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The Regulation of Energy Metabolism Pathways Through L-Carnitine Homeostasis

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1. Introduction

Diabetes mellitus is a widespread metabolic disease characterized by hyperglycemia and associated with severe complications, including cardiovascular disease and dyslipidemia. The cardiovascular risk is considerably increased in diabetic patients, and novel cardioprotective strategies are sought that could target both diabetic and cardiovascular outcomes. L-carnitine is a conditionally essential amino acid that plays an important role in cellular energy metabolism. Initial pharmacological studies suggest that preconditioning-like manipulation of substrate metabolism through the regulation of L-carnitine homeostasis could be an effective approach for the prevention of myocardial infarction and the treatment of heart failure, especially for patients with type 2 diabetes. Contradictory data exist concerning the pharmacological outcomes of L-carnitine treatment. It is known that L-carnitine supplementation is beneficial and increases overall glucose utilisation in animals and humans.

Interestingly, a state of limited L-carnitine availability also induces compensatory mechanisms that alter the pathways of energy metabolism. In some experimental studies, a decrease in L-carnitine concentration was achieved by the administration of 3-(2,2,2-trimethylhydrazinium)-propionate (mildronate). In addition to its cardioprotective and anti-atherosclerotic effects, it was shown recently that mildronate, by decreasing L-carnitine concentration, decreases fed and fasted blood glucose concentrations and prevents diabetic complications in an experimental model of type 2 diabetes, Goto-Kakizaki (GK) rats, and an obesity model using Zucker rats. Recently presented mildronate-induced gene expression profiles suggest the peroxisome proliferator-activated receptors (PPARs) as possible nuclear factors underlying the effects of mildronate and decreased L-carnitine availability on FA metabolism. The effects of mildronate might depend on dosing and the length of treatment, which could differentially compensate for the decreased L-carnitine availability and thereby inhibit FA metabolism by activating glucose utilisation. Thus, it is possible that a limited availability of tissue L-carnitine might improve the metabolic flexibility of muscle tissues and enhance the preconditioning-like adaptive responses.

2. Effects of L-carnitine

L-carnitine is a conditionally essential amino acid that plays an important role in cellular energy metabolism. In heart and skeletal muscle, L-carnitine aids the translocation of long-

chain FA into the mitochondria for subsequent beta-oxidation, which is a major energy source in muscle cells. L-carnitine is absorbed from dietary products and biosynthesised from lysine and methionine; its excretion is efficiently regulated by renal re-absorption. The physiological range of L-carnitine concentrations in various tissues is maintained by complex transporter systems.

2.1 L-carnitine in pathways of energy metabolism

The homeostasis of cellular energy metabolism relies on the availability of two major sources of substrates: FA and carbohydrates. Through catabolic processes, both substrates provide a metabolite, acetyl coenzyme A (acetyl-CoA), which further undergoes oxidation in the mitochondrial machinery to provide cellular metabolism with adenosine-5'-triphosphate (ATP) molecules, the "molecular unit of currency" of intracellular energy transfer (Koves et al., 2008a). The role of L-carnitine in regulating cellular energy metabolism is schematically summarised in Fig. 1. First, L-carnitine is a substrate of the enzyme L-carnitine palmitoyltransferase-1 (CPT I), which catalyses the rate-limiting reaction in the transfer of FA into the mitochondria (Kuhajda et al., 2007). The key mechanism for regulation of CPT I activity is allosteric inhibition by malonyl CoA (Cuthbert et al., 2005).

