein intake. © 2003 Elsevier Inc. All rights reserved.

Keywords: Skeletal muscle loss; Alanine; Isoleucine; trans-linoleic acid; Mead's acid

1. Introduction

We have been following a number of different individuals with indications of accelerated skeletal muscle loss, as evidenced by elevated plasma levels of the amino acid alanine with

* Corresponding author. Tel.: +1-909-558-4400 x85068; fax: +1-909-558-4095.
E-mail address: rwhubbard@sph.llu.edu (R. Hubbard).

0271-5317/03/$ – see front matter © 2003 Elsevier Inc. All rights reserved.
doi:10.1016/S0271-5317(03)00026-5

concurrent decreases in urinary alanine, isoleucine, and leucine levels as compared to our long-term reference ranges [1]. These findings also appeared to be associated with high lipid oxidation [2]. Also, we had studied the possibility that this skeletal muscle loss might be related to essential fatty acid deficiency [3], and concluding that current human intake of trans fatty acids most likely increases our essential fatty acid requirement [4].

The metabolic consequences of dietary trans fatty acids have been repeatedly studied with respect to the effect of these isomers on coronary heart disease [5,6,7]. The effect of trans fatty acids increase low density lipoproteins (LDL) and decrease high density lipoproteins (HDL) cholesterol levels [8,9] and lipoprotein (a) (Lp_a) [10,11]. In man, trans fatty acids appear to impair biosynthesis of long chain polyunsaturated fatty acids and even growth [12]. Physiological reactions to trans fatty acid ingestion leading to preeclampsia appear to be a potential associated risk in labor and delivery [13]. In addition the pathophysiological effects of trans fatty acids have been studied in retinal, liver and neural tissue as well as in platelet aggregation [14]. The primary adverse metabolic action of trans unsaturated fatty acid is the competitive inhibition of delta-6-desaturase [15]. This is well outlined by Simopoulos [16] relative to human metabolism and diabetes and by Sugano and Ikeda [17] in murine trans fatty acid metabolism. Also, the earlier work of Hill, et al [18] demonstrated an increased frequency of essential fatty acid deficiency in the rat caused by trans fatty acid ingestion.

There is a continuing unavoidable exposure to dietary trans fatty acids in our society from the partial hydrogenation of vegetable oils. Estimated intakes of trans fatty acids have been evaluated as mean percentage of energy ingested as trans fatty acids is 2.6% and mean percentage of total fat as trans fatty acids is 7.4% in the US population [19]. A Canadian margarine study suggests that only 11% of the trans fatty acid intake is from margarines and the majority of dietary trans fatty acids come from hidden fats in fast foods and bakery products [20]. European intake of trans fatty acids appears to be somewhat lower than the US [21].

The possible impairment of growth and body maintenance by trans fatty acids has been presented by Koletzko and coworkers [12,22] primarily in lipid metabolism, but in this study we present a relationship between amino acid metabolism relating to skeletal muscle breakdown versus cis and trans linoleic and Meads acid levels (the term Mead's acid refers to 5,8,11 eicosatrienoic acid which is synthesized from oleic acid, and which increases in amount when there is an essential fatty acid deficiency [23]). A preliminary report of our work on this topic has been presented [24]

2. Methods

An apparently healthy group of 17 women and 15 men, ages from 38 to 83 years (19 omnivores and 13 vegetarians) were recruited at Pacific Health Education Center public meetings. The subjects were active and asymptomatic, but 16 subjects had at least one of the major risk factors for coronary vascular disease. Adequacy of dietary intake was evaluated by food frequency questionnaire [25]. The blood plasma and 24-hr urine specimens were measured for amino acid content [26] and compared with the adult reference ranges in use at Loma Linda University Medical Center and at University of California Irvine Medical

Table 1
Summary of selected urinary and plasma amino acid values

Amino acids:	Reference range		Subjects (n = 32)			
	Low	High	Mean	Lower 95% confidence limit of mean	Upper 95% confidence limit of mean	% Out of range**
Urinary isoleusine (μG/24 hrs)	15	100	5.45*	3.98	6.96	100.0%
Urinary leucine (μG/24 hrs)	40	260	14.22*	9.28	19.16	93.8%
Urinary lysine (μG/24 hrs)	130	930	104.44*	43.60	165.28	87.5%
Urinary glycine (μG/24 hrs)	310	2840	942.16*	735.73	1148.58	9.4%
Urinary alanine (μG/24 hrs)	130	630	259.38*	205.48	313.26	15.6%
Plasma glycine (nG/mL)	130	450	266.75#	242.59	290.91	0.0%
Plasma alanine (nG/mL)	140	480	439.44*	394.66	484.22	37.5%

