INSULIN RESISTANCE: MOLECULAR MECHANISM

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ABSTRACT

Insulin resistance in skeletal muscle is present in humans with type 2 diabetes (non-insulin dependent diabetes mellitus) and obesity and in rodents with these disorders. Malonyl CoA is a regulator of carnitine palmitoyl transferase 1 (CAP I), the enzyme that controls the transfer of long chain fatty acyl CoA into mitochondria, where it is oxidized. In rat skeletal muscle, the formation of malonyl CoA is regulated acutely (in minutes) by changes in the activity of acetyl CoA carboxylase (ACC), the enzyme that catalyzes malonyl CoA synthesis and is a source of its precursor, cytosolic acetyI CoA. Increase in cytosolic citrate leading to an increase in the concentration of malonyl CoA, occur when muscle is presented with insulin and glucose, or when it is made inactive by denervation. In contrast, exercise lowers the concentration of malonyl CoA by activating an AMP – activated protein kinase, which phosphorylates and inhibits ACC. A numbers of reports have linked sustained increases in the concentration of malonyl CoA in muscle to insulin resistance. In this paper, we review these reports, as well all the notion that changes in malonyl CoA contributed to the increases in long chain fatty acyl CoA (LCFA CoA), diacyglycerol and triglyceride contest and changes in protein kinase C activity, observed in insulin resistant muscle. It is our hypothesis that dysregulation of the malonyl CoA regulatory mechanism, if it leads to sustained increases in the concentration of malonyl CoA and cytosolic LCFA CoA, could pay a key role in the pathogenesis of insulin resistance in muscle.

KEY WORDS: Insulin resistance; Malonyl CoA; Acetyl CoA carboxylase; Diabetes; Obesity.

INTRODUCTION

Insulin resistance in muscle is associated with, any may contribute to the pathogenesis of such disorders as obesity, type 2 diabetes, hypertension and endogenous hypertriglyceridemia. In addition, it occurs in a variety of situations when muscle activity is decreased (Table 1). Despite this, the biochemical basis for insulin resistance in muscle in most of these situations is not known. Muscle also becomes insulin resistant after denervation.

Table 1: Conditions associated with insulin resistance in Skeletal Muscle

Impaired glucose tolerance Type 2 diabetes Hypertension Hypertriglyceridemia	Syndrome x
Obesity	
Spinal cord injury Denervation Inactivity	Decreased muscle work

Muscle metabolism after denervation

Work from our laboratory has focused on two states in which insulin action is altered in muscle in association with a change in contractile activity: denervation and exercise (2,3) the stimulation of both glucose incorporation into glycogen and glucose transport by insulin are depressed in denervated muscle whereas they are both enhanced after exercise. Insulin binding to its receptor and its ability to activate the insulin receptor tyrosine kinase are not changed in either situation. Thus, the site at which insulin action is altered by exercise and denervation appears distal to the insulin receptor. One such site could be the diacyglycerol protein kinase C (DAG-PKC) signalling system. Although its precise role is unclear, increase in its content have been observed in insulin-resistant skeletal muscle of the genetically obese, hyperinsulinemic (fa/fa) rat and ob/ob mouse in denervated muscle, the increase in DAG content is associated with two to three fold increase in membrane-associated PKC activity (2,3).

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Figure 1. Effect of Denervation (den) and Prior Exercise o Insulin action in Rat Muscle

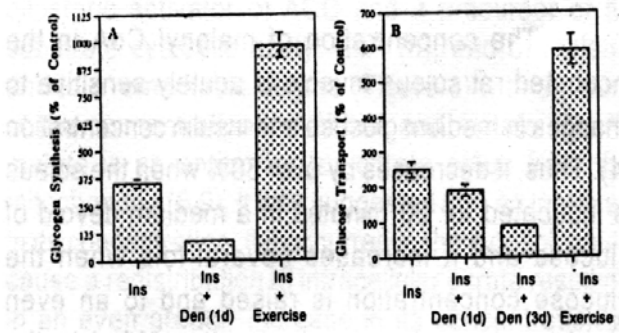
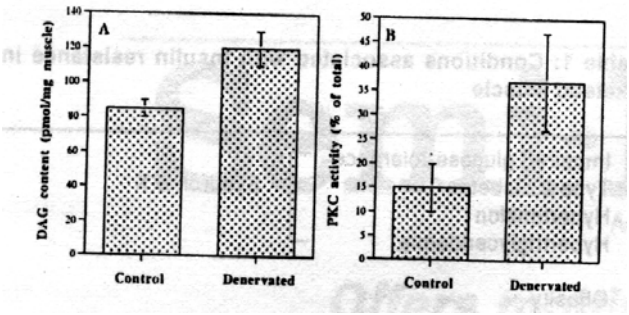