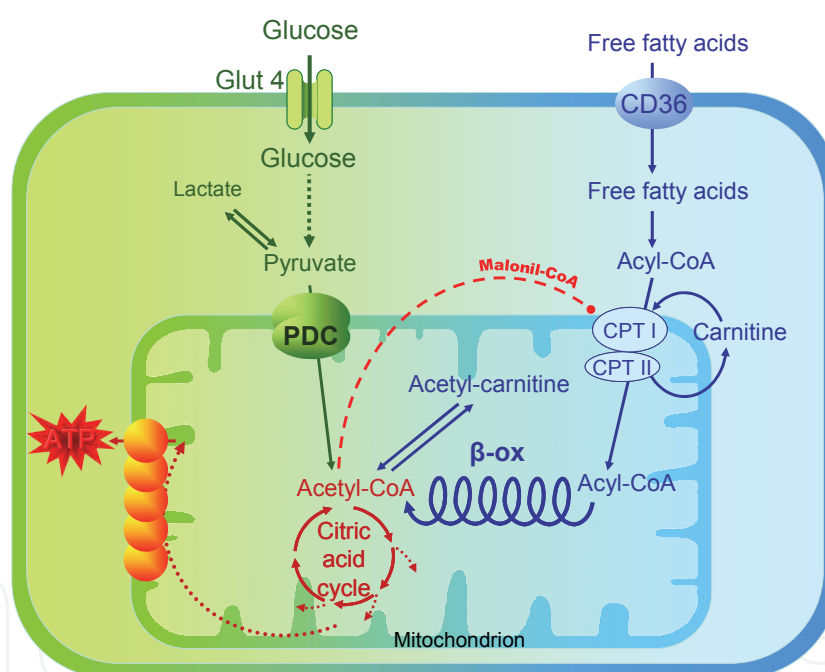


Fig. 1. The cellular pathways of energy metabolism

In the CPT I-catalysed reaction, L-carnitine and acyl-CoA-activated long-chain FA interact to form long-chain acyl-L-carnitine esters that are then shuttled across the mitochondrial membranes by L-carnitine/acyl-L-carnitine translocase (CACT) (Bremer, 1983). Inside the mitochondrial matrix, acyl-L-carnitine is converted back to L-carnitine and long-chain FA by the enzyme CPT II. Thus, L-carnitine aids the import of long-chain FA to the mitochondria for β -oxidation, which is the major provider of energy for muscle cells.

In addition to its role in FA transport pathways, L-carnitine is also involved in the export of acetyl groups out of the mitochondria (Stephens et al., 2007). In the mitochondrial matrix, lumen of the endoplasmic reticulum and peroxisomes, L-carnitine acetyltransferase (CrAT;

EC 2.3.1.7) catalyses the reversible transfer of acetyl groups between acetyl-CoA and L-carnitine (Ramsay et al., 2001). By catalysing its reaction in a reversible manner, CrAT regulates the cellular pool of CoA, which serves as a carrier of activated acetyl groups in the oxidation of energy metabolism substrates and in the synthesis of FA and lipids (Ramsay et al., 2001). Glucose metabolism pathways are also regulated through the homeostasis of the ratio of acetyl-CoA/CoA. An increase in this ratio is sensed by pyruvate dehydrogenase kinase, which phosphorylates and thereby leads to the inhibition of the pyruvate dehydrogenase complex (PDC), the key rate-limiting step in carbohydrate oxidation (Lopaschuk, 2001; Rebouche, 2004), with L-carnitine being a pivotal regulator of these pathways. For example, during high-intensity exercise, L-carnitine enters the CrAT-catalysed reaction and buffers the excess acetyl groups formed. As a result, a pool of free CoA is maintained for the continuation of the PDC and citric acid cycle reactions (Stephens et al., 2007). It can be concluded that L-carnitine is important for the regulation of both long-chain FA and carbohydrate metabolism. L-carnitine-dependent pathways are strongly involved in the regulation of the adaptive responses related to the overall homeostasis of cellular energy metabolism.

2.2 The bioavailability of L-carnitine

The homeostasis of L-carnitine (Fig. 2) is maintained through biosynthesis, efficient reabsorption in the kidneys and food intake, particularly from meat and dairy products (Rebouche, 2004). The physiological range of different L-carnitine concentrations in various tissues is maintained by complex transporter systems (Ramsay et al., 2001). The biosynthesis of L-carnitine from the amino acids lysine and methionine proceeds in the liver, kidney and testis, with γ -butyrobetaine (GBB) being an intermediate precursor (Bieber, 1988). It has been estimated that in the case of a non-vegetarian diet, only about 25% of the necessary L-carnitine is biosynthesised, and 75% is ingested from food (Longo et al., 2006). Interestingly, the blood plasma concentration in women is about 20% lower than in men (Cederblad, 1976). Additionally, in vegetarians, the L-carnitine concentration in the blood serum is 20-30% lower than that in the reference population (Delanghe et al., 1989).

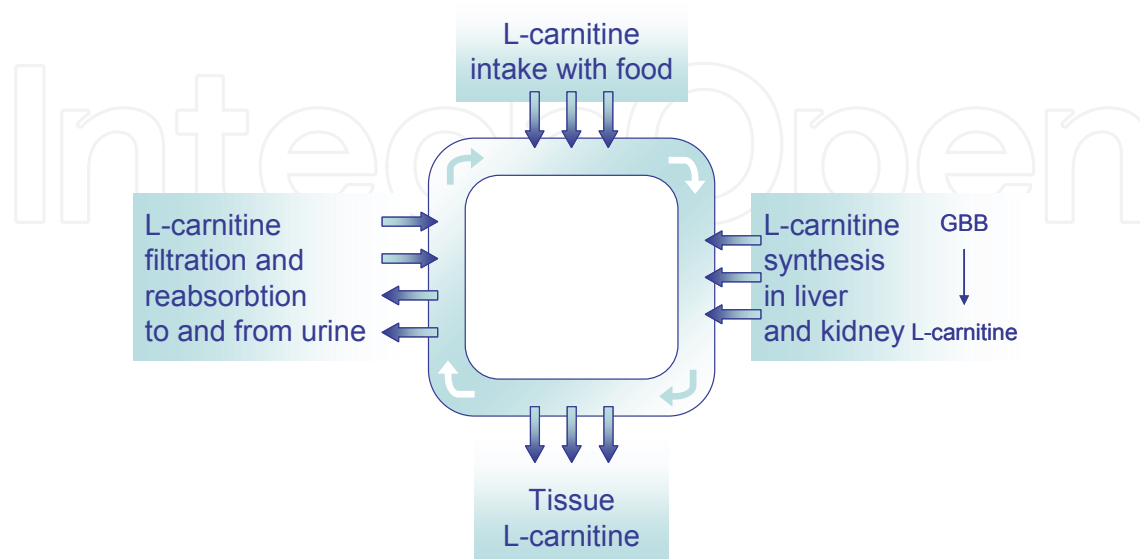


Fig. 2. The body L-carnitine pools and turnover.