Note: * represents a significant difference from the mid reference range ($p < 0.0005$).
represents a difference of marginal significance $p = 0.056$.
**indicates the percentage of results that fell outside the reference range on the side consistent with the shift of the mean of the subjects. The expectation would be 2.5% and an observed percentage of 12.5% or more would be significant ($P < 0.05$).

Center [27]. Plasma fatty acids were analyzed by the methods of Moser et al., [28] at the Kennedy Krieger Laboratories at Johns Hopkins University, Baltimore Md. Consent forms were approved by the University of Southern California, School of Medicine, Department of Preventive Medicine through Dr. Barth. All subjects were advised of their rights and signed a copy of the consent form. All statistical analyses were carried out by the Microsoft Excel 2000 statistical package (copyright 1985-99 Microsoft Corp.) and verified with SYSTAT Version 10 (copyright SPSS 2000).

3. Results

Sample amino acid means compared to the mid points of the adult reference ranges showed significantly decreased means for urinary isoleucine, leucine, lysine and alanine by one sample t-test (all p values <0.0005)(Table 1). Plasma amino acids were essentially normal except for alanine levels that were significantly increased ($p < 0.0001$), suggesting mobilization of alanine from muscle (Table 1). A related increase of plasma glutamine was also found (Pearson $r = 0.416$ $p < 0.02$).

Analysis of the plasma fatty acids in the study sample (Table 2) revealed negative correlations between the percentages of cis-linoleic versus trans-linoleic acid ($r = -0.546$ $p < 0.005$) and versus Mead's Acid ($r = -0.571$ $p < 0.005$).

Table 3 shows a forward selection, multiple regression model (F-test $p < 0.0001$) predicting the relationship of cis-linoleic acid levels with trans-linoleic acid, Mead's acid, urinary alanine and plasma glycine levels. Low levels of cis-linoleic acid are associated with high levels of both trans-linoleic acid ($p < 0.05$), and Mead's acid ($p = 0.0001$) and low levels of both urinary alanine ($p < 0.05$) and plasma glycine ($p = 0.047$). A plot of measured

Table 2
Pearson correlation coefficients, means and SD's (n = 31)

	cis-linoleic acid (*cis-18:2w6*) [mean; SD = 29.61%; 4.42%]
trans-linoleic acid (trans-18:2w6) [mean; SD = 0.25%; 0.09%]	−0.546*
Mead's acid (cis 20:3w9) [mean; SD = 0.10%; 0.04%]	−0.571*

Note: * indicates a significant correlation ($p < 0.005$)

cis-linoleic acid versus its predicted value from the multiple regression model gives a multiple $R^2 = 0.623$ (Figure 1). Plotting each of the four variables against cis-linoleic acid shows that they predict better as a group in the multiple regression rather than separately (Figure 2). Figure 2 shows that the two most important predictors of the cis-linoleic acid fraction are the trans-linoleic acid fraction (F-test, $p = 0.002$) and Mead's acid fraction (F-test, $p = 0.0008$). While, the relationships of cis-linoleic acid with urinary alanine or plasma glycine are not significant. Trans to Mead's acid comparisons are only marginally significant a $r = 0.320$ and $p = 0.079$ possibly because increases of trans-linoleic acid cause an associated drop in cis-linoleic acid, which then subsequently causes an increase in Mead's acid levels.

4. Discussion

In the 1980's our plasma alanine reference range was 140-480 ng/mL, but in our present study the subjects present with an alanine range of 265-659 ng/mL. The high plasma alanine levels and the low urinary levels of several of the essential amino acids and alanine are readily identifiable in this study. These deviations of body fluid amino acid values are expressive of inadequate protein intake, but dietary intake is more than adequate. In effect, this is starvation in the seemingly well fed. This closely relates to secondary wasting and cachexia in older subjects, involving complex interactions of the gastrointestinal, endocrine and immune systems, that emerge as metabolic dysregulation and the loss of lean body mass [30].