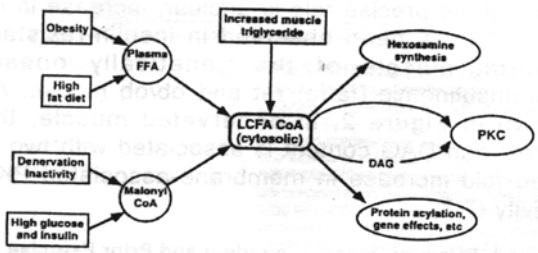
Figure 2. Effect of Denervation on Diacyglycerol mass ar distribution of Protein Kinase C (PKC).



The malonyl CoA fuel-sensing mechanism and insulin resistance

In searching for the basis for this increase in DAG mass in the various situations associated with insulin resistance in muscle, we have focused our attention on factors that could increase the concentration of cytosolic long chain fatty acyl-CoA (LCFA – CoA), a substrate for DAG synthesis. One of these factors, malonyl CoA, is both an intermediate in the pathway of fatty acid synthesis and a physiological inhibitor of carnitine palmitoyltransferase I (CPT I).(Figure 3).

Figure 3. Interrelations between Concentration of Cytosolic LCFA-CoA and development of Insulin Resistance



The concentration of malonyl CoA in the incubated rat soleus muscle is acutely sensitive to changes in medium glucose and insulin concentration (4). Thus, it decreases by over 50% when the soleus is incubated for 20 minutes in a medium devoid of glucose and it increase several-fold when the glucose concentration is raised and to an even greater extent with insulin is also added to the medium. Likewise, we have observed 1.5-3 increase in malonyl CoA in muscle after denervation (inactivity) and 50% decrease of malonyl CoA after stimulating (contraction). Winder and his co-workers (6) also have reported 40-60% decreases in malonyl CoA in rat muscle during voluntary exercise. Cumulatively, these findings suggest that the concentration of malonyl CoA in muscle can be regulated by its fuel supply and energy expenditure. How this regulations takes place in not known (7).

Figure 4 : Effect of Insulin and Glucose in the Incubate Soleus Muscle

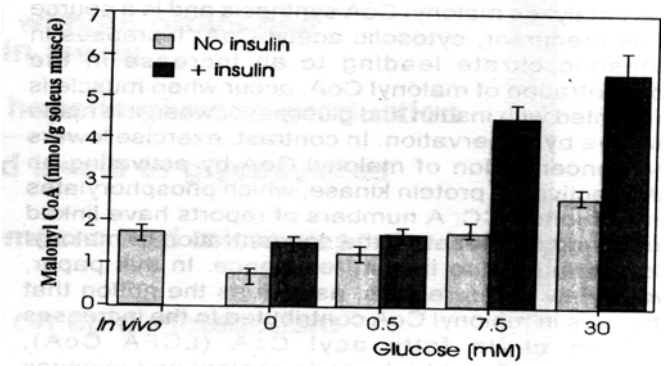


Figure 5 : Effects of Denervation and Electrically-Induce Contractions on Malonyl CoA

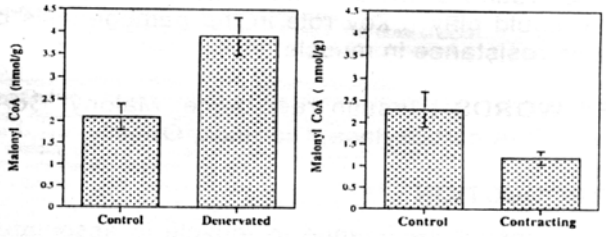
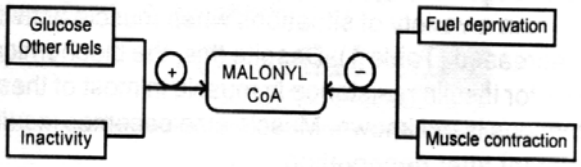