After oral intake, the absorption of L-carnitine proceeds by active transport and passive diffusion (Ramsay et al., 2001). It has been shown experimentally that the absolute bioavailability of L-carnitine after a peroral dose of 1-6 g was only 5-18%, while the intake of L-carnitine by food resulted in an absorption of up to 75% (Evans et al., 2003). When the peroral dose was higher than 6 g, a specific smell was noted, possibly due to an increased concentration of trimethylamine in sweat (Hathcock et al., 2006).

Interestingly, the L-carnitine concentration that is required to support long-chain FA oxidation spans a wide range among different tissues of the same species and in the same tissue across species (Long et al., 1982; McGarry et al., 1983) because different tissues express different isoforms of CPT I, which are, in turn, differentially sensitive to L-carnitine. For example, the rat isoforms of CPT I in the liver (CPT IA) and muscle (CPT IB) have K_m values for L-carnitine of 30 and 500 μM , respectively (McGarry et al., 1983). In the heart, both CPT I isoforms, CPT IA and CPT IB, are present, and as a result, the average K_m for L-carnitine in the heart is 200 μM (McGarry et al., 1983). Therefore, the liver is much less sensitive to lower L-carnitine concentrations than the muscles and the heart. Additionally, in cases of a reduced L-carnitine concentration, a compensatory increase in CPT I is observed (Uenaka et al., 1996). In a recent publication, it was demonstrated that systemic L-carnitine deficiency causes severe hypoglycaemia in mice (Kuwajima et al., 2007). Similarly, after treatment with sodium pivalate, glucose oxidation was increased in L-carnitine-deficient hearts (Broderick, 2006). Thus, the bioavailability of L-carnitine changes along with the amounts of its dietary intake, and its requirements could be different in different tissues, reflecting the energy needs under certain conditions.

2.3 Effects of L-carnitine supplementation

During the last decades, the intake of L-carnitine as a food supplement has become very popular even though scientific evidence of its efficacy is not always available. Undoubtedly, L-carnitine is effective for the treatment of inherited disorders related to functional anomalies in a specific organic cation/carnitine transporter (OCTN2), in drug-induced L-carnitine deficiency and in cases of excessive excretion of L-carnitine because of renal tubular disorders and in patients on haemodialysis (Angelini et al., 2006; Fornasini et al., 2007; Lheureux et al., 2005). The safety profile of L-carnitine allows its frequent use in cocktails of food additives that are aimed to increase the oxidation of FA to eliminate body fat and thus produce cosmetic effects. However, thus far, the body weight-reducing effect of L-carnitine supplementation has not been proven clinically (Saper et al., 2004). A similar situation is characteristic for the use of L-carnitine for improved physical activity. The ineffectiveness of L-carnitine might be related to its low bioavailability during normal homeostasis (Hathcock et al., 2006). As a result, additional L-carnitine does not reach the cellular mitochondria where it could bring about the desired effects and drive the processes of FA metabolism. Recent developments in the use of L-carnitine to stimulate the oxidation of FA are linked to novel approaches to increase the bioavailability of L-carnitine (Stephens et al., 2007).

In addition to being an important cofactor for the L-carnitine mediated transport of long-chain FA into the mitochondrial matrix, as a pharmacological agent, L-carnitine enhances the glycolytic processing of carbohydrates (Foster, 2004; Mingrone, 2004; Stephens et al., 2007). The suggested mechanisms of pharmacological action of L-carnitine are increased

PDC activity, regulation of acetyl- and acyl-cellular trafficking and changes in the acetyl CoA/CoA ratio and expression of key glycolytic and gluconeogenic enzymes (Mingrone, 2004; Uziel et al., 1988). L-carnitine also stimulates glucose oxidation in intact fatty acid-perfused rat heart (Broderick, 2006). The beneficial effects of L-carnitine supplementation in animal models of diabetes also have been demonstrated (Malone et al., 1999; Malone et al., 2006; Sena et al., 2008). L-carnitine supplementation is believed to be beneficial for diabetic patients (Mingrone, 2004). In type 2 diabetic patients, L-carnitine increases the amount of whole-body glucose utilisation and glucose oxidation (Capaldo et al., 1991; De et al., 1999). However, for use in the management of diabetic complications, L-carnitine is listed between antioxidants for which no established benefit has been found (Golbidi et al., 2011), and the effects of L-carnitine in healthy subjects or in type 2 diabetic patients are still controversial (Gonzalez-Ortiz et al., 2008). Nevertheless, additional intake of L-carnitine is recommended for diabetic patients with late complications (Poorabbas et al., 2007; Power et al., 2007).