The data from Table 2 suggests that trans fatty acids may cause an increased essential fatty

Table 3
Multiple regression model predicting the plasma fraction of cis-linoleic acid (n = 31). F-test $p < 0.0001$; adjusted squared multiple R = 0.623

Variable	Coefficient	Standardized coefficient	Probability (two-tailed)
Constant	0.29837	0.0000	0.0000
trans-linoleic acid fraction	−13.33685	−0.2571	0.0485
Mead's acid fraction	−62.81198	−0.5894	0.0001
Urinary alanine	0.00007	0.2396	0.0473
Plasma glycine	0.00028	0.4301	0.0014

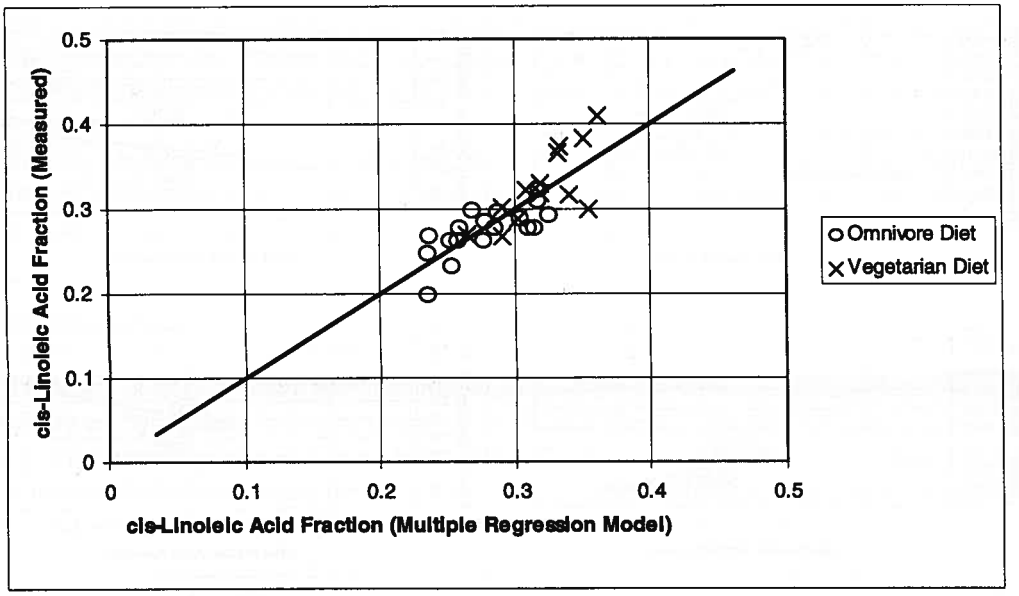

Fig. 1. Plot of the measured plasma fraction of cis-linoleic acid against its predicted value from a multiple regression model composed of the plasma fractions of trans-linoleic acid and Mead's acid plus the levels of plasma glycine (nG/mL plasma) and urinary alanine (uG/24 Hr urine sample). Multiple R squared = 0.623. Note Vegetarians have higher predicted and observed cis-linoleic acid plasma fractions.

acid requirement that in turn appears to correlate with an increased Mead's acid production. Because cis-linoleic acid is in the middle of this chain of cause and effect it is significantly correlated with both ends. Trans-linoleic acid and Mead's acid, however, are not as well correlated due to the compounding error of the multiple links between them. Sugano and Ikeda [17] in experimental animals, demonstrated that trans-fatty acids exacerbate essential fatty acid deficiency by interfering with the metabolism of linoleic (18:2n-6) and alpha-linolenic (18:3n-3) acids and hence with eicosanoid and prostaglandin production.

The multiple regression model (Table 3) of cis-linoleic acid versus trans-linoleic acid, Mead's acid, urinary alanine and plasma glycine percentages indicates a high probability that the two amino acids are metabolically interrelated to trans-fatty acid levels. Visualizing the measured variability of the percent cis-linoleic acid as a pie graph, trans-linoleic acid and Mead's acid account for large pieces of the pie while alanine and glycine are only slivers, accounting for only an insignificant fraction of the total cis-linleic variability. However, multiple regression, in effect, cuts the variability pie into pieces with each variable only having to predict its unique piece. Furthermore, even though other amino acids may have also been out of range, they would not have been included in the model unless they were associated with a unique piece of the cis-linoleic acid variability. As it is, the other amino acids only echo the predictive contribution of alanine and glycine and add no new information to the model. The decreased plasma glycine levels can be due to decreased dietary protein intake/utilization, which is known to decrease glycine synthesis [31].

