

Effect of L-Carnitine on Insulin Sensitivity and Responsiveness in Skeletal Muscle, Adipose tissue and Hepatic tissues

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Received: 21 Dec 2014
Accepted: 25 May 2015
Available online: 5 July 2015

Keywords

- L-Carnitine
- Insulin Sensitivity
- Insulin Responsiveness

Abstract

Background and objectives: L-carnitine (LC) is a non-protein amino acid includes both diet and endogenous synthesis. There are no studies on the effect of LC on glucose uptake and net glucose output in muscle and hepatic tissues. So, the aim of the present work is to study the metabolic aspects of LC and to study some mechanisms that may be of help in treatment of insulin resistance. **Methods:** Twenty four male rats were divided into two main groups, twelve rats per each group. In vivo group was divided into control one and L-Carnitine - treated one, It was given in the dose of 3 gm carnitine/kg once every day orally for 4 weeks where fasting serum glucose and plasma free fatty acids (FFA) were measured. In vitro group included experiments on epididymal fat pad (EFP), hemidiaphragm and liver. **Results:** LC caused a highly significant decrease in fasting plasma FFA level (4.9 ± 1.5 mg/ dl). However, it caused a significant increase in insulin-stimulated glucose uptake by EFP in the presence of supraminimal and supramaximal insulin concentrations (2.5 ± 0.8 and 3.4 ± 0.4) (mg/ gm wet tissue /hour) and also by hemidiaphragm (4.9 ± 0.3 and 6 ± 0.97) (mg/ gm wet tissue /hour). Also, it increased net glucose output by liver slices in the presence of both insulin concentrations ($- 4.9 \pm 1.1$ and $- 4 \pm 1.42$) (mg/ gm wet tissue /hour). It decreased FFA release from EFP in the presence of both insulin concentrations (0.5 ± 0.21 and 0.4 ± 0.2) (mg/ gm wet tissue /hour), hemidiaphragm (0.5 ± 0.1 and 0.5 ± 0.16) (mg/ gm wet tissue /hour) and liver slices (0.58 ± 0.19 and 0.63 ± 0.27) (mg/ gm wet tissue /hour). **Conclusion:** LC increased plasma FFA level. It potentiated the effect of insulin on glucose uptake in EFP and hemidiaphragm. It decreased hepatic insulin sensitivity and responsiveness and enhanced the suppressive effect of insulin on FFA release from different peripheral tissues.

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INTRODUCTION

L-carnitine (LC) belongs to a group of food factors known as non-nutrient supplements. It is synthesized in the body from the lysine and methionine [1]. Several clinical conditions of LC deficiency have been described, including the genetic deficiency of LC, hemodialysis, muscular and liver disorders, kidney and cardiovascular diseases and diabetes mellitus [2].

In presence of L-carnitine deficiency, a common feature appears to be the increased serum concentration of triacylglycerol [3-6]. Therefore, a relevant aspect of the L-carnitine supplementation is a lipid-lowering effect observed in the experimental animals [7-12] and humans [13-17].

On the other hand, diabetes promotes LC deficiency [18,19] and increased blood triacylglycerol. However, studies investigating the effect of LC supplementation on the blood levels of triacylglycerol in type 1 diabetes are absent. So, the aim of the present work is to clarify:

1) The effect of LC on serum glucose level and plasma FFA level in rats 2) the effect of LC on basal and insulin-stimulated glucose uptake by different tissues 3) the effect of LC on FFA release in presence or absence of insulin in such tissues

MATERIALS AND METHODS

Experimental animals

Twenty four healthy male Sprague Dawley rats, 80 ± 5 days old with average weight of 250 ± 50 grams old were used in the study. They were obtained from Medical Experimental Research Center of Mansoura University, Egypt. The experimental protocol was approved by the Institutional Animal Ethics Committee of Mansura University. Rats were housed in polycarbon cages

and were exposed to a 12 h light-dark cycle at a room temperature of 21-24°C and 50 - 60% relative humidity.

