British Journal of Nutrition (2006), **95**, 947–954
DOI: 10.1079/BJN20061756

Effects of dietary *cis* and *trans* unsaturated and saturated fatty acids on the glucose metabolites and enzymes of rats

Claudio A. Bernal[1,2]*, Jordi Rovira[3], María E. Colandré[1], Roser Cussó[3] and Joan A. Cadefau[3]

[1]*Cátedra Bromatología y Nutrición, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, Santa Fe, Argentina*
[2]*Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Santa Fe, Argentina*
[3]*Departamento de Ciencias Fisiológicas I, Facultad de Medicina, Universidad de Barcelona, Instituto de Investigaciones Biomédicas August Pi i Sunyer (IDIBAPS), Barcelona, Spain*

(Received 20 July 2005 – Revised 12 January 2006 – Accepted 24 January 2006)

The aim of the present study was to examine whether the level of dietary *cis* fatty acid (*c*FA), or the isomers (*trans* or *cis*) and/or the saturation of the fatty acids at high dietary fat levels altered the intracellular glucose metabolites and certain regulatory enzyme activities in the skeletal muscle and liver of rats. The animals were fed for 30 d on either a recommended control diet (7 % *c*FA, w/w) or a high-fat diet (20 % fatty acids, w/w). The high-fat diet was enriched with either *c*FA, *trans* fatty acid (*t*FA), a moderate proportion of saturated fatty acid (MSFA), or a high proportion of saturated fatty acid (HSFA). The most striking findings were observed in the gastrocnemius muscle with a HSFA diet. There was a significant increase in glucose-6-phosphate (306 %), glucose-1-phosphate (245 %), fructose-6-phosphate (400 %), fructose-1,6-bisphosphate (86 %), glyceraldehyde-3-phosphate (38 %), pyruvate (341 %), lactate (325 %), citrate (79 %) and the bisphosphorylated sugars as compared with the *c*FA diet. These changes were paralleled by an increase in muscle triacylglycerol content (49 %) and a decrease in glucose (39 %). In addition, the amount of *c*FA and the other types of fatty acid (i.e. *t*FA and MSFA) led to no great differences in glucose metabolism as compared with the respective control group. These data support the hypothesis that glucose changes induced by a HSFA diet are a multifaceted abnormality. Glucose and lactate transport and intracellular glucose metabolism could be the key biochemical defects involved in this detrimental effect on glucose metabolism.

***Trans* fatty acids: Saturated fatty acids: Dietary fat: Glucose metabolism**

Many epidemiological and experimental studies (Lichtenstein *et al.* 1998; Grundy *et al.* 2002; Riccardi *et al.* 2004) suggest that the amount and type of fats consumed influence the development and subsequent progression of several major diseases, including CHD, obesity, diabetes and cancer. It is widely known that a high intake of saturated fatty acids (SFA) (Storlien *et al.* 1991; Alsaif & Duwaihy, 2004) and *trans* fatty acids (*t*FA) (Clevidence *et al.* 1997; Ascherio *et al.* 1999) has an adverse impact on the plasma lipid profile, and increases the risk of developing CVD and type 2 diabetes.

The effect of diets high in fatty acids on glucose metabolism in human subjects and in experimental animal models is well documented in the literature. For example, Uusitupa *et al.* (1994) reported that in normal female subjects a high-fat diet (total fat energy 40 %) reduces the glucose tolerance; similarly, in their thorough review Lichtenstein & Schwab (2000) showed that impaired glucose tolerance and insulin resistance are induced by high-fat feeding in experimental animals. Such effects are also influenced by the dietary fatty acid composition. Animals fed on high-SFA diets develop glucose intolerance (Wang *et al.* 2002) and impairment of insulin action in both skeletal muscle and liver (Storlien *et al.* 1991; Oakes *et al.* 1997). Kim *et al.* (1996) reported that when rats are fed a high-saturated fat diet, glycolysis is suppressed and glycogen synthesis is altered in skeletal muscle. They hypothesised that these changes might lead to the development of insulin resistance. However, whereas *n*-3 PUFA improve insulin action, *n*-6 PUFA have a slight negative impact on insulin sensitivity (Storlien *et al.* 1991; Mohan & Das, 2001; Taouis *et al.* 2002).

Several studies (Judd *et al.* 1994; Zock & Katan, 1997) have shown that *t*FA increase both total and LDL-cholesterol levels, resembling some of the dietary high proportion of SFA (HSFA) effects. Although a number of conflicting results have been described, some studies indicate that *t*FA decreases HDL-cholesterol (Judd *et al.* 1994) and increases plasma lipoprotein (a) levels (Aro *et al.* 1997). The specific effect of dietary *t*FA on the glucose metabolic pathway, as compared with

Abbreviations: *c*FA, *cis* unsaturated fatty acid; F-1,6-P_2, fructose-1,6-bisphosphate; F-2,6-P_2, fructose-2,6-bisphosphate; F-6-P, fructose-6-phosphate; G-1-P, glucose-1-phosphate; G-1,6-P_2, glucose-1,6-bisphosphate; G-6-P, glucose-6-phosphate; HSFA, high saturated fatty acid; MSFA, moderate saturated fatty acid; PFK-1, 6-phosphofructo-1-kinase; SFA, saturated fatty acid; *t*FA, *trans* unsaturated fatty acid.