The link between skeletal muscle loss and an increased need for cis-linoleic acid because

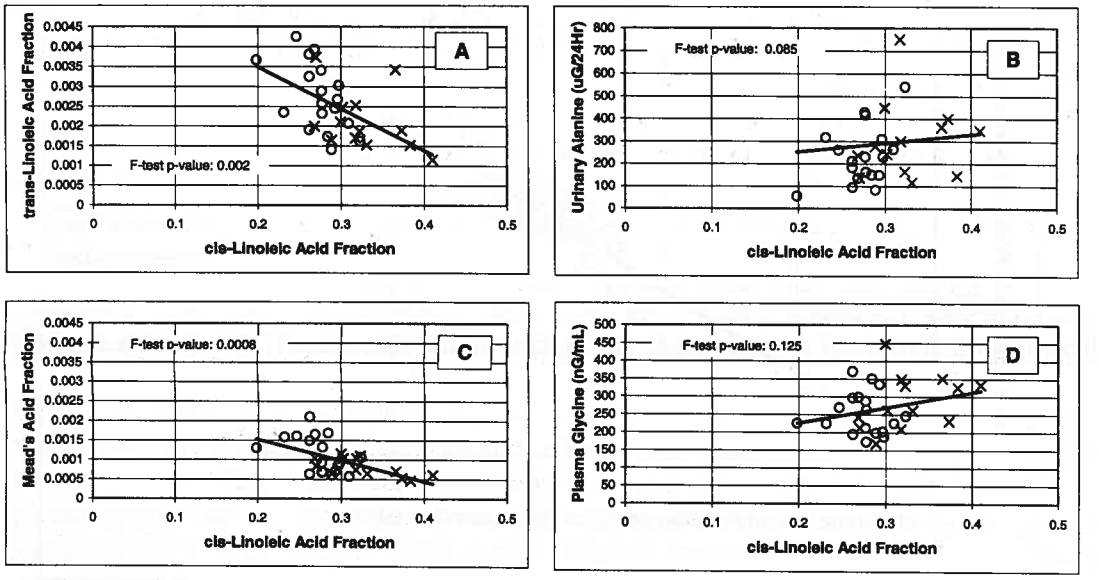

Fig. 2. Individual simple linear regression plots (Omnivore [O] and Vegetarian [X]) of the terms in the multiple regression model against plasma fraction of cis-linoleic acid. Note that in isolation plasma glycine and urinary alanine are not significantly related to the fraction of cis-linoeic acid as indicated by the F-tests.

of trans fatty acid intake, is strengthened by the previous proposal by Chevilotte et al [31] that fatty acids (omega-6 fatty acids) regulate uncoupling protein (UCP-2) gene expression in skeletal muscle involving at least protein kinase A, and the nuclear receptor PPARbeta. Also, omega-6 rather than omega-3 fatty acids increase food intake and weight gain [32]. The related PPARalpha regulates metabolism of amino acids in liver in addition to oxidation of fatty acids [33]. PUFA also coordinately regulates the expression of enzymes involved in carbohydrate and lipid metabolism [34].

The persistent dietary intake of trans fatty acids by the American public, is associated with a continuing chain of health risk. Salmeron et al [35] state that trans fatty acids increase while polyunsaturated fatty acids decrease the risk of type-2 diabetes in women. This is similar to the risks of diabetes described earlier by Simopoulos [16]. The work of Ozanne et al [36] on the effects of maternal low protein diet in rats and the resulting effects on fatty acid desaturase activity in the offspring, links fetal growth retardation and insulin resistance. This is further evidence of the inter-relationship of restricted dietary protein intake and essential fatty acid metabolism albeit in the offspring. In pilot programs [1,3] we have used supplemental omega-6 essential fatty acids to prevent loss of lean body mass in three subjects with amyotrophic lateral sclerosis (ALS), three that were failure to thrive seniors, four subjects with multiple sclerosis, four subjects with chronic fatigue syndrome, four children with anticonvulsant medication resistant seizures and one child with developmental delay. Significant improvements in both humeral and neuronal symptoms were very evident in these pilot programs. Details of these experiments are to be presented elsewhere.

