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## Dietary conjugated linoleic acid has limited effects on tissue protein anabolism in sedentary and exercising adult rats

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**Abstract** – The effects of conjugated linoleic acid isomers (CLA) and endurance training on lean body mass are expected to result from their action on tissue protein metabolism. The aim of this study was to analyze their effects on protein metabolism in 2 muscles, the small intestine and liver of adult rats. Four-month-old male Wistar rats were fed diets containing either no CLA, *cis*-9, *trans*-11 CLA isomer (1 g.100 g<sup>-1</sup>), *trans*-10, *cis*-12 CLA isomer (1 g.100 g<sup>-1</sup>) or both isomers (1 g.100 g<sup>-1</sup> each) for 6 weeks. Half of the rats were subjected to endurance training by running on a treadmill. At the end of this period, the rats were injected with a flooding dose of <sup>13</sup>C-valine to determine protein synthesis rates in the post-absorptive (experiment 1) and in the post-prandial (experiment 2) states. No effect of CLA or endurance training were detected in the small intestine. Training reduced food intake and protein synthesis rates in the liver but no effect was found on the protein synthesis rates in muscles. In the post-absorptive state, protein synthesis rate was increased by feeding the *trans*-10, *cis*-12 CLA isomer alone in the liver (+9%) or in combination with the *cis*-9, *trans*-11 isomer in the gastrocnemius (+30%), mostly in sedentary rats. In the post-prandial state, the *cis*-9, *trans*-11 CLA isomer tended to reduce the protein synthesis rate in the gastrocnemius muscle. However, no effect of CLA was found on muscle protein amounts. In conclusion, CLA isomers would have limited but differential effects on tissue protein metabolism in adult rats.

conjugated linoleic acid / protein metabolism / exercise / muscle / liver / intestine

### 1. INTRODUCTION

Mixtures of conjugated linoleic acid isomers (CLA) were initially shown to reduce the percentage of whole body fat and/or the mass of fat pads, in mice [1] and then in rats, hamsters, pigs and hu-

mans (see [2, 3] for reviews). However, in the latter species, the effects were less marked than in mice. This fat-lowering effect appeared to be mainly related to lipid metabolism alterations induced by the *trans*-10, *cis*-12 isomer. The effects of CLA feeding on lean body mass and on its main constituents, muscle and protein masses are less consistent. In some studies,

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CLA feeding had no effect on these parameters in mice [4], rats [5–10], hamsters [9, 11] and humans [12]. In other studies, CLA increased body protein concentration or lean body percentage without modification of absolute amounts, in mice [13–15], rats [16] and humans [17, 18]. It has also been shown to increase lean mass, protein levels and muscle mass in mice [19–21], rats [22], pigs [23] and humans [1, 24, 25]. The discrepancies in the results, even within a specific species, suggest that several factors such as genotype, age, diet, or physical activity may interfere with CLA effects. It has been suggested that CLA could be most effective in reducing fat mass and increasing lean mass when combined with enhanced physical activity in humans [2] and endurance training in mice [21]. Whether or not the differential effects of CLA isomers that were reported for lipid metabolism [26, 27] occur in the control of lean body mass, cannot be determined, because CLA isomer mixtures were used in all the experiments quoted above except two [1, 11]. In addition, the mechanisms responsible for the effects of CLA on lean body mass are unknown, mostly because their effects on the balance between protein synthesis and degradation, which controls tissue protein content and lean body mass, have not yet been described. Furthermore it can be assumed that their effects on protein metabolism can be detected more precociously on protein turnover than on protein contents.

The purpose of this study was therefore to determine whether feeding the two main isomers of CLA separately or together can modify protein metabolism in skeletal muscles or in splanchnic organs in adult rats in spite of limited effects on body composition [5]. A possible interaction with endurance training that was recently shown to enhance CLA effects on lean body mass in mice [21], was simultaneously tested. Since protein synthesis and degradation rates depend on nutritional

state [28], their responses to CLA feeding and endurance training could be different in the post-absorptive and in the post-prandial states. Consequently, the effects of CLA feeding and endurance training were studied in two separate experiments, the first in the post-absorptive state and the second in the post-prandial state.