Figure 6 : The Malonyl CoA Fuel Sensing System



Malonyl CoA Insulin Resistance

A number of lines of evidence suggest an association between sustained elevations in the concentration of malonyl CoA and insulin resistance in skeletal muscle. Thus, high level of malonyl CoA have found in denervated muscle and muscle from a wide variety of hyperglycemic and/or hyperinsulinemic rodents, including the Zucker rat, the KKAy mouse, rats infused with glucose for 1-4 days, and the GK rat, which is hyperglycemic due to impaired glucose-mediated insulin secretion (Table 2) (8-10). Furthermore, in the KKAy mouse decreases in insulin resistance, induced by treatment with the thiazolidinedione, pioglitazone, correlate closely with decrease in the concentration of malonyl CoA in muscle and liver (11).

Table 2 : Lipid metabolites and Protein Kinase C (PKC) in Muscle of insulin Resistant Rodents

	Plasma		Muscle				
Rodent	Insulin	Glucose	TG	DAG	LCFA CoA	Malonyl CoA	Altered PKC distribution And/or activity
KKAy mouse	+	+	+	+	ND	+	ND
Fa/fa rat	+	0	+	+	ND	+	Yes
Glucose-infused rat	+	+	+	ND	+	+	Yes
Fat-fed rat	0	0	+	+	±	Yes	
Denervated rat	0	0	ND	+	ND	+	Yes

TG= triglycerides; DAG = diacylglycerol; ND= not detected

Regulation of Malonyl CoA levels in Muscle

A major factor that could regulate the concentration of malonyl CoA in skeletal muscle is acetyl CoA carboxylase (ACC), the enzyme that catalyzes the carboxylation of cytosolic acetyl CoA to form malonyl CoA. In the liver, the predominant. ACC is a 265 kDa (now referred to as ACCά) (8). In general, insulin and glucose increase the activity of the 265kDa ACC in liver by inhibiting 5'-AMP kinase and by inducing its synthesis, whereas glucagan, catecholamines and long-chain fatty acids have the opposite effects (8-10). Citrate is thought to be the major precursor of the cytosolic acetyl CoA, from which malonyl CoA is synthesized and it is an allosteric activator of ACC, in vitro (8). However, its role in ACC regulation, in vivo, is unclear (8). In contrast to the ACC regulation, in vivo, is unclear (8). In constrant to the ACC of liver, the dominant enzyme in skeletal muscle is a 280 kDa protein (now reffered as ACCβ), which is similar to minor ACC component of liver (Table 3).

Figure 7 : Possible Mechanisms for Regulation of Acety CoA Carboxylase

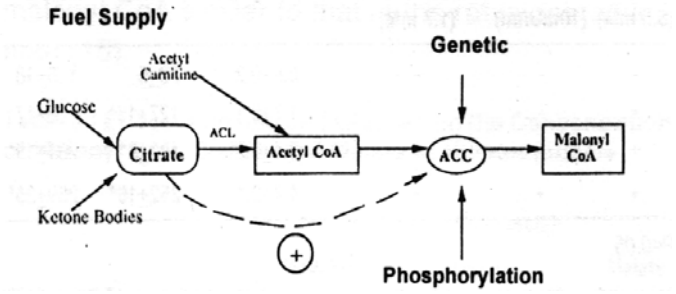


Table 3: Characteristics of Accα and ACCβ

	Accα	ACCβ
Apparent molecular mass, kDa	265	275-280
Km acetyl CoA, mM	74	167
Chromosomal location of gene	17	12
Possible mitochondria-binding sequence at		
NH^2 terminus	No	Yes
Role in fatty acid synthesis	Yes	?
Role in fatty acid oxidation	?	Yes
Activity altered by nutritional state	Yes (liver)	No (muscle)

Cytosolic Citrate links the Malonyl CoA Fuel-sensing Mechanism to the Glucose-Fatty acid cycle

Recent studies have shown that substantial increase in malonyl CoA occur in rat soleus muscle when it is incubated for 20 minutes with a medium enriched in glucose and insulin or glucose and acetoacetate (Table 4) (7). These increase in malonyl CoA were observed in the absence of stable changes in the activity of acetyl CoA carboxlase (ACC), the rate limiting enzyme for malonyl CoA formation; however, they correlated with increases in the whole cell concentration of citrate (7), an allosteric activator of ACC and a precursor of its substrate cytosolic acetyl CoA (8), and of malate, and to a remarkable degree with the sum of the