From this study and our prior reports [1,3,4], and the work of Sugano and Ikeda [17] and Hill [18], we believe there is an increased requirement for essential fatty acids caused by the

intake of trans fatty acids. In turn this is related to skeletal muscle breakdown from decreased protein absorption/utilization that subsequently impedes protein synthesis. Any obstruction of essential fatty acids is certainly going to effect prostaglandin synthesis, which means that these major controllers of body physiology can be significantly altered. We are still only part of the way towards identifying what this means. However, we now have more than enough evidence to know that we must significantly decrease our continued consumption of trans fatty acids in our food chain [30].

Acknowledgments

The authors acknowledge the financial support of the Department of Pathology, School of Medicine, Loma Linda University, and both Pacific Health Education Center, and the San Joaquin Community Hospital, from Bakersfield California, and Vespro LLC, Lenexa Kansas. The assistance of Ann Moser, for her advise on fatty acid analysis and data from the Kennedy Krieger Institute, is gratefully acknowledged.

References

[1] Hubbard RW, Westengard J, Sanchez A, Barth J. Chronic gluconeogenesis lowers urinary amino acids. Exp Biol 1999;13(5):935, #688.19.
[2] Hubbard RW, Westengard J, Sanchez A, Barth J. Chronic gluconeogenesis with high lipid oxidation in a normal control population. Exp Biol 1999;13(5):935, #688.20.
[3] Hubbard RW, Horning M. Daytime gluconeogenesis in the young and old apparently caused by essential fatty acid deficiency. Exp Biol 1997;11(3):A160, #935.
[4] Chisholm A, Mann J, Skeaff M. Trans fatty acids: a cause for concern? Inter J Food Sci and Clin Nutr 1995;46:171–6.
[5] Mann GV. Metabolic consequences of dietary trans fatty acids. Lancet 1994;343:1268–71.
[6] Willett WC, Stampfer MJ, Manson JE, Colditz GA, Speizer FE, Rosner BA, Sampson LA, Hennekens CH. Intake of trans fatty acids and risk of coronary heart disease among women. Lancet 1993;341:581–5.
[7] Bolton-Smith C, Woodward M, Fenton S, Brown CA. Does dietary trans fatty acid intake relate to the prevalence of coronary heart disease in Scotland? Eur Heart J 1996;17:837–45.
[8] Mensink RP, Temme EHM, Hornstra G. Dietary saturated and trans fatty acids and lipoprotein metabolism. Ann Med 1994;26:461–4.
[9] Noakes M, Clifton PM. Oil blends containing partially hydrogenated or inter-esterified fats: differential effects on plasma lipids. Am J Clin Nutr 1998;68:242–7.
[10] Nestel P, Noakes M, Belling B, McArthur R, Clifton P, Janus E, Abbey M. Plasma lipoprotein lipid and Lp(a) changes with substitution of elaidic acid for oleic in the diet. J Lipid Res 1992;33:1029–36.
[11] Lichtenstein AH. Trans fatty acids and blood lipid levels, Lp(a), parameters of cholesterol metabolism, and hemostatic factors. Nutr Biochem 1998;9:244–8.
[12] Koletzko B. Trans fatty acids may impair biosynthesis of long-chain polyunsaturates and growth in man. Acta Paediatr 1992;81:302–6.
[13] Williams MA, King IB, Sorensen TK, Zinghein RW, Troyer BL, Zebelman AM, Luthy DA. Risk of preeclampsia in relation to elaidic acid trans fatty acid in maternal erythrocytes. Gynecol Obstet Invest 1998;46:84–7.
[14] Chardigny JM, Sebedio JL, Grandgirard QA. Possible physiological effects of trans polyunsaturated fatty acids. Editors Sinclair A, Gibson R., Third International Congress of the American Oil Chemists Society, Champaign, Illinois 1992, pgs 148–52.