Because FA metabolism is the main source of energy for muscle cells, including cardiomyocytes, the effects of L-carnitine have been evaluated for the treatment of different cardiovascular diseases (Ferrari et al., 2004). The evidence for the cardioprotective effectiveness of L-carnitine is also not unambiguous. Treatment with L-carnitine was protective against ischemia-reperfusion injury (Cui et al., 2003), but another experimental study provided evidence that L-carnitine worsens ischemic injury under conditions where L-carnitine does not facilitate glucose metabolism (Diaz et al., 2008). For example, in the CEDIM 2 trial, L-carnitine therapy led to a reduction in early mortality without affecting the risk of death and heart failure at 6 months in patients with anterior acute myocardial infarction, resulting in a non-significant finding with respect to the primary end-point (Tarantini et al., 2006).

Even though the clinical and experimental evidence for the effects of supplementary L-carnitine intake are not always convincing, it is a very popular food additive due its safety profile, antioxidant-type activity and suggested effects on energy metabolism pathways.

3. Metabolic consequences of pharmacologically decreased availability of L-carnitine

Mildronate (3-(2,2,2-trimethylhydrazinium)propionate dihydrate) (Fig. 3) represents the only pharmacological agent that effectively reduces L-carnitine concentration and in turn induces protective effects against diabetes and cardiovascular diseases. The mechanism of action of mildronate is believed to involve a decreased tissue concentration of L-carnitine (Dambrova et al., 2002), even though other possible interactions between mildronate and pathways of energy metabolism signalling cannot be excluded.

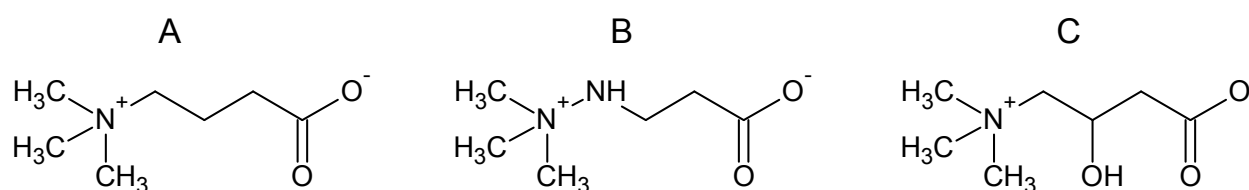


Fig. 3. Structural formulas of GBB (A), mildronate (B) and carnitine (C).

3.1 Inhibitory effect of mildronate on L-carnitine content

Mildronate is known to be an inhibitor of L-carnitine biosynthesis (Simkhovich et al., 1988), its transport into tissues and its reabsorption in the kidney (Kuwayama et al., 1999). The K_i for GBB hydroxylase enzyme inhibition by mildronate has been determined to be 19 μM (enzyme prepared as described by (Tars et al., 2010)). The EC_{50} for OCTN2 inhibition by mildronate was calculated to be 21 μM (Grube et al., 2006). At a dose of 100 mg/kg, the mildronate concentration in rat blood plasma reached 20 μM after a single administration; however, after 3 days of repeated administration, the mildronate concentration remained at 20 μM even 24 h after the last administration (Liepinsh 2006). Mildronate is actively transported into tissues by OCTN2 (Grigat et al., 2009), and the mildronate concentration in liver and heart reaches 500-1000 μM (Liepinsh et al., 2009a). Thus, mildronate concentrations are able to completely inhibit L-carnitine biosynthesis and substantially decrease L-carnitine transport into tissues and reabsorption in the kidneys. As a result, mildronate (100 mg/kg) induces a significant reduction of the L-carnitine concentration in blood plasma and different tissues by 60-70% (Liepinsh et al., 2009a). In our recent human study, it was shown that mildronate also decreases L-carnitine concentrations by 20% in healthy human non-vegetarian volunteers (Liepinsh et al., 2011a). A less-pronounced effect of mildronate on L-carnitine content in humans could be explained by meat consumption, which is the most important source of L-carnitine (Rebouche, 2004). Compared to a human non-vegetarian diet, in standard laboratory animal feed, the content of L-carnitine is very low (unpublished data). Overall, after long-term treatment, the plasma concentration of mildronate reaches a level that is able to induce changes in the homeostasis of L-carnitine. However, it must be noted that to our knowledge, an acute mildronate treatment does not induce any metabolic or gene expression changes, and therefore, long-term treatment (at least 10-14 days) is necessary to achieve regulatory effects. In addition, all changes induced by mildronate are highly related to the decrease in the L-carnitine concentration in tissues. The modulation of L-carnitine pathways by mildronate then changes the activity of cellular enzymes and receptor systems in a manner that could maintain sufficient energy metabolism and induce adaptive-like changes.

By inhibiting the biosynthesis of L-carnitine, mildronate increases the concentration of its substrate, γ -butyrobetaine (GBB, Fig. 3.), which is mostly known as a bio-precursor of L-carnitine (Bremer, 1983). We have investigated the effects of mildronate administration (4-12 weeks, 100 mg/kg) on both L-carnitine and GBB contents in rat plasma and tissues and have shown that in concert with a decrease in L-carnitine concentration, the administration of mildronate causes a significant increase in GBB concentration (Liepinsh et al., 2009a). There have been speculations regarding the cholinergic activity of GBB; however, only the methyl-ester of GBB produces this activity (Dambrova et al., 2004). The physiological and pharmacological consequences of increased GBB concentration remain to be discovered.