## 2. MATERIAL AND METHODS

### 2.1. Animals, diets and endurance training

All procedures were performed according to current legislation on animal experimentation in France. In each experiment, 48 male Wistar rats aged 4 mo were purchased from Iffa-Credo/Charles River (L'Arbresle, France). They were maintained in individual wire-bottom cages at 21 °C with 12:12-h light-dark cycle (lights on at 2000 h) and free access to tap water. The adaptation period lasted 7 d. The 48 rats were then randomly divided into 2 groups. One group ( $n = 24$ ), termed the trained rats, was exercised by treadmill running. The animals were progressively adapted during the first week to run for one hour at approximately 22 m/min (i.e. no more than 50%  $\text{VO}_2$  max). They were trained for 6 wk, 6 d/wk during the dark period (at 1500 h). The other group ( $n = 24$ ), the sedentary rats, had no exercise.

In each group, six animals were assigned to one of the four diets studied: the control diet, the control diet with 1% *cis*-9, *trans*-11 CLA (diet c9,t11), the control diet with 1% *trans*-10, *cis*-12 CLA (diet t10,c12) and the control diet with 1% *cis*-9, *trans*-11 and 1% *trans*-10, *cis*-12 CLA (diet Mix). The 4 diets had the same basal composition ( $\text{g}\cdot\text{kg}^{-1}$ ): casein 180, maize starch 430, sucrose 210, cellulose 20, mineral mixture 50, vitamin mixture + DL methionine 10 and oil mixture 100. They differed in the composition of the oil mixture (Tab. I). The CLA isomers provided as

**Table I.** Composition of the oil mixtures.

Diets	Control	c9,t11 (g per kg of diet)	t10,c12	Mix
Oleisol <sup>1</sup>	98.4	88.4	88.4	78.4
Linseed oil	1.6	1.6	1.6	1.6
<i>Cis</i> -9, <i>trans</i> -11 CLA <sup>2</sup> %		10		10
<i>Trans</i> -10, <i>cis</i> -12 CLA <sup>3</sup> %			10	10
Total fat	100	100	100	100

<sup>1</sup> Sunflower oil rich in oleic acid.

<sup>2</sup> The fatty acid composition of the *cis*-9, *trans*-11 source was the following (%): oleic acid 1.5, *cis*-9, *trans*-11 CLA 96.1, *trans*-10, *cis*-12 CLA 1.4, other CLA 0.3 (Natural Lipids, Hovdebygda, Norway).

<sup>3</sup> The fatty acid composition of the *trans*-10, *cis*-12 CLA source was the following (%): palmitic acid 0.8, stearic acid 0.3, oleic acid 0.3, *cis*-9, *trans*-11 CLA 3.0, *trans*-10, *cis*-12 CLA 93.2, other CLA 1.4 (Natural Lipids, Hovdebygda, Norway).

triglycerides (Natural Lipids, Hovdebygda, Norway), were substituted for sunflower oil. The diets were fed at the beginning of the dark period (0800 h), in a semi-liquid form in order to have better control of food intake. The rats were fed these diets ad libitum for 6 wk. During this period, daily food intake was measured 5 d/wk and the rats were weighed 3 times/wk. The mean initial body weights of the rats were nearly identical in all groups within each experiment (mean  $\pm$  SEM: 434  $\pm$  1 and 465  $\pm$  1 g in experiments 1 and 2, respectively).

## 2.2. Determination of tissue protein turnover

Variations in lean body mass depend mainly on variations in protein amounts that result from the difference between protein synthesis and degradation rates. Protein synthetic activity in an organ can be assessed by its fractional synthesis rate (FSR, %·d<sup>-1</sup>) which is the percentage of tissue protein that is synthesized per day. An alteration in the overall efficiency of protein synthesis mechanisms can be detected by comparing translational efficiency [29]. Protein degradation rates were not determined directly but in muscles their

variations could be detected because they result from the difference between protein synthesis and protein gain, which could be assessed. This reasoning cannot be applied to the liver and intestines because of protein export.