* **Corresponding author:** Professor Claudio Bernal, fax +54 342 4575221, email cbernal@fbcb.unl.edu.ar

SFA and/or *cis* fatty acid (*c* FA) at high fat levels, has not been determined. Dietary *t* FA might exert different effects on the glucose oxidation pathway, as they do on lipid metabolism. In this regard, Louheranta *et al.* (1999) showed that a *t* FA diet has no effect on glucose and insulin metabolism as compared with a MUFA diet in young healthy women. Alternatively, Alstrup *et al.* (1999) demonstrated that in mouse β cells *t* FA potentiates glucose-stimulated insulin secretion more than *c* FA of the same carbon-chain length. This is probably due to a differential effect on glucose oxidation. Cromer *et al.* (1995) demonstrated that *t* FA might inhibit glucose oxidation in adipocytes isolated from rats.

Therefore, high levels of dietary fatty acid might alter glucose tolerance. This effect appears to be dependent on the type of fatty acid. We can also assume that a suppression of intracellular glucose metabolism by competition for substrate oxidation might precede and/or cause insulin resistance in skeletal muscle. On the basis of these assumptions, we aimed to evaluate the glucose metabolites and some key enzyme activities involved in skeletal muscle and liver glucose metabolism on an experimental animal model. The variables were: (1) the amount of dietary *c* FA; (2) the isomers (*trans* or *cis*) and/or the degree of saturation (high or moderate) of dietary fat.

Materials and methods

Materials

Nutrients for diet preparations were chemical grade, with the exception of maize oil (Mazola, Argentina), sucrose, cellulose, and maize starch, which were commercial grade obtained from a local source. The same maize oil was used to prepare both isomerised and saturated fats, as previously described (Colandré *et al.* 2003). Biochemical reagents, enzymes and standards were purchased from Sigma Chemical Co. (St Louis, MO, USA) or Roche Molecular Biochemicals (Barcelona, Spain). Plasma glucose kits were purchased from Wiener Co. (Rosario, Argentina). Catalytic palladium in charcoal was from Alpha Aesar Co. (Ward Hill, MA, USA). Solvents and reagents used for quantifying fatty acids were chromatography grade. All the other chemicals used were of the highest analytical grade.

Animals, diets and tissue preparation

All the animal studies were conducted in accordance with the principles of our School of Biochemistry regulations, compiled using the *Guide to the Care and Use of Experimental Animals of the Laboratory* (Institute of Laboratory Animal Resources *et al.* 1996). Male Wistar rats weighing 80–100 g were supplied by the Comisión Nacional de Energía Atómica (Buenos Aires, Argentina). They were housed in animal quarters under controlled conditions (23 ± 2°C and 12 h light–dark cycle) in individual cages. The animals had free access to water and a standard diet until reaching a weight of 120–130 g. After this, they were randomly divided into five weight-matched groups and fed each diet *ad libitum* for 30 d.

All diets were nutritionally adequate and differed either in the amount of *c* FA, or in the fatty acid composition (Table 1). The control diet was based on the American Institute of Nutrition Ad Hoc Committee recommendation (AIN-93G diet formulated for the growth, pregnancy and lactation phases of rodents; Reeves *et al.* 1993). It contained 70 g fat/kg. All other diets were enriched by replacing carbohydrate with fat, to attain 200 g/kg (38·5 % energy as fat). These enriched fat diets contained 30 g maize oil/kg to exceed the essential fatty acid recommendations, and fats with different compositions (170 g/kg). The *c* FA diet was enriched with 170 g *c* FA/kg (from maize oil). The *t* FA diet was enriched with isomerised maize oil (170 g/kg) containing 300 g *t* FA/kg. The moderate proportion of SFA (MSFA) diet was enriched with MSFA based on a blend of maize oil (119 g/kg) and hydrogenated maize oil (59 g/kg), and the HSFA diet was enriched with SFA from hydrogenated maize oil (170 g/kg).

The preparation of the experimental dietary fats and the methodology for evaluating their fatty acid composition was recently described (Colandré *et al.* 2003). The fatty acid composition of the experimental fats used is shown in Table 2. Each diet was freshly prepared every 3 d during the experimental period.

Body weight and food consumption were measured throughout the experimental period. After 30 d of dietary treatment, rats were anaesthetised with acepromazine (1 mg/kg body weight) and ketamine (100 mg/kg body weight) and blood was collected in heparinised tubes. After blood

Table 1. Composition of the experimental diets*

Ingredient	Control diet	*c* FA diet	*t* FA diet	MSFA diet	HSFA diet
Maize starch (g/kg diet)	529·5	399·5	399·5	399·5	399·5
Casein (g/kg diet)	200	200	200	200	200
Sucrose (g/kg diet)	100	100	100	100	100
Maize oil (g/kg diet)	70	200	30	149	30
Isomerised maize oil (g/kg diet)	–	–	170	–	–
Hydrogenated maize oil (g/kg diet)	–	–	–	51	170
Fibre (g/kg diet)	50	50	50	50	50
Mineral mixture (g/kg diet)	35	35	35	35	35
Vitamin mixture (g/kg diet)	10	10	10	10	10
L-Cystine–L-methionine (g/kg diet)	3·0	3·0	3·0	3·0	3·0
Choline (g/kg diet)	2·5	2·5	2·5	2·5	2·5
Energy (kJ/100 g)	1656·9	1928·8	1928·8	1928·8	1928·8

c FA, *cis* fatty acid; *t* FA, *trans* fatty acid; MSFA, moderate level of saturated fatty acid; HSFA, high level of saturated fatty acid.
* Vitamin and mineral mixtures were prepared according to Reeves *et al.* (1993).