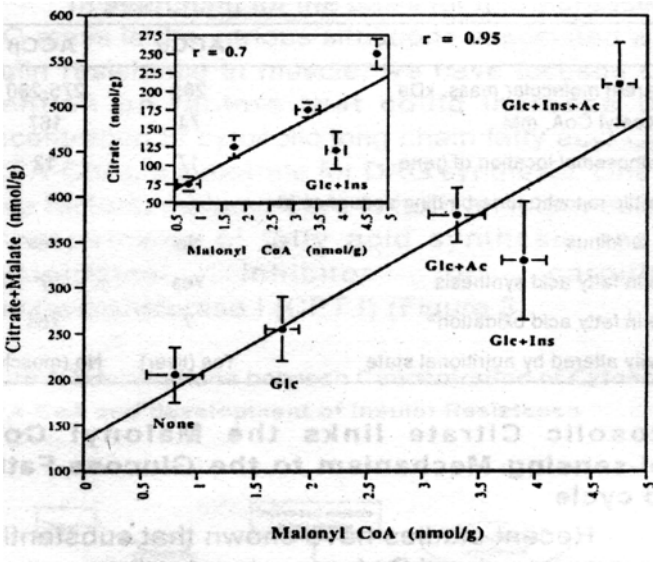
concentrations of citrate and malate (7). As malate is an antiporter for citrate efflux from the mitochondria (8,9), it was suggested that an increase in its concentrations, if it occurred in the cytosol, could cause a redistribution of intracellular citrate resulting the cytosol.

Table 4: Effect of Acetoacetate on Malonyl CoA, citrate and Malate in Incubated Rat Soleus Muscle

Additional to incubation media			Tissue Concentration (nmol/g)		
Glucose (5.5 mM)	Insulin (10mU/ml)	Acetoacetate 1.7 mM)	Malonyl CoA	Citrate	Malate
-	-	-	0.9±0.2	79±5	135±18
+	-	-	1.7±0.1*	127±13*	135±18
+	-	+	3.4±0.2*	182±8*	201±19*
+	+	+	4.7±0.1*	252±16*	259±25*

**P <0.05*

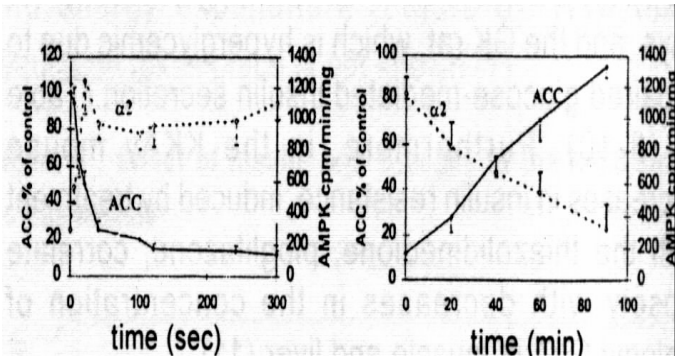
Figure 8 : Relationship between concentration of Malonyl CoA and concentrations of Sum of Citrate and Malate in Soleus Muscles



When muscle is made to contract by electrical stimulation, citrate and malate levels both increase within seconds-minutes; however, malonyl CoA levels are decreased rather than increased. Similar decreases in malonyl CoA have been shown to occur during voluntary exercise. ACCβ activity is diminished in these situations, apparently due do phosphorylation by the α1 isoform of AMP-dependent protein kinase.

The decrease in ACCβ activity during contraction and the increase in activity during recovery are associated with reciprocal changes in the activity of the α2 (but not the α1) isoform of AMPK. Such phosphorylation markedly diminishes the activation of ACCβ by citrate and this presumably accounts for the fact that the correlation between changes in cell citrate and malonyl CoA, so strong in resting muscle, is lost. Another conclusion from these finding is that a dual mechanism for regulating ACCβ is present in skeletal muscle, and that one of these mechanisms (i.e. activation by citrate) is superceded by other (inhibition by ACC phosphorylation) when both are operative (5).

Figure 9 : Effect of Electrically Induced Contractions of Rat Gastrocnemois Muscle on Concentration of ACC and AMP- Activated Protein Kinase



Operation of the Malonyl CoA Fuel-sensing Mechanism in Muscle in vivo

Increase in the concentrations of malonyl CoA, citrate and malate in muscle, similar to those observed during incubation with insulin and glucose, occur in rats undergoing a euglycemic hyperinsulinemic clamp or a prolonged glucose infusion. Furthermore, the activity of ACCβ is not increased in these situations, suggested that the apparent change in cytosolic citrate is regulatory (Table 5) (12,13)

Table 5: Effect of a Euglycemic-Hyperinsulinemic clamp and Glucose infusion on the Concentration of Malonyl CoA, Citrate and Malate in Rat Muscle.