The animals were studied in the fasting state, and these tissues were obtained:

- **Adipose Tissue:** was obtained from epididymal adipose tissue
- **Skeletal Muscle:** was obtained from hemidiaphragm
- **Liver Slices:** were obtained from right lobe of the liver

These tissues were obtained from the rats after overnight fast. Rats were anaesthetized with an intraperitoneal injection of 50 mg per kg body weight sodium thiopental. The skin over the abdomen was removed. The lower abdominal cavity was widely exposed, then displacing the testes into the abdominal cavity by gentle pressure over the scrotum. Each testicle was elevated with the forceps until the main spermatic blood vessels could be seen together with the pampiniform plexus of veins. The distal part of the epididymal fat pads, distal to the entrance of the blood vessels, was dissected from right and left sides. Thin liver slices were obtained using sharp scalpel. Diaphragm was dissected carefully and divided into two halves excluding the central tendon. These tissues were washed in Krebs Ringer Bicarbonate Buffered Solution (KRBBBS), dry by filter paper then placed immediately in the incubation medium (**Krebs and Henseleit, 1950**).

Incubation Procedures

3 ml of the incubation medium was placed in each dry and clean bottle and the weight of each with its contents was determined. Different tissues (epididymal fat pad, hemidiaphragm, liver slices)

were placed in the weighed bottles. The bottles were reweighed to determine the weight of the tissues. For each set of experiments, 3 ml of the incubation medium was placed in a given incubation bottle without added tissue and is considered as control for changes in level of glucose and free fatty acids (FFA). Insulin and/or LC was/or were added to incubation medium. The supraminimal concentration of insulin was (100 μ U per ml) and the supramaximal concentration was (10 mU per ml). The incubation bottles were gassed again with carbogen for 30 seconds each. Then they were shut off with tightly fitting rubber stopper and placed for one hour in the metabolic shaker at 37° C with a shaking rate of 100 r.p.m. After incubation was off, the bottles were taken outside the shaker, cooled under running tap water, and kept inverted while still closed to include the condensed vapour on the upper wall of the flasks into the medium. The covers of the bottles were opened, the incubated tissues were removed and then the solution was taken from medium for assay.

Chemicals and Drugs Used

- **LC** was obtained from (MEPACO Egypt). It was given in the dose of 3 gm carnitine/kg once every day orally for 4 weeks (20)
- **Insulin:** actrapid insulin, in the form of ampoule containing 10 ml in a concentration of 100 IU per ml

Experimental Groups

I. In Vivo Experiments

- **Control group** containing six rats
- **L-Carnitine -treated group** containing six rats. The rats received L-carnitine in the dose of 3 gm

carnitine / kg once every day orally for 4 weeks [30]

After one week, the rats were sacrificed

- Blood samples were taken from the fasting rats through intracardiac blood sampling method for determination of serum glucose and plasma free fatty acids (FFA) level

II. In Vitro Experiments

A. Experiments on Epididymal Fat Pad (EFP)

1. Studying glucose uptake
2. Studying glucose uptake in the presence of LC supplementation 2 mM in 5 mg/mL concentration
3. Studying glucose uptake in the presence of supraminimal concentration of insulin (100 μ U per ml).
4. Studying glucose uptake in the presence of LC and supraminimal concentration of insulin (100 μ U per ml)
5. Studying glucose uptake in the presence of supramaximal concentration of insulin (10 mU per ml)
6. Studying glucose uptake in the presence of LC and supramaximal concentration of insulin (10 mU per ml)

B. Experiments on Hemidiaphragm

1. Studying glucose uptake (Control group)
2. Studying glucose uptake in the presence of LC
3. Studying glucose uptake and free fatty acids (FFA) release in the presence of supraminimal concentration of insulin (100 μ U per ml)
4. Studying glucose uptake in the presence of LC and supraminimal concentration of insulin (100 μ U per ml)