Table 2. Fatty acid composition of experimental fats
(Mean values)

Fatty acids	Experimental maize oil (weight percentage (w/w) of the total fatty acid methyl esters)		
	Non-treated	Isomerised	Hydrogenated
16 : 0	10·78	10·87	11·23
c 16 : 1	0·20	0·10	0·15
18 : 0	2·24	2·61	59·33
*t*18 : 1	ND	26·47	10·47
*c*18 : 1	31·36	25·41	17·22
t,*t*18 : 2	ND	2·09	ND
t,*c*18 : 2 + *c*, *t*18 : 2	0·48	1·35	ND
c,*c*18 : 2	52·85	29·57	0·50
t,*c*,*c*18 : 3	0·06	0·03	ND
c,*c*,*c*18 : 3	0·75	0·41	ND
20 : 0	0·52	0·52	0·68
*t*20 : 1	0·12	0·07	ND
*c*20 : 1	0·24	0·13	0·22
22 : 0	0·20	0·18	0·12
24 : 0	0·20	0·19	0·08
% *Trans* fatty acids	0·66	30·00	10·47
% Saturated fatty acids	13·94	14·38	71·44

c, *cis*; *t*, *trans*; ND, not detected.

collection, samples of gastrocnemius muscle and liver were taken. Plasma was separated by centrifugation at 3000 ***g*** for 10 min. Sample tissues were immediately frozen at −80°C and stored until analysis. For glycolytic metabolites and enzyme analysis, a portion of each sample was lyophilised and stored under dry conditions at −20°C.

Analysis of muscle and liver glycogen concentrations

Glycogen was extracted from about 4–5 mg dry tissue by acid treatment at 100°C (Adamo & Graham, 1998). The glucose produced was measured using the enzymic method with fluorimetric techniques (Lowry & Passonneau, 1972).

Analysis of metabolite concentrations

Plasma glucose levels were determined using commercial kits (Wiener, Rosario, Argentina).

ATP, creatine, phosphate creatine, glucose, glucose-6-phosphate (G-6-P), glucose-1-phosphate (G-1-P), fructose-6-phosphate (F-6-P), fructose-1,6-bisphosphate (F-1,6-P_2), dihydroxyacetone-phosphate, glyceraldehyde-3-phosphate, lactate, pyruvate and citrate were extracted by acid treatment from 3–5 mg dry muscle and liver tissue in ice-cold 0·5 M-perchloric acid. After centrifugation and neutralisation, samples of the extracts were used. Measurements were carried out using enzymatic methods with fluorimetric techniques (Lowry & Passonneau, 1972).

About 5–10 mg dry tissue powder was homogenised in 5 volumes of 50 mM-NaOH and kept at 90°C for 10 min. The extracts were neutralised with ice-cold acetic acid and the soluble materials were used to measure fructose-2,6-bisphosphate (F-2,6-P_2) (van Schaftingen *et al.* 1982) and glucose-1,6-bisphosphate (G-1,6-P_2) (Passonneau *et al.* 1969). To minimise the effect of blood and connective tissue on the freeze-dried tissue, the total creatine content (sum of phosphate creatine and creatine) for a given individual sample was determined. It was then used to adjust individual metabolite concentrations (Sabina *et al.* 1984).

Determination of liver and muscle triacylglycerol content

Fresh liver and muscle tissue samples were homogenised in 10 volumes of saline solution and used to measure triacylglycerol, by the method of Laurell (1966).

Analysis of enzyme activities

Powdered muscle and liver were homogenised in 70 volumes (w/v) of ice-cold extraction buffer (containing 50 mM-tri(hydroxymethyl)-aminomethane-HCl, 4 mM-EDTA, 30 mM-KF, 30 mM-β-mercaptoethanol; pH 7·0). The homogenate was centrifuged at 12 000 ***g*** for 15 min at 4°C and the supernatant fraction was used to measure the activities of 6-phospho-fructo-1-kinase (PFK-1) (Cadefau *et al.* 1990). Citrate synthase activity in liver and muscle was assessed according to Bass *et al.* (1969).

Statistics

We used the unpaired Student's *t* test to determine the statistical differences between dietary levels of *cis* unsaturated fats (control diet *v.* *c* FA diet). Comparisons of fatty acid composition at high level of dietary fats (200 g/kg) were established by one-way ANOVA (1 × 4). When ANOVA showed significant differences between dietary groups, the Tukey test was performed. All differences were considered significant at $P<0·05$. Values are expressed as means with their standard errors of five or six animals per group.