3.2 Metabolic changes in fatty acid metabolism

3.2.1 The effects on CPT I and mitochondrial fatty acid metabolism

Mildronate-decreased tissue L-carnitine concentrations induce changes in the energy metabolism pathways, especially in mitochondrial long-chain FA oxidation (Fig. 4). These changes are exclusively related to CPT I activity and FA transport into the mitochondria. If the L-carnitine content is lowered by mildronate treatment, a subsequent decrease in CPT I activity and a decreased CPT I-driven FA oxidation rate is observed (Kuka et al., 2011;

Simkhovich et al., 1988). Interestingly, due to the differences in L-carnitine content and the K_m values of CPT I for L-carnitine, at least a 60-70% reduction in L-carnitine content in the heart is necessary to reduce CPT I-dependent long-chain FA transport and oxidation.

Additionally, if the L-carnitine content is lowered significantly below the K_m value of CPT I, a compensatory increase in CPT I expression is observed (Uenaka R 1996). After long-term mildronate treatment, a 2 to 2.5-fold increase in the expression of CPT IA and CPT IB was found in the heart and liver (Degrace et al., 2007; Liepinsh et al., 2008). However, the increase in CPT I protein expression cannot compensate for significantly reduced L-carnitine availability, and therefore, subsequent changes in metabolic pathways occur. Although the expression of CPT I after mildronate treatment is increased, the overall FA oxidation rate in mitochondria is decreased (Kuka, et al. 2011; Simkhovich et al., 1988).

In a recent study, the effects of mildronate, L-carnitine and a combination of both substances on myocardial infarction were compared (Kuka et al., 2011). Mildronate treatment markedly decreased the L-carnitine concentration of the heart tissue and significantly decreased the infarct size. Administration of mildronate together with L-carnitine restored the L-carnitine concentration, and therefore, CPT I activity was similar to that in control samples; as a consequence, the infarct size was not reduced. Therefore, we conclude that the decrease of L-carnitine concentration is the main mechanism of action of mildronate, and this decrease is necessary to induce changes in cellular energy metabolism.

3.2.2 The modulation of PPARs and fatty acid metabolism-related gene expression

Peroxisome proliferator-activated receptors (PPARs) are central regulators of energy metabolism that mediate adaptive metabolic responses to increased systemic lipid availability following activation by the binding of endogenous or dietary lipids or lipid derivatives (Grimaldi, 2007; Sugden et al., 2009). Several genes that are induced by mildronate treatment, particularly CPT I (Degrace et al., 2007; Liepinsh et al., 2008), suggest PPARs as possible nuclear factors involved in the mildronate-induced effects on glucose and FA metabolism (Fig. 4). In a very recent study to elucidate the molecular mechanism of mildronate, PPAR- α and PPAR- γ nuclear content and the expression of glucose and FA metabolism genes were determined in obese Zucker rats (Liepinsh et al., 2011b). The hearts of obese Zucker rats had a significant impairment in the PPAR- α system, and the response to increased FA and triglyceride (TG) was disrupted. Thus, the expression of PPAR- α and its target genes in obese Zucker rat heart tissue was reduced compared to their expression in lean animals. Mildronate treatment restored the sensitivity of PPAR- α , which is manifested as significantly increased nuclear expression of PPAR- α in heart tissue and the expression of genes involved in FA metabolism. In cell culture, we found that mildronate and L-carnitine did not change PPAR activity *in vitro*. Thus, PPAR activation is instead induced as a result of a reduced FA oxidation rate and some FA accumulation.

Interestingly, increased expression of FA metabolic genes, particularly CPT-I, did not compensate for a reduction in the L-carnitine content, and therefore, the CPT I-dependent FA oxidation rate in heart tissue was significantly reduced. However, PPAR- α activation also induces genes that are involved in intra-mitochondrial FA β -oxidation. Thus, mildronate treatment could stimulate mitochondrial β -oxidation of FA transported into the mitochondria by CPT I (mildronate induces partial reduction in CPT I activity) and carnitine-acylcarnitine transport system. This could be another mechanism to protect the mitochondria from lipid overload and the accumulation of FA in mitochondria. To test this hypothesis, mitochondrial respiration measurements were performed on energy substrates

like palmitoyl-CoA and palmitoyl-L-carnitine. CPT I-dependent mitochondrial oxidation of palmitoyl-CoA was reduced in mildronate-treated rat hearts, while palmitoyl-L-carnitine oxidation in mildronate-treated heart mitochondria was even faster than in the control group (unpublished data). In contrast, the expression of PPAR- α and target genes in the liver of obese Zucker rats was not significantly affected by treatment. The effects of mildronate on PPAR- γ and PPAR- δ and the expression of their target genes are rather unclear and should be clarified in future studies. In conclusion, mildronate-dependent gene expression is, to some extent, dependent on the activation of PPAR- α in heart tissue but not in liver tissue.