The synthesis rates of tissue proteins as a whole were determined at the end of the 6-wk experimental period. In experiment 1, rats were in the post-absorptive state: food had been withdrawn at the end of the dark period, 13 to 16 h before measurements performed between 0900 h and 1200 h. In experiment 2, the rats were in the post-prandial state: daily food was offered at the beginning of the dark period, 5 to 8 h before measurements and was still available at the time of killing, between 1300 h and 1600 h. In trained rats, the determination of protein synthesis rates occurred at the same times as in sedentary rats. For each trained rat, it was 18 h 30 min–19 h after the end of the one-hour bout of exercise in experiment 1 and 23 h 15 min–23 h 45 min in experiment 2. Tissue protein synthesis rates were measured using the flooding dose method [30]. Briefly, each rat was injected with a flooding dose of L-<sup>13</sup>C valine (150  $\mu$ moles·100 g<sup>-1</sup> body, 50 atom%) in a lateral tail vein. General anesthesia

was induced by intraperitoneal injection of pentobarbital sodium just before killing by exsanguination, which occurred 15 min after the  $^{13}\text{C}$  valine injection. The liver, small intestine, gastrocnemius (plus plantaris) and soleus muscles were quickly excised and chilled on ice to stop tracer incorporation. The liver was cut into small pieces, rinsed in cold saline and wiped. The small intestine was rinsed with cold trichloroacetic acid (0.12 M). All tissues were weighed and frozen in liquid nitrogen 3–5 min after exsanguination. This extra time was not allowed for in the calculation of incorporation time. In vivo fractional synthesis rates (FSR,  $\% \cdot \text{d}^{-1}$ ) of tissue proteins were calculated according to the following:  $\text{FSR} = 100 \times (\text{EP} - \text{EN}) / (\text{EA} \times t)$  where EP and EA are the  $^{13}\text{C}$  enrichments of protein-bound valine and of free valine respectively, at the end of the incorporation time. EN is an estimation of the natural  $^{13}\text{C}$  enrichment of protein-bound valine (before flooding dose injection); it was determined in 4 additional rats fed the same diets as the experimental rats. The  $^{13}\text{C}$  enrichments were determined by gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS) for protein-bound valine and by GC-MS for tissue-free valine according to the methods described [31]. Tissue protein and RNA concentration were determined according to the bicinchoninic acid method [32] and the method of Manchester and Harris [33], respectively. Translational efficiency was calculated as the amount of protein synthesized (mg) per day per mg RNA.

### 2.3. Statistical analyses

Values in the text are given as means  $\pm$  SEM. The experimental schedule allows to determine the effects of diet, endurance training and their interaction on protein metabolism in each nutritional state but not those of nutritional state. They were ana-

lyzed in each experiment separately using a two-way ANOVA (StatView statistical software package, version 5, SAS Institute, Cary, NC, USA). When diet effect was significant, the 4 diets were compared by contrast analysis. Differences were considered to be significant at  $P < 0.05$  in two-sided tests.

## 3. RESULTS

In both experiments, final body weight and food intake were not affected by CLA feeding but significantly ( $P < 0.01$ ) reduced (–11%) by endurance training (Tab. II).

### 3.1. Protein metabolism during the post-absorptive state (experiment 1)

In the gastrocnemius muscle, CLA feeding had no detectable effect on protein amount (Tab. III). However protein FSR in the rats fed the Mix diet was significantly higher ( $P < 0.01$ ) than in the rats fed the diets lacking the *trans*-10, *cis*-12 isomer (diets Control and c9,t11) but no difference with that in the rats fed the t10,c12 diet was found. There was also significant interaction between CLA feeding and endurance training for FSR and translational efficiency: the effect of the Mix diet was significant in the sedentary rats only (Fig. 1A for FSR; not shown for translational efficiency).

In the soleus muscle, the only significant effect observed was this interaction between CLA feeding and endurance training for FSR and translational efficiency (Tab. III and Fig. 1B).

In the small intestine, no significant effect of CLA feeding on protein amount, FSR and translational efficiency was detected (Tab. IV). No effect of training on intestinal protein amount and FSR was

**Table II.** Effects of CLA feeding and endurance training on body weights, food intakes and weight gains in the rats of experiment 1 (post-absorptive state) and experiment 2 (post-prandial state).