Results

No differences could be observed between the diets in either average daily food energy intake (control diet 273·6 (SE 13·1) kJ/d, *c* FA diet 265·0 (SE 6·8) kJ/d, *t* FA diet 247·7 (SE 4·2) kJ/d, MSFA diet 254·9 (SE 11·5) kJ/d, HSFA diet 270·7 (SE 21·2) kJ/d; $P>0·05$) or body-weight gain (control diet 133·9 (SE 6·6) g/30 d, *c* FA diet 139·2 (SE 4·1) g/30 d, *t* FA diet 136·3 (SE 4·3) g/30 d, MSFA diet 133·6 (SE 6·5) g/30 d, HSFA diet 138·7 (SE 4·3) g/30 d; $P>0·05$). No differences could be found between the groups in the levels of metabolites related to the energetic content of the tissues, specifically ATP in muscle and liver and total creatine in muscle (Table 3). These metabolites also reflected storage conditions and manipulation of the samples.

When levels of *c* FA were considered, no differences were found in plasma glucose concentrations under fasted conditions, or in muscle and liver triacylglycerol content (Table 3). However, the type of fatty acid in the high-fat diets affected plasma glucose concentrations and muscle triacylglycerol content. These were both higher in the HSFA diet ($P<0·05$ *v.* *c* FA). Liver triacylglycerol content was higher in *t* FA, MSFA and HSFA diets ($P<0·05$ *v.* *c* FA).

Compared with the recommended levels of fat, high levels of *c* FA significantly decreased pyruvate concentration and increased F-6-P, G-1,6-P_2 and F-2,6-P_2 in muscle (Table 4). No differences were observed in all the other metabolites

Table 3. Plasma glucose and triacylglycerol (TG), and ATP and creatine content in muscle and liver†
(Mean values with their standard errors of five or six animals per group)

	Control diet		*c*FA diet		*t*FA diet		MSFA diet		HSFA diet	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Plasma glucose (mM)	6·50	0·17	6·11	0·11	6·61	0·28	5·85‡	0·22	7·56*	0·44
Gastrocnemius TG (μmol/g wet tissue)	1·45	0·34	1·86	0·04	2·40	0·57	1·50	0·04	2·77*	0·21
Liver TG (μmol/g wet tissue)	8·10	0·06	8·02	0·71	17·09*	1·48	14·27*	1·38	13·29*	0·89
Gastrocnemius ATP (μmol/g dry tissue)	25·56	1·62	28·50	2·44	26·56	2·15	25·92	1·25	26·59	1·07
Liver ATP (μmol/g dry tissue)	7·08	0·39	6·79	0·31	7·11	0·31	7·44	0·25	8·25	0·26
Gastrocnemius total creatine (μmol/g dry tissue)	119·7	4·4	115·5	6·1	117·5	3·2	113·7	5·9	115·9	2·9

*c*FA, *cis* unsaturated fatty acid; *t*FA, *trans* unsaturated fatty acid; MSFA, moderate saturated fatty acid; HSFA, high saturated fatty acid.
* Mean value was significantly different from that for the *c*FA diet ($P<0·05$).
† The comparison of *cis* fatty acid levels was not significantly different, by unpaired Student's *t* test, at $P<0·05$. Comparison of fatty acid composition at high dietary fat levels was established by ANOVA (1 × 4) followed by the Tukey test. For details, see p. 949.
‡ *n* 3.

analysed in muscle or liver. In addition, no changes in muscle PFK-1 and citrate synthase or in hepatic PFK-1 and citrate synthase activities were observed after 30 d of the high-*c*FA-level diet.

The effects of different compositions of fatty acids at high fat levels on muscle and liver metabolites are shown in Fig. 1, Fig. 2, Fig. 3 and Fig. 4. Thus, Fig. 1 shows a significant reduction in glucose levels and a significant increase of G-6-P, F-6-P and F-1,6-P_2 in the gastrocnemius muscle of rats fed a HSFA diet as compared with a *c*FA diet. In addition, muscle G-6-P and F-6-P were increased in HSFA *v.* tFA and *v.* MSFA diets; and muscle F-6-P in the HSFA diet *v.* the MSFA diet. No differences were observed in muscle and liver glucose, G-6-P, F-6-P and F-1,6-P_2 between the *c*FA, tFA and MSFA diets. In gastrocnemius muscle, the HSFA diet significantly increased the G-1-P levels as compared with any other type of high-fat-level diet. The glycogen level was slightly reduced by the HSFA diet, reaching statistical significance only between the HSFA diet and the MSFA diet (Fig. 2) in muscle. No differences were detected in hepatic G-1-P and glycogen content between the high-dietary fat groups. The HSFA diet increased the muscle pyruvate, lactate and citrate levels *v.* *c*FA, tFA or MSFA diets. The *t*FA diet increased the muscle pyruvate levels *v.* the *c*FA diet. Surprisingly, the HSFA diet also significantly increased the citrate levels in liver, with no changes in the other metabolites analysed (Fig. 3). G-1,6-P_2 and F-2,6-P_2 were not changed by any type of fatty acid at high levels of fat intake either in muscle or liver. However, in muscle both bisphosphorylated sugars were significantly higher in HSFA diets with regard to the control diet (Fig. 4).