5. Studying glucose uptake and free fatty acids (FFA) release in the presence of supramaximal concentration of insulin (10 mU per ml)

6. Studying glucose uptake in the presence of LC and supramaximal concentration of insulin (10 mU per ml)

C. Experiments on liver slices

1. Studying net glucose uptake (Control group)

2. Studying net glucose uptake in the presence of LC

3. Studying net glucose uptake the presence of supraminimal concentration of insulin (100 μ U per ml)

4. Studying net glucose uptake in the presence of LC and supraminimal concentration of insulin (100 μ U per ml)

5. Studying net glucose uptake in the presence of supramaximal concentration of insulin (10 mU per ml)

6. Studying net glucose uptake in the presence of LC and supramaximal concentration of insulin (10 mU per ml)

Incubation Medium

The incubation medium employed was Krebs Ringer Bicarbonate Buffered Solution (KRBBS).

Measured parameters

Serum glucose level, plasma FFA level, tissue specific glucose uptake and FFA release

Tissues Glucose Uptake

It was calculated as mg per gm wet tissue weight per hour. The glucose uptake was calculated using the following equation:

$$\text{Glucose uptake} = \frac{M \times V \times 1000}{N \times 100} \text{ mg per gm wet}$$

tissue per hour

Where:

N= Net weight of tissue in Gm

V= volume in ml of the incubation medium in the flask (3 ml)

M= difference in the concentration of glucose per 100 ml of the medium between the control and the tissue bottles

Determination of Free Fatty Acids (FFA)

The principal based on the colorimetric method already described for chloroform solutions of fatty acids.

The method adapted in the present work is that of Dunucomb (1964). The principal based on the colorimetric method already described for chloroform solutions of fatty acids.

Statistical analysis:

Values were expressed in the form of mean (+/-) SD which are done by using excel program for figures and SPSS (SPSS, Sigma Plot Software, Inc, Chicago, IL) program statistical package for social science version 16. To test the normality of data distribution K-S (Kolmogorovo-Smirnov) test was done only significant data revealed to be nonparametric.

N.B: all tested data revealed to be parametric

The description of data was done in the form of mean (+/-) SD for quantitative data and frequency & proportion for qualitative data. The analysis of the data was done to test statistical significant difference between groups. For quantitative data student t-test was used to compare between groups. Paired sample t-test to compare one group at different time.

To test the association between variables correlation co-efficiency test was done.

N.B: P is significant if lower than or = 0.05 at confidence interval 95 %. The level of significance as the follow: P higher than 0.05 non significant, P

lower than 0.05 significant, P lower than 0.01 highly significant, P lower than 0.001 very highly significant

RESULTS

Results are illustrated in tables 1, 2 and 3 and figures 1, 2 and 3

Table 1: Effect of L-Carnitine on fasting serum glucose level (mg/dl) and Fasting Plasma Free Fatty Acids (FFA) (mg/dl) level

		Control group	L-Carnitine -treated group
Fasting Serum Glucose Level (mg/dl)	Mean	99	97
	±SD	± 5.21	±4.42
	P		NS
Fasting Plasma Free Fatty Acids (FFA) (mg/dl) level	Mean	22	4.92
	±SD	± 2.05	± 1.54
	P		0.000

P: as compared with control group. NS: denotes non significant

Table 2: Effect of LC on glucose uptake (mg/gm wet tissue/h.) by epididymal fat pad, hemidiaphragm and net glucose uptake by liver slices in basal conditions and in the presence of supraminimal (100 µU per ml) and supramaximal concentrations of insulin (10 mU per ml)