Diets	Experiment 1			
	Sedentary rats		Trained rats	
	Final body weight (g)	Food intake (g · d <sup>-1</sup> )	Final body weight (g)	Food intake (g · d <sup>-1</sup> )
Control	522 ± 25	20.16 ± 0.83	469 ± 15	18.71 ± 0.35
c9,t11	520 ± 17	20.96 ± 0.53	448 ± 21	18.34 ± 0.68
t10,c12	530 ± 22	20.78 ± 0.81	470 ± 17	18.26 ± 0.75
Mixed	469 ± 15	20.70 ± 0.37	466 ± 16	19.05 ± 0.72
Diets	Experiment 2			
	Sedentary rats		Trained rats	
	Final body weight (g)	Food intake (g · d <sup>-1</sup> )	Final body weight (g)	Food intake (g · d <sup>-1</sup> )
Control	558 ± 6	21.07 ± 0.14	498 ± 11	18.57 ± 0.20
c9,t11	562 ± 22	20.80 ± 0.66	503 ± 11	18.63 ± 0.38
t10,c12	541 ± 21	20.16 ± 0.90	502 ± 12	18.17 ± 0.58
Mixed	536 ± 6	19.36 ± 0.71	481 ± 18	17.32 ± 1.13

Means ± SEM. Number of animals = 6 in all groups except in experiment 2, in the sedentary rats fed the control diet and in the trained rats fed the t10,c12 diet where it was 5. Anova indicated no significant effect of diet but a significant effect of training ( $P < 0.01$ ) for final body weight and food intake, in the two experiments.

shown. However, translational efficiency was significantly ( $P < 0.001$ ) higher (+32%) in the trained rats than in the sedentary rats. Furthermore, there was a significant interaction ( $P = 0.04$ ) between CLA feeding and endurance training since translational efficiency was significantly higher with the c9,t11 diet than with the Mix diet, in the trained rats only (Fig. 1C).

In the liver, feeding the t10,c12 diet significantly ( $P = 0.03$ ) increased both FSR (+10%) and translational efficiency (+11%) when compared to the three other diets. Endurance training significantly ( $P < 0.01$ ) decreased (-9%) protein FSR but had no effect on its protein amount (Tab. IV). The significant interaction between CLA feeding and endurance training corresponded to the fact that feeding the Mix diet decreased FSR and translational efficiency only in the trained rats (FSR,  $P = 0.03$ , Fig. 1D; translational efficiency,  $P = 0.05$ , not shown).

### 3.2. Protein metabolism during the post-prandial period (experiment 2)

Both diet and endurance training effects on protein metabolism were less pronounced in the post-prandial state than in the post-absorptive state.

In the gastrocnemius muscle, no effect of diet or endurance training on muscle protein amount, or translational efficiency was detected (not shown). However, the diets tended to have a significant ( $P = 0.07$ ) effect on protein FSR which was significantly lower with the c9,t11 diet than with the Control (-17%) or t10,c12 (-13%) diets in sedentary and trained rats. With the Mix diet, it was not significantly different from the other 3 diets (Fig. 2).

In the soleus muscle, no effect of diet or endurance training was observed on protein synthesis rates (data not shown).

**Table III.** Effects of conjugated linoleic acid intake and endurance training on protein amount and fractional synthesis rates in the gastrocnemius and soleus muscle of post-absorptive rats (experiment 1).

	Diet				Endurance training		Residual standard deviation	Significant factors*
	Control	c9,t11	t10,c12	Mix	Sedentary	Trained		
Number of rats	12	11	12	11	23	23		
Gastrocnemius								
Protein amount, g	0.556	0.572	0.553	0.550	0.579	0.536	0.064	T
Fractional synthesis rate, %·d <sup>-1</sup>	5.84 <sup>a</sup>	6.24 <sup>a</sup>	6.79 <sup>ab</sup>	7.59 <sup>b</sup>	7.01	6.19	1.42	D, D×T
Translational efficiency, mg prot/(d.mg RNA)	12.3	14.0	14.4	15.6	14.4	13.7	4.0	D×T
Soleus								
Protein amount, mg	34.6	32.8	34.8	32.4	34.5	32.8	3.9	
Fractional synthesis rate, %·d <sup>-1</sup>	9.99	10.00	10.30	10.80	10.00	10.53	1.77	D×T
Translational efficiency, mg prot/(d.mg RNA)	12.2	12.0	12.8	13.3	12.6	12.5	4.0	D×T

Means of the main effects, diet or endurance training.