Fig. 5 shows regulatory enzyme activities in the muscle and liver of rats fed high fat levels with different compositions of fatty acid. No differences in PFK-1 and citrate synthase

Table 4. Effect of *cis* unsaturated fat level on glycolytic metabolites contents and enzyme activities in the muscle and liver
(Mean values with their standard errors of five or six animals)

	Muscle				Liver			
	Control diet†		*c*FA diet‡		Control diet†		*c*FA diet‡	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Glycogen content (μmol glucose/g dry tissue)	125·9	3·3	131·7	4·5	887·1	76·0	793·2	46·8
Glucose content (μmol/g dry tissue)	2·91	0·36	2·88	0·32	26·3	1·4	25·4	2·2
G-1-P content (μmol/g dry tissue)	0·14	0·02	0·11	0·02	0·17	0·02	0·18	0·02
G-6-P content (μmol/g dry tissue)	0·71	0·16	1·12	0·13	1·39	0·16	1·02	0·08
F-6-P content (μmol/g dry tissue)	0·14	0·01	0·20*	0·02	0·38	0·04	0·26	0·02
F-1,6-P_2 content (μmol/g dry tissue)	3·71	0·66	2·75	0·35	0·18	0·01	0·23	0·02
DHAP content (μmol/g dry tissue)	0·28	0·05	0·21	0·07	0·08	0·01	0·09	0·03
Gly-3-P content (μmol/g dry tissue)	0·04	0·01	0·03	0·01	0·05	0·01	0·06	0·01
Lactate content (μmol/g dry tissue)	6·27	0·59	4·98	0·40	19·4	1·50	18·6	1·8
Pyruvate content (μmol/g dry tissue)	0·34	0·03	0·22*	0·03	0·35	0·07	0·26	0·01
Citrate content (μmol/g dry tissue)	0·43	0·04	0·53	0·06	0·55	0·09	0·50	0·09
F-2,6-P_2 content (nmol/g dry tissue)	4·27	0·27	6·57*	0·68	25·0	2·5	26·0	3·0
G-1,6-P_2 content (nmol/g dry tissue)	233·4	41·3	455·7*	46·3	185·0	26·3	203·6	23·3
PFK-1 activity (U/g dry tissue)	196·9	10·3	196·2	19·7	3·19	0·23	3·14	0·17
Citrate synthase activity (U/g dry tissue)	62·6	6·8	64·5	6·3	18·1	0·9	17·9	1·8

*c*FA, *cis* unsaturated fatty acid; G-1-P, glucose-1-phosphate; G-6-P, glucose-6-phosphate; F-6-P, fructose-6-phosphate; F-1,6-P_2, fructose-1,6-bisphosphate; DHAP, dihydroxyacetone-phosphate; Gly-3-P, glyceraldehyde-3-phosphate; F-2,6-P_2, fructose-2,6-bisphosphate; G-1,6-P_2, glucose-1,6-bisphosphate; PFK-1, 6-phosphofructo-1-kinase.
* Mean value was significantly different from that for the control diet ($P<0·05$). For details, see p. 949.
† Dietary fat 70 g/kg
‡ Dietary fat 200 g/kg.

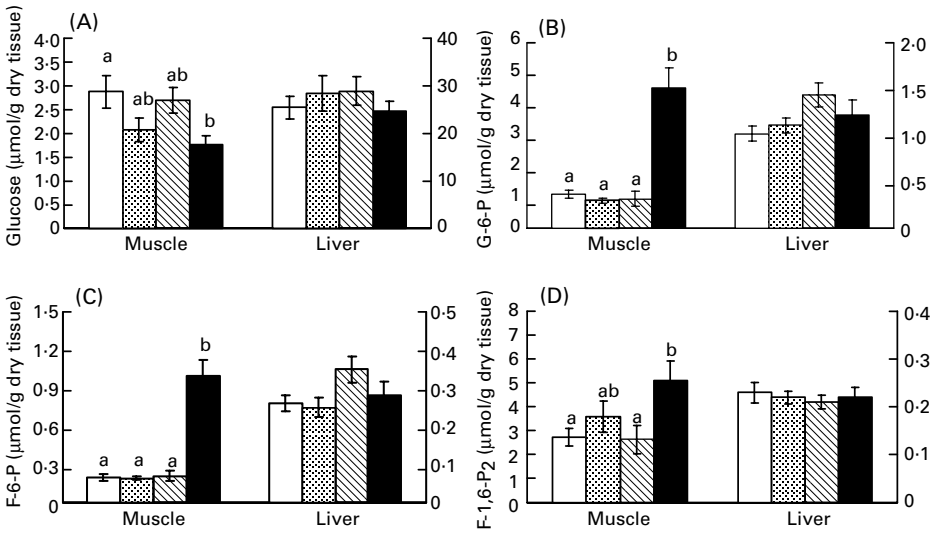

Fig. 1. Glucose (A), glucose-6-phosphate (G-6-P) (B), fructose-6-phosphate (F-6-P) (C) and fructose-1,6-bisphosphate (F-1,6-P_2) (D) content in the muscle and liver of animals fed high levels of fat. Values are means of five to six animals, with their standard errors represented by vertical bars. (□), *Cis* unsaturated fatty acid diet; (▤), *trans* unsaturated fatty acid diet; (▧), moderate-saturated fatty acid diet; (■), high-saturated fatty acid diet (see p. 949). [a,b] Mean values with unlike letters were significantly different ($P<0{\cdot}05$) by Tukey's test, after significant differences were found by one-way ANOVA (1 × 4).