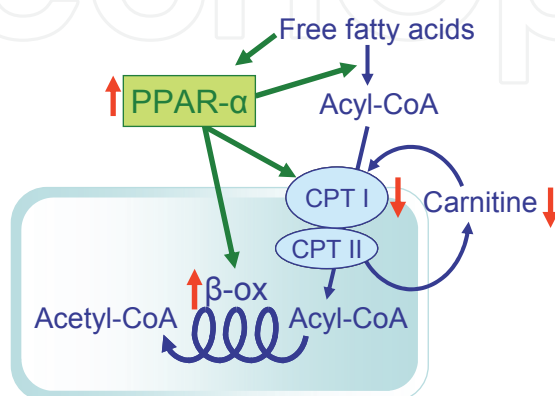


Fig. 4. The effects of pharmacologically decreased availability of L-carnitine on PPAR- α activity and fatty acid metabolism pathways. The red arrows indicate mildronate-induced changes; green arrows indicate stimulating effects of PPAR- α .

3.2.3 Metabolic flexibility and protection against lipid overload

Healthy subjects, depending on the availability of glucose or FA, are able to switch appropriately between the available energy substrates (Fig. 5A). At a postprandial state, the glucose and insulin concentrations are increased, and glucose oxidation dominates over FA oxidation. In contrast, in a fasted state, the glucose concentration is significantly lower, and energy metabolism is switched to FA. As shown in Fig. 5B, in obese subjects, lipid overload induces a metabolic inflexibility that is characterised by the reduced ability of cells to appropriately move between energy metabolism substrates (Larsen et al., 2008). Thus, an increase in circulating lipids stimulates FA influx into the mitochondria (Koves et al., 2008b; Petersen et al., 2004). Then, the increased concentration of long-chain FA and intermediates of FA metabolism, in particular acetyl-CoA, mediates the inhibition of the PDC and CPT I activity and induces the impairment of the citric acid cycle and respiratory chain functions (Koves et al., 2008b; Wang et al., 2007). These changes result in a pathologically decreased oxidation rate of both glucose and FA and deteriorate the mitochondrial capacity to produce ATP (Kelley et al., 2002). Therefore, lipid overload-induced metabolic inflexibility and lipotoxicity is primarily associated with FA accumulation in the mitochondria.

Mildronate treatment prevents lipid overload by decreasing the L-carnitine content in tissues and subsequently decreasing CPT I-mediated long-chain FA transport and pathological accumulation in the mitochondria (Asaka et al., 1998; Kirimoto et al., 1996). In obese Zucker rats, mildronate induced a moderate increase in the FA and TG concentrations, and it significantly improved insulin sensitivity and reduced blood glucose and insulin concentrations (Fig. 5C) (Liepinsh et al., 2011b). Thus, mildronate treatment, by

decreasing L-carnitine content, improves metabolic flexibility and adaptive responses to hyperglycaemia- and hyperlipidaemia-induced metabolic disturbances.

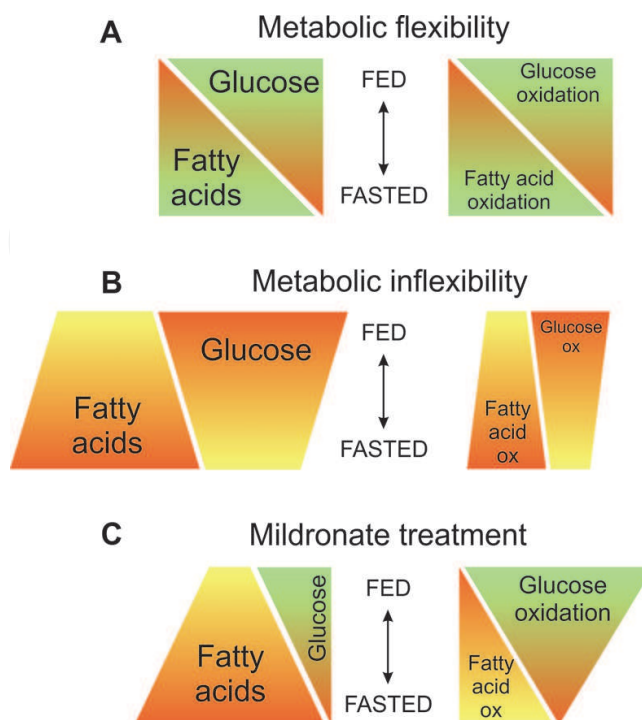


Fig. 5. Metabolic flexibility, inflexibility and mildronate treatment induced protection against lipid overload.