\* Significant factors ( $P \leq 0.05$ ): D: diet, T: endurance training, D×T: interaction diet × endurance training. <sup>a,b</sup> within diet factor, the values with a common superscript cannot be considered as different ( $P < 0.05$ ).

In the small intestine, neither diet nor endurance training effects on protein amount or protein synthesis rates could be detected (data not shown).

In the liver, none of these parameters were affected by diet but they were significantly lower ( $P < 0.01$ ) in the trained rats than in the sedentary rats being respectively (means  $\pm$  SEM,  $n = 23$ )  $2.90 \pm 0.06$  and  $3.35 \pm 0.07$  g for the protein amount,  $55.5 \pm 1.9$  and  $63.3 \pm 1.4\%$ ·d<sup>-1</sup> for the fractional synthesis rate,  $15.0 \pm 0.5$  and  $17.4 \pm 0.4$  mg protein/(d.mg RNA) for translational efficiency.

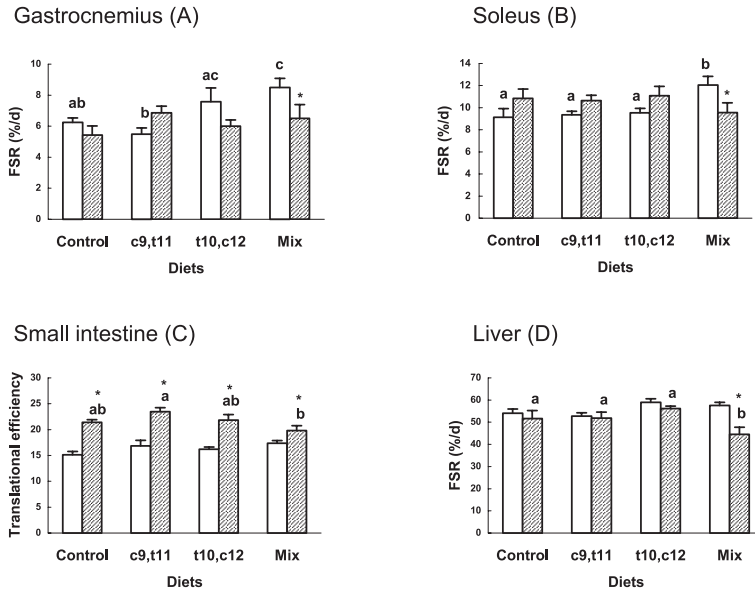
## 4. DISCUSSION

### 4.1. Effect of CLA feeding

In this study, few effects of CLA isomer feeding on protein metabolism were observed. The most pronounced effects were

a stimulation of protein synthesis by the Mix diet in muscle and by the t10,c12 diet in the liver during the post-absorptive period. This stimulatory effect was mainly observed in muscles of the sedentary rats. This difference in gastrocnemius FSR protein, between the sedentary rats fed the Mix diet ( $8.5 \pm 0.6\%$ ·d<sup>-1</sup>) and the c9,t11 diet ( $5.5 \pm 0.4\%$ ·d<sup>-1</sup>) was similar to the difference observed previously for 7-wk-old rats fed ad libitum or a 50% energy restricted diet for 4 weeks:  $8.6$  vs.  $5.7\%$ ·d<sup>-1</sup> [34]. Such a difference in non specific protein synthesis rates was expected to have an impact on muscle protein amounts except if a stimulation of protein degradation was associated. The lack of diet effect on muscle protein amount suggests that both protein synthesis and degradation were stimulated by the Mix diet. The effect of this diet on muscle protein turnover could be a specific effect of the isomer





**Figure 1.** Effect of CLA feeding and endurance training on fractional protein synthesis (%.d<sup>-1</sup>) rates in muscles and liver and on translational efficiency (mg protein/(mg RNA×d)) in the small intestine of post-absorptive rats (experiment 1). Means ± SEM, n = 6. Diet Control: control diet; diet c9,t11: control diet with 1% *cis*-9, *trans*-11 CLA isomer; diet t10,c12: control diet with 1% *trans*-10, *cis*-12 CLA isomer, diet Mix, control diet with 1% of both isomers. □ Sedentary rats ▨ Trained rats; n = 6 for most groups except sedentary rats fed the Control diet and trained rats fed the t10,c12 diet where n = 5. \* Significantly different from sedentary rats (P < 0.05). abc Within either sedentary rats or trained rats, the bars sharing the same superscripts cannot be considered as different at P < 0.05.

mixture or an enhancement of the effect of the *trans*-10, *cis*-12 isomer by the *cis*-9, *trans*-11 isomer as suggested by the results in gastrocnemius. In the latter, FSR were indeed the highest with the 2 diets containing the *trans*-10, *cis*-12 isomer, in particular when both isomers were fed simultaneously (Mix diet).