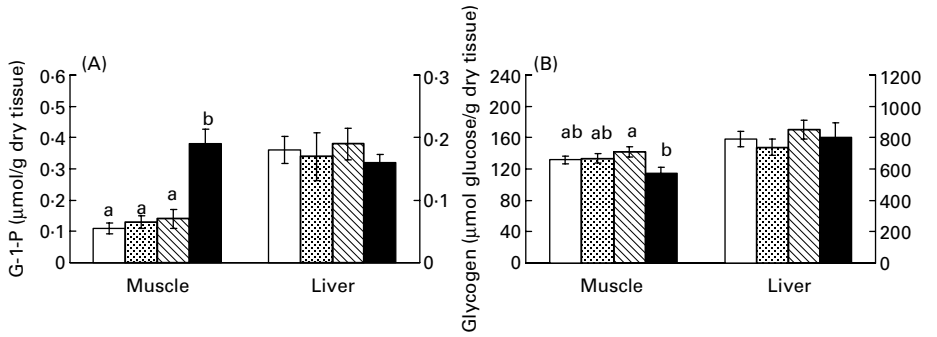

Fig. 2. Glucose-1-phosphate (G-1-P) (A) and glycogen (B) content in the muscle and liver of animals fed high levels of fat. Values are means of five to six animals, with their standard errors represented by vertical bars. (□), *Cis* unsaturated fatty acid diet; (▤), *trans* unsaturated fatty acid diet; (▧), moderate-saturated fatty acid diet; (■), high-saturated fatty acid diet (see p. 949). [a,b] Mean values with unlike letters were significantly different ($P<0{\cdot}05$) by Tukey's test, after significant differences were found by one-way ANOVA (1 × 4).

activities in muscle and liver were observed between the dietary groups. However, the muscle F-1,6-P_2:F-6-P ratio, used as an index of flux through PFK-1, was significantly reduced by the HSFA diet, mainly with regard to the control diet. The mean values of the F-1,6-P_2:F-6-P ratio in gastrocnemius muscle were: control diet, 26·5 (SE 1·21); *c* FA diet, 13·37 (SE 0·64) ($P<0{\cdot}05$ *v.* control diet); *t* FA diet, 18·31 (SE 2·37); MSFA diet, 12·69 (SE 0·90) ($P<0{\cdot}05$ *v.* control diet); HSFA diet, 5·80 (SE 1·09) ($P<0{\cdot}05$ *v.* control diet).

Discussion

Dietary fats have been shown to lead to insulin resistance (Storlien *et al.* 1997) and glucose metabolism changes. It is widely recognised that both the amount and type of dietary fatty acids modify insulin sensitivity in the muscle and liver of experimental animals (Storlien *et al.* 1986). In addition, intracellular glucose metabolism suppression might precede and/or cause insulin resistance in the skeletal muscle of rats fed a high-fat diet (Kim *et al.* 2000). In view of these assumptions, we evaluated the glucose metabolites and key enzyme activities in skeletal muscle and liver on an experimental dietary model. The variables were: (1) the amount of dietary *c* FA; (2) the isomers (*trans* or *cis*) and/or the saturation of fatty acid at high dietary fat levels.

The levels of dietary *c* FA did not change the muscle and liver triacylglycerol content under our experimental conditions. In addition, dietary *c* FA levels did not lead to pronounced changes in glucose metabolism. The most remarkable changes at high levels of *c* FA are the increases in F-6-P, F-2,6-P_2 and G-1,6-P_2 in muscle. High levels of the allosteric activators of PFK-1 (F-2,6-P_2 and G-1,6-P_2) could maintain the glycolytic pathway, increasing muscle glucose utilisation. On the other hand, citrate, an allosteric inhibitor of PFK-1, is not influenced by dietary levels of *c* FA.

Our most striking finding is that the type of dietary fatty acid, rather than the level of fat content *per se*, may be a decisive factor in the detrimental effects of dietary fat on muscle glucose metabolites. Animals fed the HSFA diet showed significantly increased muscle triacylglycerol content associated