[15] Booyens J, Van Der Merwe CF. Margarines and coronary artery disease. Med Hypothesis 1992;27:241–4.
[16] Simopoulos AP. Omega-6/Omega-3 fatty acid ratio and trans fatty acids in non-insulin-dependent diabetes mellitus. Lipids and Syndromes of Insulin Resistance Annals of the New York Academy of Sciences 1997;827:327–38.
[17] Sugano M, Ikeda I. Metabolic interactions between essential and trans-fatty acids. Curr Opin Lipidol 1996;7:38–42.
[18] Hill EG, Johnson SB, Holman RT. Intensification of essential fatty acid deficiency in the rat by dietary trans fatty acids. J Nutr 1997;109:1759–65.
[19] Allison DB, Egan K, Barraj LM, Caughman C, Infante M, Heimbach JT. Estimated intakes of trans fatty and other fatty acids in the US population. J Am Diet Assoc 1999;99:166–74.
[20] Ratnayake WMN, Pelleltier G, Hollywood R, Bacler S, Leyte D. Trans fatty acids in Canadian margarines: recent trends. JAOCAS 1998;75:1587–94.
[21] van Poppel G, on behalf of the Transfair Study Group. Intake of trans fatty acids in Western Europe: the Transfair study. Lancet 1998;351:1099.
[22] Decsi T, Koletzko B. Do trans fatty acids impair linoleic acid metabolism in children? Ann Nutr Metab 1995;39:36–41.
[23] Fulco AJ, Mead JF. Metabolism of essential fatty acids: VIII. Origin of 5,8,11-eisosatrienoic acid in the fat deficient rat. J Biol Chem 1959;234:1411–16.
[24] Hubbard RW, Westengard J, Sanchez A. Trans-linoleic acid and its metabolic comparisons in an adult group of omnivores and vegans. Exp Biol 2002;16(5),A988, 727.10.
[25] DeHoog S. The assessment of nutritional status. In: Mahan LK, Escott-Stump S, editors. Krause's Food Nutrition and Diet Therapy. New York: WB Saunders, 1996. p. 351–86.
[26] Hubbard RW, Chambers JG, Sanchez A, Slocum R, Lee P. Amino acid analysis of plasma: studies in sample preparation. J Chrom 1988;431:163–9.
[27] Shaw, K, Hubbard, RW. A cooperative plasma and urine amino acid reference range project between Children's Hospital of Los Angeles and Loma Linda University. Unpublished results 1987.
[28] Moser A, Jones DS, Raymond GV, Moser HW. Plasma and red blood cell fatty acids in peroxisomal disorders. Neurochem Res 1999;24:187–96.
[29] Raiten DJ. Introduction: Clinical trials for the treatment of secondary wasting and cachexia. J Nutr 1999;1229:223S–4S.
[30] Yu YM, Yang RD, Matthews DE, Wen ZM, Burke JF, Bier DM, Young VR. Quantitative aspects of glycine and alanine nitrogen metabolism in post absorptive young men: effects of level of nitrogen and dispensable amino acid intake. J Nutr 1985;115:399–410.
[31] Chevilotte E, Rieusset J, Roques M, Desage M, Vidal H. The regulation of uncoupling protein-2 gene expression by omega-6 polyunsaturated fatty acids in human skeletal muscle cells involves multiple pathways, including the nuclear receptor peroxisome proliferator-activated receptor beta. J Biol Chem 2001;276:10853–60.
[32] Nakamura MT, Cho HP, Clarke SD. Regulation of hepatic delta-6-desaturase expression and its role in the polyunsaturated fatty acid inhibition of fatty acid synthase gene expression in mice. J Nutr 2000;130: 1561–5.
[33] Kersten S, Mandard S, Escher P, Gonzalez FJ, Tafuri S, Desvergne B, Wahli W. The peroxisome proliferators-activated receptor alpha regulates amino acid metabolism. FASEB J 2001;15:1971–8.
[34] Jump DB, Clarke SD, Thelen A, Liimatta M. Coordinate regulation of glycolytic and lipogenic gene expression by polyunsaturated fatty acids. J Lipid Res 1994;35:1076–84.
[35] Salmeron J, Hu FB, Manson JE, Stampfer MJ, Colditz GA, Rimm EB, Willet WC. Dietary fat intake and risk of type 2 diabetes in women. Am J Clin Nutr 2001;73:1019–26.
[36] Ozananne SE, Martensz ND, Petry CJ, Loizou CL, Hales CN. Maternal low protein diet in rats programmed fatty acid desaturase activities in the offspring. Diabetologia 1998;41:1337–42.