Tissue	Control	L-Carnitine	Supraminimal Insulin (100 µU per ml)	Supraminimal Insulin (100 µU per ml)	Supramaximal Insulin (10 mU per ml)	L-Carnitine and Insulin (10 mU / ml)
EFP	1.25	1.45	1.91±0.42	2.52±0.81	2.61±0.53	3.41±0.42
Mean ±SD	±1	± 0.63				
P		NS				
P1				0.01		
P2						0.01
Diaphragm	2	2.12	2.91±0.79	4.91±0.32	4.31±1.04	6±0.97
Mean ±SD	±0.61	±0.8				
P		NS				
P1				0.02		
P2						0.02
Liver	1.92	2.31±1.04	2.3±1.32	-4.91±1.13	2.91±0.66	-4±1.42
Mean ±SD	±0.89	NS				
P		NS				
P1				0.000		
P2						0.000

The results are Mean±SD. P: as compared with control. P1: as compared with supraminimal insulin, P2: as compared with supramaximal insulin. NS: denotes non significant

Table 4: Effect of LC on free fatty acids (FFA) release (mg/gm wet tissue/h.) from epididymal fat pad, hemidiaphragm and liver slices in basal conditions and in the presence of supraminimal (100 μ U per ml) and supramaximal concentrations of insulin (10 mU per ml)

Tissue	Control	L-Carnitine	Supraminimal Insulin (100 μ U per ml)	L-Carnitine Supraminimal Insulin (100 μ U per ml)	Supramaximal Insulin (10 mU per ml)	L-Carnitine and Supramaximal Insulin (10 mU per ml)
EFP	1.31 \pm 0.59	1.12 \pm 0.43	0.71 \pm 0.21	0.53 \pm 0.21	0.53 \pm 0.21	0.41 \pm 0.23
Mean \pmSD						
P		NS				
P1				0.02		
P2						0.01
Diaphragm	1.22 \pm 0.39	1 \pm 0.14	0.71 \pm 0.23	0.52 \pm 0.13	0.61 \pm 0.24	0.51 \pm 0.16
Mean \pmSD						
P		NS				
P1				0.02		
P2						0.01
Liver	1 \pm 0.26	0.92 \pm 0.24	0.71 \pm 0.14	0.58 \pm 0.19	0.72 \pm 0.19	0.63 \pm 0.27
Mean \pmSD						
P		NS				
P1				0.03		
P2						NS

P: as compared with control P1: as compared with supraminimal insulin P2: as compared with supramaximal insulin

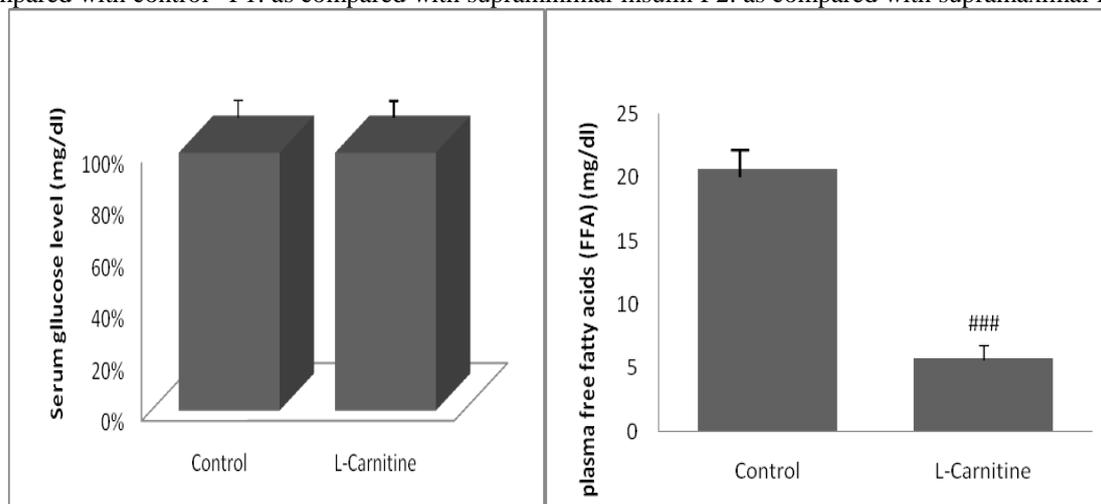


Fig. 1. Effect of L-Carnitine on fasting serum glucose level (mg/dl) (a) and fasting plasma free fatty acids (FFA) (mg/dl) (b) ### denotes that P = 0.000