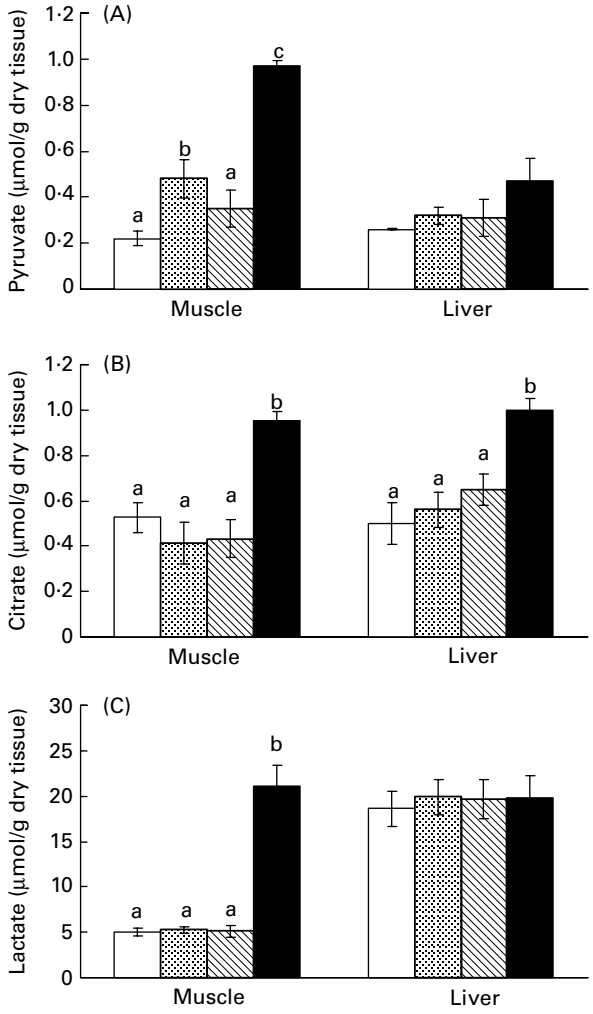

Fig. 3. Pyruvate (A), citrate (B) and lactate (C) content in muscle and liver of animals fed high levels of fat. Values are means of five to six animals, with their standard errors represented by vertical bars. (□), *Cis* unsaturated fatty acid diet; (▤), *trans* unsaturated fatty acid diet; (▧), moderate-saturated fatty acid diet; (■), high-saturated fatty acid diet (see p. 949). [a,b] Mean values with unlike letters were significantly different ($P<0·05$) by Tukey's test, after significant differences were found by one-way ANOVA (1 × 4).

with significantly elevated plasma glucose levels, and with changes in muscle glucose metabolites. Thus, the high levels of muscle G-6-P, G-1-P, F-6-P, F-1,6-P_2, glyceraldehyde-3-phosphate, lactate, pyruvate and citrate in rats fed a HSFA diet are associated with low muscle glucose and normal glycogen muscle stores, and reveal a glucose metabolic defect. Several mechanisms might partially explain the impairment of glucose metabolism. If the intracellular glucose–fatty acid cycle (Randle *et al.* 1963) is operating in gastrocnemius muscle, the high availability and oxidation of muscle triacylglycerol content in a HSFA diet could lead to increased levels of acetyl CoA. In turn, this could reduce the pyruvate dehydrogenase activity, and therefore increase the pyruvate and lactate levels in rats fed a HSFA diet. It has been shown that a high-fat diet significantly increases pyruvate dehydrogenase kinase-4 isoform expression, with a corresponding decrease in pyruvate dehydrogenase activity (Pehleman *et al.* 2005). The inhibition of pyruvate dehydrogenase activity could be at least partly responsible for the increases in intermediary glycolytic metabolites, including glycerol-3-phosphate. Since glycerol-3-phosphate is a regulatory metabolite in triacylglycerol synthesis (Maggs *et al.* 1995), it is reasonable to suggest that raised levels of glycerol-3-phosphate could increase triacylglycerol synthesis, impairing the glucose metabolism. The higher availability and probably the oxidation of triacylglycerol in the muscle of HSFA-fed animals could also increase the muscle citrate levels, thereby reducing the PFK-1 and hexokinase activities. The very low F-1,6-P_2:F-6-P ratio in the HSFA diet indicates that flux through PFK-1 is reduced. The high citrate level and the acidic environment caused by lactate production might counteract the effect of PFK-1 activation by bisphosphorylated sugars (Andrés *et al.* 1990).

The elevated levels of plasma glucose, associated with low glucose concentration in gastrocnemius muscle, might suggest a deficiency in glucose transport in the muscle of rats fed a HSFA diet. These data are clearly consistent with a reduction of GLUT-4 gene expression in the muscle of animals fed 80 % fat (% energy) for 7 weeks described by Kahn & Pedersen (1993). In addition, a HSFA diet might inhibit the GLUT by increasing muscle diacylglycerol concentrations (Montell *et al.* 2001). Thus, in the presence of increased levels of fatty acids, a high concentration of diacylglycerol activates a protein kinase (protein kinase C-θ), causing the serine phosphorylation of insulin receptor substrate-1 (Yu *et al.* 2002). Serine phosphorylated insulin receptor substrate-1 cannot recruit phosphatidyl-inositol-3-kinase and thus inhibits GLUT-4 translocation.

The huge accumulation of muscle lactate does not exclude the possibility that both an overproduction of lactate and a decrease in trans-sarcolemmal lactate transport could be present, as was described in experimental diabetes induced by

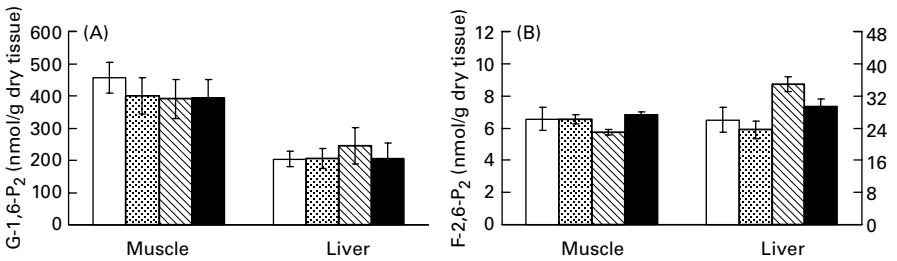

Fig. 4. Glucose-1,6-bisphosphate (G-1,6-P_2) (B) and fructose-2,6-bisphosphate (F-2,6-P_2) (B) content in the muscle and liver of animals fed high levels of fat. Values are means of five to six animals, with their standard errors represented by vertical bars. (□), *Cis* unsaturated fatty acid diet; (▤), *trans* unsaturated fatty acid diet; (▧), moderate-saturated fatty acid diet; (■), high-saturated fatty acid diet (see p. 949). [a,b] Mean values with unlike letters were significantly different ($P<0·05$) by Tukey's test, after significant differences were found by one-way ANOVA (1 × 4).