The increase in muscle and liver protein turnover in the post-absorptive state would result in a slight increase in energy expenditure. Slight (+3–4%) [14, 19] and more elevated increases (+7–16%) [35, 36] in energy expenditure have been reported in mice fed a CLA mixture, and in obese rats (+12% with the *trans*-10, *cis*-12 CLA) [37], where they resulted in a decrease in fat mass. However it was only in the

trained group that feeding a CLA mixture increased energy expenditure in Balb/C mice [21].

It is likely that the stimulation of muscle and liver protein turnover during the post-absorptive period by feeding the *trans*-10, *cis*-12 CLA in the present experiments, did not result in a marked increase in energy expenditure because no effect was observed on body composition in adult rats fed ad libitum [5, 27]. However, these rats probably never experienced a post-absorptive period as long as that created when protein synthesis rates were determined i.e. 13–16 h. This suggests that the still debated fat-lowering effect of *trans*-10, *cis*-12 CLA could be enhanced by long post-absorptive periods, in agreement with



**Table IV.** Effects of conjugated linoleic acid intake and endurance training on protein amount and fractional synthesis rates in the small intestine and liver of post-absorptive rats (experiment 1).

	Diet				Endurance training		Residual standard deviation	Significant factors*
	Control	c9,t11	t10,c12	Mix	Sedentary	Trained		
Number of rats	12	12	12	12	23	23		
Small intestine								
Protein amount, g	0.882	0.865	0.908	0.923	0.905	0.884	0.134	
Fractional synthesis rate, %·d <sup>-1</sup>	74.0	77.4	76.5	77.2	75.3	77.3	6.1	
Translational efficiency, mg prot/(d.mg RNA)	18.3	20.2	19.0	18.6	16.4	21.6	1.9	T, D×T
Liver								
Protein amount g	2.64	2.57	2.70	2.84	2.66	2.72	0.33	
Fractional synthesis rate %·d <sup>-1</sup>	52.9 <sup>a</sup>	52.3 <sup>a</sup>	57.5 <sup>b</sup>	51.1 <sup>a</sup>	55.8	51.0	5.4	D, T, D×T
Translational efficiency mg prot/(d.mg RNA)	12.9 <sup>a</sup>	13.2 <sup>ab</sup>	14.3 <sup>b</sup>	12.5 <sup>a</sup>	13.0	13.5	1.46	D, D×T

Means of the main effects, diet or endurance training

Significant factors ( $P \leq 0.05$ ): D: diet, T: endurance training, D×T: interaction diet × endurance training. <sup>a,b</sup> within diet factor, the values with a common superscript cannot be considered as different ( $P < 0.05$ ).

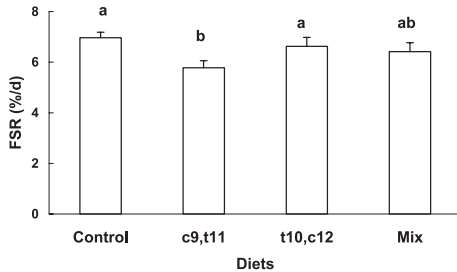
the results obtained in restricted rats [7] which usually experience such a feeding pattern.