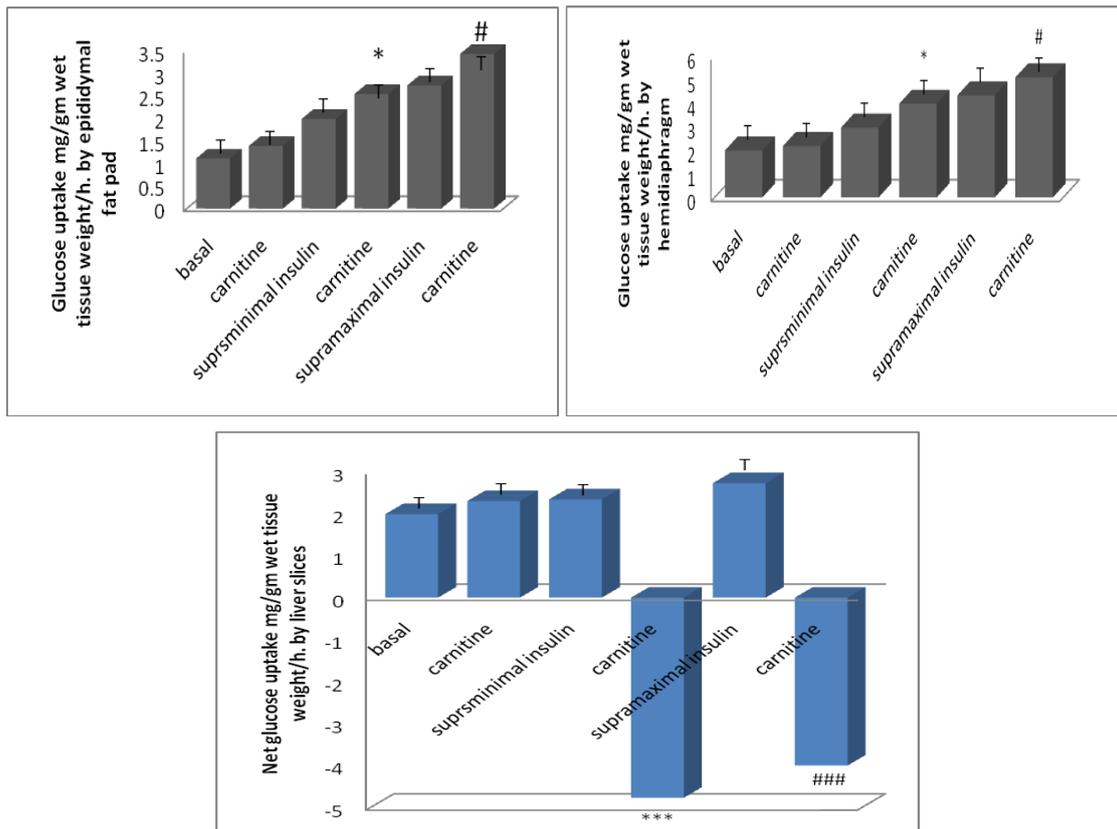


Fig. 2. Effect of L-Carnitine on glucose uptake (mg/gm wet tissue/h.) by epididymal fat pad, hemidiaphragm and net glucose uptake by liver slices in basal conditions and in the presence of supraminimal (100 μ U per ml) and supramaximal concentrations of insulin (10 mU per ml) *: Significant as compared with supraminimal insulin #: Significant as compared with supramaximal insulin