3.2.4 Comparison of mildronate to other inhibitors of fatty acid oxidation

Different approaches are used to inhibit FA oxidation and to switch energy metabolism from FA to glucose oxidation (Table 1). The most similar to the mildronate mechanism of action is the direct inhibition of CPT I or targeting malonyl-CoA, which is a potent endogenous inhibitor of CPT I (Anderson et al., 1995; Cuthbert et al., 2005). Because CPT I is the rate-limiting enzyme in FA oxidation, the inhibition of CPT I is an effective way to achieve a decrease in the long-chain FA oxidation rate. Several irreversible and reversible inhibitors of CPT I, such as etmoxir and teglicar, were synthesised and tested experimentally (Table 1). A significant myocardial infarction-lowering effect for etmoxir was demonstrated; however, its toxicity strongly limits its usage in clinic. Teglicar, both *in vitro* and in animal models, reduces gluconeogenesis and improves glucose homeostasis (Table 1) (Conti et al., 2011). Another approach is to inhibit malonyl-CoA decarboxylase, an enzyme that degrades malonyl-CoA to acetyl-CoA. The inhibition of this reaction by selective inhibitors leads to the accumulation of malonyl-CoA which inhibits CPT I and related FA oxidation. The inhibitors of CPT II have also been considered to be potentially useful for the treatment of type 2 diabetes (Rufer et al., 2009). Overall, the direct inhibition of CPT I or CPT II as well as an increase in malonyl-CoA and reduction in L-carnitine concentrations are similarly effective ways to target CPT I-dependent FA oxidation. As a result, cardiac metabolism is switched from FA to glucose oxidation.

In contrast, trimetazidine and ranolazine inhibit 3-ketoacyl coenzyme-A thiolase, which catalyses the last step in mitochondrial FA β -oxidation. A potential benefit offered by

trimetazidine and ranolazine in myocardial dysfunction is the ability to increase the utilisation of glucose and lactate, improving oxygen consumption of the myocardium. Overall, free FA inhibitors effectively act as metabolic modulators in the protection of ischemic myocardium both in non-diabetic and diabetic subjects.

Name	Mechanism of action	Effect on myocardial infarction	Effect on blood glucose metabolism	Benefit against diabetes complications	Literature
Mildronate	Lowered L-carnitine conc. and CPT I dependent FA oxidation	↓	↑	+	(Liepinsh et al., 2009b; Sesti et al., 2006)
Trimetazidine	Inhibition of 3-KAT in mitochondrial FA β-oxidation	↓	↑	+	(Monti et al., 2006; Mouquet et al., 2010)
Ranolazine	Inhibition of 3-KAT in mitochondrial FA β-oxidation	↓	↑	+	(Hale et al., 2008; Morrow et al., 2009)
Etmozir	Inhibition of CPT I and dependent FA oxidation	↓	↑	?	(Gunther et al., 2000; Wolf et al., 1988)
Teglicar	Inhibition of liver CPT IA and dependent FA oxidation	?	↑	?	(Conti et al., 2011)

3-KAT – 3-ketoacyl coenzyme-A thiolase, FA – fatty acids, CPT I – L-carnitine palmitoyl transferase, ↑ – stimulate, ↓ – decrease, + – beneficial effect, ? – under investigation.

Table 1. Comparison of mildronate to other inhibitors of fatty acid oxidation.

3.3 Metabolic changes in glucose metabolism

3.3.1 Switch to glucose metabolism

It is generally accepted that better survival of ischemic myocardium cells can be achieved by stimulating glucose oxidation, either directly or secondarily, to partially inhibited FA oxidation (Lopaschuk, 2001). The beneficial effects of metabolic modulators in cardiac tissue are based on the increased efficiency of the obtained ATP per mole of oxygen for glucose oxidation compared to FA oxidation and, more importantly, the increased coupling of glycolysis to glucose oxidation resulting in a net reduction of the proton burden in the ischemic tissue (Schofield et al., 2001; Taegtmeier et al., 1998). Moreover, insulin resistance and lowered glucose uptake and oxidation have been associated with the progression of cardiovascular pathologies such as coronary heart disease and heart failure (Kamalesh, 2007;

Taegtmeyer et al., 1998). A shift of the energy substrate preference from FA oxidation to glucose oxidation was suggested as a basis for the cardioprotective effects of mildronate long before it was demonstrated experimentally (Asaka et al., 1998; Dambrova et al., 2002). Indeed, the reduction of L-carnitine content by mildronate treatment leads to a decrease in the CPT I-dependent FA oxidation rate in mitochondria, and in turn, due to a Randle cycle, a substantial increase is expected in the rate of glucose uptake, as was found in mouse hearts after mildronate treatment (Liepinsh et al., 2008). Thus, in cases of partially decreased FA oxidation, changes in glucose metabolism could play a compensatory role. In contrast to mouse and diabetic rat models, where a significant blood glucose-lowering effect was observed, the glucose concentration was not significantly decreased in rats treated with mildronate at doses up to 400 mg/kg (Liepinsh et al., 2009a). These results suggest that mildronate treatment does not directly stimulate but rather facilitates glucose metabolism and that the effect of mildronate strongly depends on substrate availability and energy requirements.