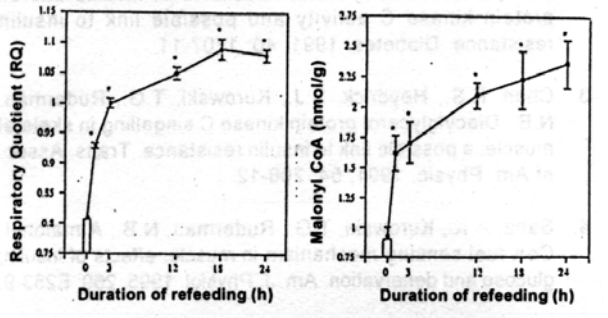
				Plasma	
	Malonyl Coa	Citrate	Malate	Insulin	Glucose
	(nmol/g muscle)			U/ml	MM
Infusions (90 min)					
Saline	1.2±0.2	111±10	115±12	25±4	5.3±0.2
Euglycemic/ Hyperinsulinemic clamp	2.5+0.4*	251±25*	131+6	232+18*	5.4±0.2
Infusions (1-4d)					
Saline (4d)	2.1±0.1	80±20	305±20	25±5	6.9±0.2
Glucose (1d)	3.5±0.2*	80±15	730±30*	237±28**	14.1±1.6
Glucose (4d)	4.7±0.3**	110±10	730±40*	153±21*+	7.5±0.3#

Values are means ± SE for 4.6 rats per experimental condition. *P < 0.05, **P<0.01, # P<0.001, compared to saline infusion group.

Changes in Malonyl CoA Modulate Fatty acid Oxidation in Skeletal muscle

Whether malonyl CoA regulates fatty acid oxidation in skeletal muscle in response to changes in nutritional state is less clear. Malonyl CoA levels in rat muscle are decreased after 24-48 hours of starvation and are increased after 24 hours of refeeding. On the other hand, it is not known whether these increase in malonyl CoA during refeeding occur quickly enough to contribute to the rapid decrease in whole body fat oxidation that occurs after a carbohydrate meal. The malonyl CoA levels increase rapidly in muscle during refeeding after a fast which is paralleled by the increases in whole body respiratory quotient (RQ) (Figure 10) (14).

Figure 10 : Changes in whole body Respiratory Quotient (RQ) and Tissue Malonyl CoA



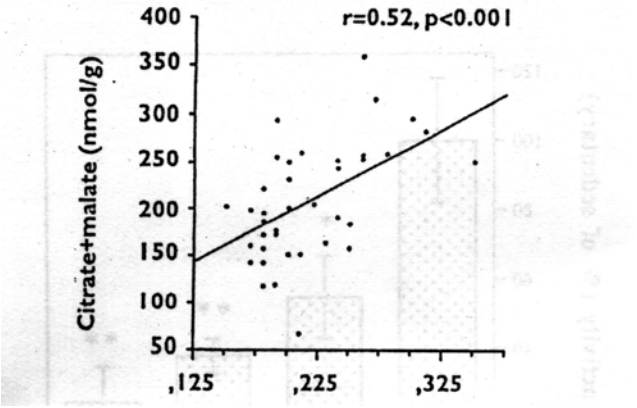
The Malonyl CoA Hypothesis and Insulin Resistance in Humans

The regulation of malonyl CoA concentration by insulin and glucose had not been studied in human skeletal muscle. To evaluate whether the malonyl CoA fuel sensing mechanism operates in humans, in conjunctions with Drs. Efendic and Bavenholm at the Karolinska Institute in Stockholm, we assessed malonyl CoA level in muscle in 16 healthy, middle aged, Swedish men, undergoing a two-step euglycemic hyperinsulinemic clamp. As shown in Table 6, no increase in the concentration of malonyl CoA was observed in muscle during the low dose insulin (0.25 mU/kg/min) infusion; however, during the high dose (1mU/kg/min) infusion, its concentration was increased from 0.2±0.01 (high dose). Although, the absolute increase in malonyl CoA was relatively small, it occurred in all but two subjects, and it was highly significant. There is a significant correlation between citrate plus malate and malonyl CoA. Thus, a mechanism for increasing malonyl CoA similar to that in the rat is operative in man (15).