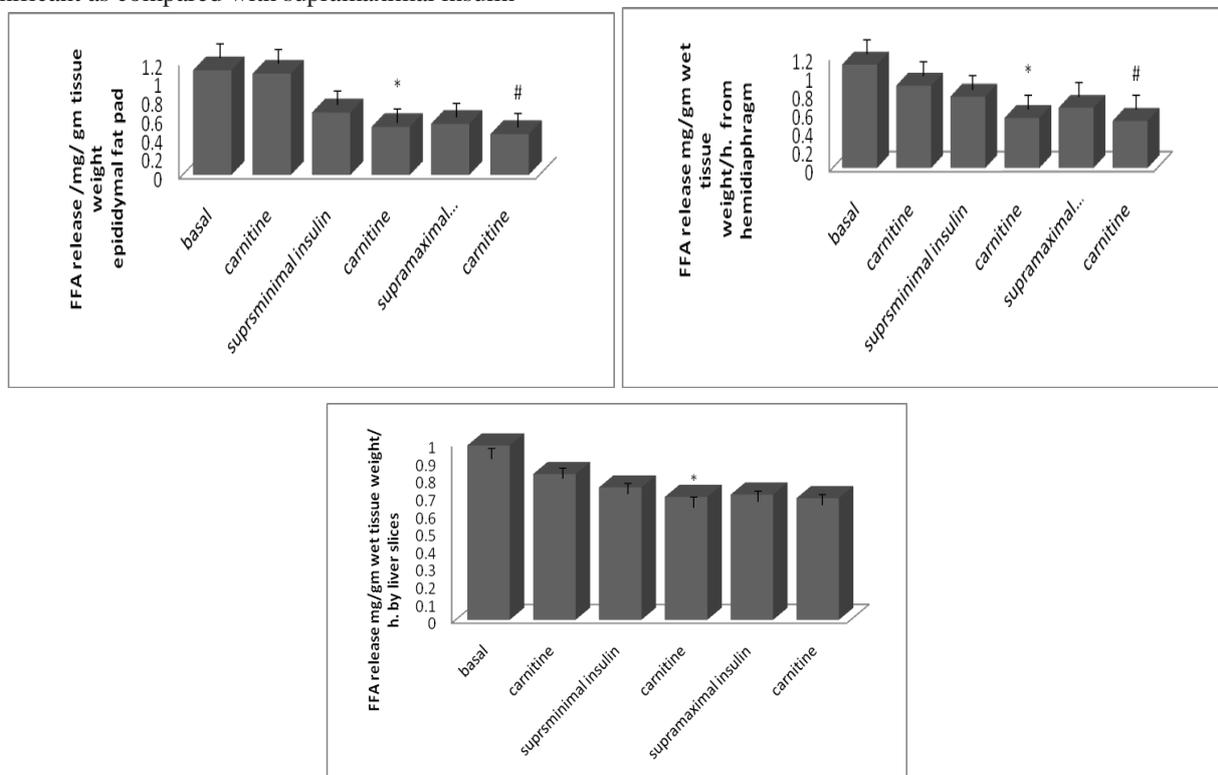


Fig. 3. Effect of L-Carnitine on free fatty acids (FFA) release (mg/gm wet tissue/h.) from epididymal fat Pad, hemidiaphragm and liver slices in basal conditions and in the presence of supraminimal (100 μ U per ml) and supramaximal concentrations of insulin (10 mU per ml) *: Significant as compared with supraminimal insulin, #: Significant as compared with supramaximal insulin

DISCUSSION

LC acts as a cofactor in beta-oxidation by facilitating long chain FA entrance into mitochondria in the form of LC esters (carnitine-palmitoyl transferase system CPT-I and II) and the exit of acetyl groups from mitochondria to the cytosol (carnitine acyl transferase system) [21, 22]. A reduction in FA transportation to mitochondria facilitates triglyceride accumulation in the cytosol, which is related with the pathogenesis of insulin resistance (IR). Although the mechanisms by which FA dysregulation leads to insulin resistance are not well understood, the following pathways could be involved: a) accumulation of diacylglycerol and acyl-CoA, which could cause defects in insulin signalling, b) the inhibition of GLUT-4 translocation by long chain acyl-CoA, c) non metabolised FA accumulation in mitochondria, which could cause mitochondrial stress and IR [23, 24]. These mechanisms may explain why LC, being a conditionally essential nutrient involved in bidirectional transportation of acyl-CoA in mitochondria, could reduce lipid overload and improve insulin-sensitivity.