Unlike that observed with *trans*-10, *cis*-12 CLA in the post-absorptive state, the *cis*-9, *trans*-11 isomer tended to reduce post-prandial protein synthesis rates in gastrocnemius, mostly in the trained group where the difference reached 25% ( $6.94 \pm 0.30$  vs.  $5.25 \pm 0.42\% \cdot d^{-1}$ ,  $P = 0.01$ ). Since this lowering effect on protein synthesis had no significant consequence on muscle protein amount, it is likely that it had the same lowering effect on protein degradation. Consequently, each isomer appears to have different effects on muscle protein metabolism in adult rats: the *trans*-10, *cis*-12 CLA stimulating protein turnover in non anabolic (post-absorptive) conditions and the *cis*-9, *trans*-11 isomer lowering it in anabolic (post-prandial) conditions. Similarly, these two isomers were shown to have different effects on lipid

metabolism and the effects differed between rats and mice [26, 27]. Such effects were consistent with limited effects on body composition in adult rats fed ad libitum [5]. Indeed protein synthesis and degradation appear to vary in a similar way and consequently this may have only minor effects on protein amounts. Furthermore, the potential increase in energy expenditure could be small if occurring mainly during the post-absorptive period which is likely to be short in ad libitum fed rats. Whether similar results would be obtained in younger rats and/or with higher CLA doses than those used in the present experiments remains a debatable point.

#### 4.2. Effect of endurance training

Calculated from the data of both experiments, gastrocnemius and soleus protein amounts were lower in the trained rats ( $534 \pm 9$  and  $32.7 \pm 0.5$  mg respectively)



**Figure 2.** Effect of CLA isomer feeding on fractional protein synthesis rates in gastrocnemius muscle of post-prandial rats (experiment 2). Means of the pooled data from sedentary and trained rats  $\pm$  SEM because there was no significant effect of endurance training. Control diet: control diet; c9,t11 diet: control diet with 1% *cis*-9, *trans*-11 CLA isomer; t10,c12 diet: control diet with 1% *trans*-10, *cis*-12 CLA isomer; Mix diet, control diet with 1% of both isomers.  $n = 12$  for most groups except in rats fed the control diet and the t10,c12 diet where  $n = 11$ . There was a tendency for a diet effect ( $P = 0.07$ ) and no significant effects of endurance training or of the interaction in the two-factor anova. <sup>ab</sup> The bars sharing the same superscripts cannot be considered as different at  $P < 0.05$ .

than in the sedentary rats ( $558 \pm 9$  and  $35.4 \pm 0.6$  mg). In contrast, the relative protein amounts of these muscles (mg of protein per 100 g empty body weight) were higher in the trained rats for gastrocnemius ( $114 \pm 2$  vs.  $106 \pm 2$  mg,  $P < 0.01$ ) but not for soleus ( $6.98 \pm 0.12$  vs.  $6.75 \pm 0.11$  mg). This indicates that the growth inhibition resulting from endurance training (and the associated decreased voluntary food intake) was lower for the gastrocnemius than for the whole body. A similar effect of training was described for the quadriceps muscle [38] and the tibialis anterior muscle [39] in rats that experienced a similar exercise-induced decrease in food intake. The absence of an endurance training effect on muscle protein synthesis rates at rest, observed in the present study was in agree-

ment with other results obtained in the tibialis anterior [39] and in the epitrochlearis [40] in rats. This conflicts with recent results in humans fed weight-maintaining diets reporting a stimulation at rest of muscle protein synthesis by endurance training [41, 42]. The reason why protein fractional synthesis rates in the liver were lower at rest in trained rats than in sedentary rats remains unclear. It was proposed that a decrease in protein synthesis in the liver [43] or in the gut would reduce amino acid utilization in these organs and increase amino acid availability in peripheral organs like muscles [44] or for energy production.

In conclusion, the effect of endurance training with the corresponding slight food restriction appears to be limited. Furthermore, endurance training interacted slightly with the effect of CLA on tissue protein metabolism. Actually in this study, the *trans*-10, *cis*-12 CLA isomer (in a mixture or alone) stimulated muscle and liver protein turnover, and this effect was more marked in sedentary rats. It is likely, however, that this did not result in any fat-lowering effect by increasing energy expenditure in adult rats. This change in protein metabolism occurred indeed mostly in situations where protein turnover was low, such as in the post-absorptive sedentary rats. On the contrary, the *cis*-9, *trans*-11 isomer tended to reduce muscle protein turnover, in particular when protein turnover was stimulated such as in the post-prandial trained animals. Further experiments, taking into account the specific effects of each isomer are necessary to verify these conclusions and more specifically to investigate whether or not they depend on anabolic conditions like those observed with resistance training.

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