Table 6: Effect of insulin and Glucose on the Concentrations of Malonyl CoA, Citrate and Malate in Human Muscle

(nmol/g muscle)			
	Molonyl CoA	Citrate	Malate
Basal	0.20±0.01	102±6	80±6
Low insulin infusion	0.20±0.01	100±7	77±9
High insulin infusion	0.24±0.01	137±7	126±9

Figure 11 : Relationship Between the Concentration of Malonyl CoA and the Sum of Citrate + Malate



One study which entailed an evaluation of the changes that occur during exercise, was carried out with Dr. Eric Richter's group at the Krough institute, in Copenhagan. Normal volunteers were

exercised at various intensities for period ranging between 10-30 minutes and biospsies taken sequentially for determination of malonyl CoA and ACC. We found a 20% decrease in malonyl CoA between resting vs. exercising values in same individuals. We also found that ACC activity was decreased by 50-80% in muscle from exercised humans. The results suggest that the responses of ACC and malonyl CoA to exercise are qualitatively similar in humans (16).

Figure 12 : Concentrations of Malonyl CoA at Rest and After Exercise

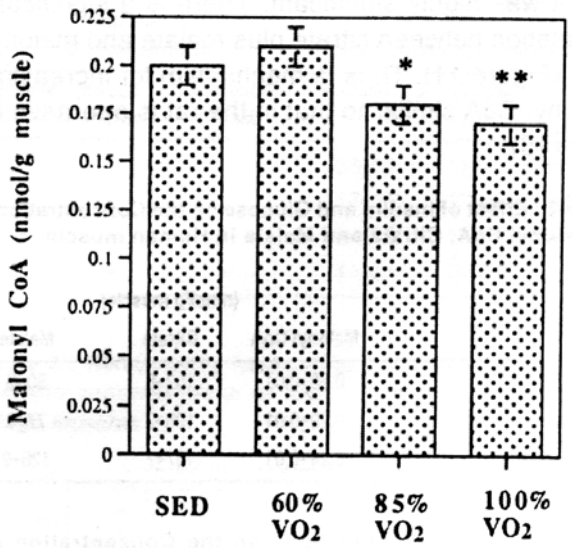
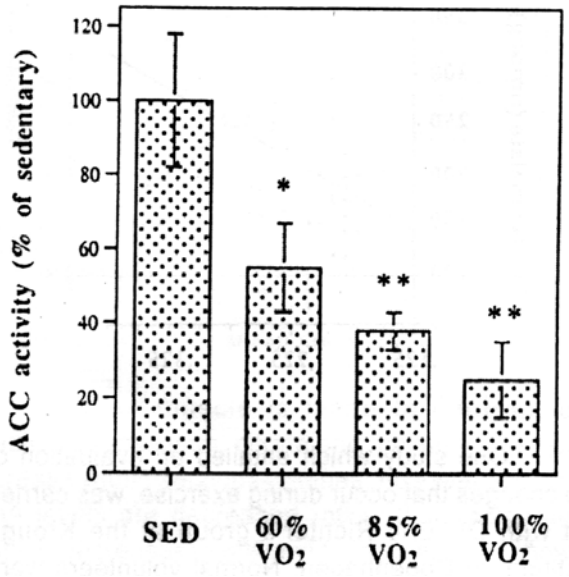


Figure 13 : Exercise Inhibits Human Skeletal Muscle Acetyl CoA Carboxylase (ACC)



Concluding Remarks

A mechanism for regulating the concentration of malonyl CoA by glucose, similar to that observed in muscle, exists in the pancreatic β-cell, where it plays a key role in the stimulation of insulin secretion by glucose and probably in the heart. An increasing body of evidence suggests that dysregulation of the malonyl CoA fuel sensing and signaling mechanism could play a role in the pathogenesis of obesity and the insulin resistance syndrome. How changes in malonyl CoA concentration relate to the formation and action of leptin, uncoupling proteins, TNFα and other molecules who have a role in the pathophysiology of obesity and insulin resistance, is now being intensively studied.

Pharmacological agents, and other therapies that lower the concentration of malonyl CoA and diminish glycerolipid sysnthesis requires a potentially fruitful area for research.

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