Although LC is known to play a beneficial role in diabetes, however, there are no studies on the effect of LC on glucose uptake and net glucose output in muscle and hepatic tissues. So, the aim of the present work is to clarify:

1) The effect of LC on serum glucose level and plasma FFA level in rats 2) the effect of LC on basal and insulin-stimulated glucose uptake by different tissues 3) the effect of LC on FFA release in presence or absence of insulin in such tissues

In this study, LC non-significantly decreased serum glucose level. This is in agreement with the work of Bazotte and Lopes-Bertolini [25] who

found a decrease in serum glucose level following LC administration.

In this study, LC has no significant effect on basal glucose uptake by epididymal adipose tissue but significantly increased glucose uptake in the presence of both supraminimal and supramaximal insulin concentrations suggesting that LC acts synergistically with insulin. This is in agreement with the work of Patel et al. [26] who demonstrated an increase in insulin-stimulated glucose uptake in isolated epididymal adipose tissue in a dose dependent manner.

The results of the present study demonstrated that LC has no significant change in basal net glucose uptake by the liver slices. However, L-carnitine increases net glucose production in the presence of insulin suggesting antagonism of insulin action.

Insulin inhibits gluconeogenesis, through the activation of the insulin receptor (IR). It acts predominantly by suppressing the expression of the genes for the key gluconeogenic enzymes phosphor-enol-pyruvate-carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase) [27, 28].

Also, in this study, LC caused a highly significant decrease in plasma FFAs level. This is in agreement with the work of Bieber [29]. This effect could be explained by the effect of LC on improvement of fatty acid oxidation.

We have investigated the direct effects of LC on FFAs release from epididymal fat pad in vitro. It was observed that LC caused a non-significant decrease in FFAs release. Addition of supraminimal and supramaximal concentrations of insulin caused a significant decrease in FFAs release. Addition of LC to the incubation medium

with insulin in both concentrations caused a more significant decrease in FFAs release.

We have also investigated the effects of LC on FFAs release from hemidiaphragm. It caused a non-significant decrease in free fatty acids (FFAs) release. However, addition of LC with insulin in both concentrations caused a more significant decrease in FFAs release suggesting potentiation of insulin action in inhibiting lipolysis.

In the present study, we have investigated the effects of LC on FFAs release from liver slices. It caused a non-significant decrease in free fatty acids (FFAs) release. Addition of supraminimal and supramaximal concentrations of insulin to the incubation medium caused a significant decrease in FFAs release. Addition of LC with insulin in both concentrations to the incubation medium caused a more significant decrease in FFAs release.

The data in this work support a direct role for LC in enhancing insulin-inhibited FFAs release in isolated epididymal adipose tissue, hemidiaphragm and liver slices. This is in agreement with the work of [30] who found that a reduction in the hepatic LC pool is the principle mechanism leading to impaired hepatic fatty acid metabolism.

In the present study, LC and insulin had the same effects on lipid metabolism in hemidiaphragm, adipose tissue, as well as, in liver slices, where both of them favor lipid storage as triacylglycerol (TAG). These effects may be located at the level of the receptor in the coupling between the receptor and the intracellular mediators resulting in the modulation of cellular lipid oxidation.

Conclusion

LC has no direct effect on basal glucose uptake or FFA release by peripheral tissues. It potentiates insulin-stimulated glucose uptake by adipose tissue and muscle tissue. It enhanced the suppressive effect of insulin on FFA release from different peripheral tissues.

Acknowledgement

Dr. Rasha Rizk Mansoura faculty of medicine, clinical pathology department is acknowledged for her contribution to the experimental part of the study.

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