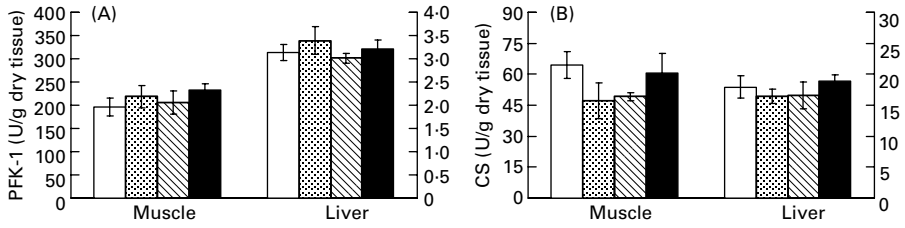

Fig. 5. 6-Phosphofructo-1-kinase (PFK-1) (A) and citrate synthase (CS) (B) activities in the muscle and liver of animals fed high levels of fat. Values are means of five to six animals, with their standard errors represented by vertical bars. (□), *Cis* unsaturated fatty acid diet; (▤), *trans* unsaturated fatty acid diet; (▧), moderate-saturated fatty acid diet; (■), high-saturated fatty acid diet (see p. 949). [a,b] Mean values with unlike letters were significantly different ($P<0{\cdot}05$) by Tukey's test, after significant differences were found by one-way ANOVA (1 × 4).

streptozotocin (Py *et al.* 2001). Moreover, Storlien *et al.* (1996) described changes in the fatty acid composition of membrane phospholipids induced by a HSFA diet. These alterations could affect membrane fluidity or permeability, supporting the plausible theory that there is a decrease in glucose and/or lactate transport.

In agreement with data recently reported by our group (Colandré *et al.* 2003), HSFA increased liver triacylglycerol content. However, this significant shift did not impact on the hepatic glucose metabolism to the same extent as observed in muscle. The significant increase in hepatic citrate levels with a HSFA diet seems to have a negligible impact on the glycolytic pathway after 30 d of feeding. It is not known whether feeding a HSFA diet for longer periods would lead to other hepatic metabolic disorders.

To our knowledge, the present study is the first to report the effect of dietary *t*FA at high fat levels on glucose metabolites in the muscle and liver of experimental animals. A noteworthy aspect of the present study is the very little impact of the muscle glucose metabolites to high fat levels enriched with *t*FA as compared with HSFA. Thus, by reinforcing the key role of the high muscle lipids availability and oxidation as a driving force to the muscle glucose metabolism alterations in a HSFA diet, we found that rats fed high levels of *t*FA resulted in normal gastrocnemius muscle triacylglycerol content and thereby in a lack of glucose metabolite alterations in this muscle. In contrast, a *t*FA (as HSFA) diet led to a significant accumulation of liver triacylglycerol content, without any significant changes in hepatic glucose metabolism. Moreover, these experimental data may be consistent with previous results from Louheranta *et al.* (1999), in which a *t*FA diet in young healthy women resulted in changes in the plasma lipid profile, with no changes in glucose and insulin metabolism. On the other hand, Alstrup *et al.* (1999) found that *t*FA potentiate glucose-stimulated insulin secretion more than the corresponding *cis* isomers.

Finally, the high-fat-level MSFA diet had a lesser effect on muscle and liver glucose metabolism. This might indicate that a certain level of dietary SFA has to be reached to induce glucose metabolic changes. Alternatively, dietary *c*FA might counteract the negative impact of SFA on glucose metabolism. If the latter is true, these results might provide some evidence of the advantageous effect of replacing SFA with *c*FA.

In short, under our experimental conditions, dietary fats had greater detrimental effects on glucose metabolism in muscle than in liver. The type of dietary fatty acid rather than the level of dietary fat content had an impact on muscle glucose metabolism. Glycolytic changes induced by the HSFA diet in muscle were a multifaceted abnormality. Glucose and lactate transport and intracellular glucose metabolism could play a key role in the biochemical defects involved in the detrimental effect of an HSFA diet on glucose metabolism. Dietary *t*FA had a slight effect on glucose metabolism compared with the HSFA diet.

Acknowledgements

We wish to thank Walter DaRú and Adolfo Larese for their technical assistance. We are also grateful to Dr Ricardo Grau and Dr Miguel A. Baltanás for their valuable collaboration and advice on the fat isomerisation and hydrogenation procedure. We are grateful to Mr Robin Rycroft for his editorial support. Finally, we are grateful for the financial support received from: the Universidad Nacional del Litoral – Cursos de Acción para la Investigación y Desarrollo (CAI + D 2000 no. 13-1-22); Secretaría de Ciencia y Técnica – UNL; the Consejo Nacional de Investigaciones Científicas y Tecnológicas (PIP no. 02 930, Resol. 127/04); the Ministerio Español de Ciencia y Tecnología – BFI2002-00 218; the FISS of the Instituto Carlos III (Red de Centros RCMN-C03708); the Agencia Española de Cooperación Internacional.

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