3.3.2 Effects on insulin signalling

Insulin is the major regulator of glucose homeostasis in the body. The insulin signalling pathway is responsible for controlling substrate preference in the heart, muscles and adipose tissues. Insulin stimulates myocardial glucose uptake and accelerates glucose oxidation (Benecke et al., 1993). Both in non-diabetic and diabetic animals, mildronate treatment significantly facilitates insulin-dependent glucose uptake and metabolism (Fig. 6). At the same time, mildronate, which decreases the blood glucose concentration in mice and diabetic rats, usually does not affect plasma insulin and C-peptide levels (Liepinsh et al., 2008; Liepinsh et al., 2009b). In addition, in study in Zucker rats mildronate along with decrease in glucose concentration also decreased pathologically elevated insulin concentration in fed Zucker rats (Liepinsh et al., 2011b). Moreover, mildronate induces a statistically significant 35% increase in insulin-stimulated glucose uptake in isolated mouse hearts (Liepinsh et al., 2008). Mildronate action could be explained by specific insulin pathway-related gene expression. During insulin-stimulated conditions, glucose transport by GLUT 4 is the rate-limiting step for glucose uptake in muscles and the heart. In genetically manipulated mice overexpressing GLUT 4, improvement in both glucose tolerance and insulin action were noted (Hansen et al., 1995). Mildronate treatment induces an increase in GLUT 4 protein expression in heart tissues, which explains the increase in insulin-stimulated glucose uptake.

The important intracellular regulators of aerobic oxidation of glucose are the enzymes of the PDC, which links the glycolysis metabolic pathway to the mitochondrial citric acid cycle (Fig. 6). A significant 2-fold increase in the expression of both PDC enzymes, E1/E2, in mildronate-treated mouse hearts compared with non-treated controls was observed. In addition, in several studies, a decrease in lactate concentration in ischemic hearts of mildronate-treated animals has been noted, which indicates that long-term mildronate treatment not only facilitates glucose uptake but also enhances the aerobic oxidation of glucose (Asaka et al., 1998; Hayashi et al., 2000b; Liepinsh et al., 2011b). Besides the direct effects of insulin on glucose homeostasis, stimulation of the insulin receptor leads to the activation of signalling pathways such as the phosphatidylinositol-3-kinase and the mitogen-activated protein kinase cascades, which are involved in cardioprotection from reperfusion injury (Jonassen et al., 2001; Youngren, 2007). Overall, the observed increase of

insulin receptor sensitivity could be important for the manifestation of the cardioprotective and antidiabetic effects of the mildronate treatment.

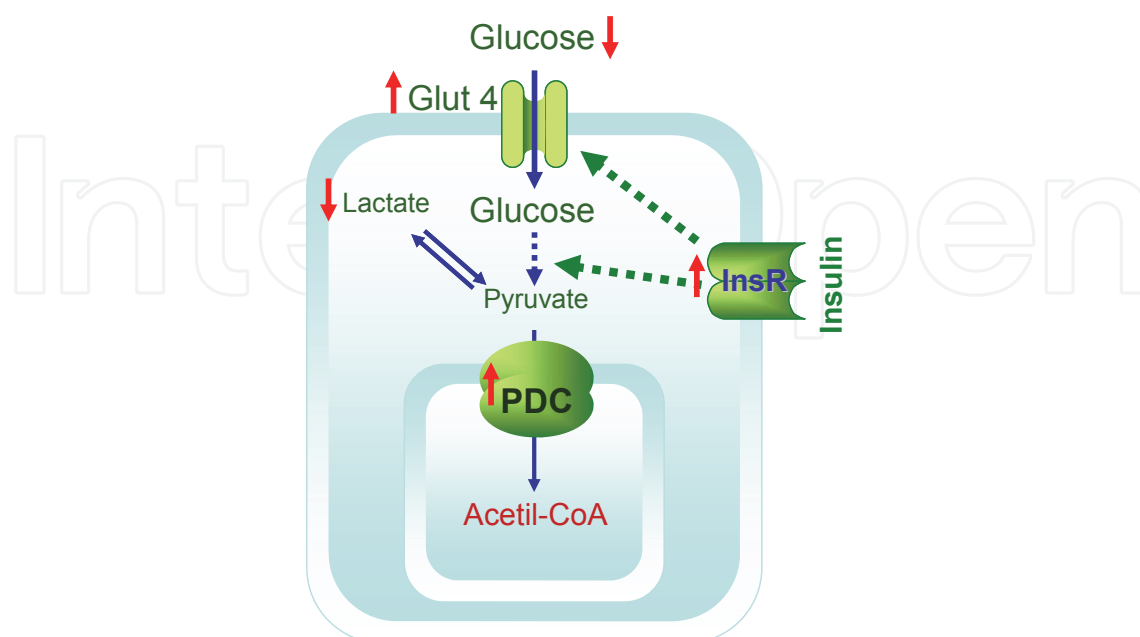


Fig. 6. The effects of pharmacologically decreased availability of L-carnitine on glucose metabolism. The red arrows indicate mildronate-induced changes; green arrows indicate stimulating effects of insulin.

4. Pharmacological effects of decreased availability of L-carnitine

4.1 Effects on myocardial infarction

Mildronate is primarily known as a cardioprotective drug, the mechanism of action of which is based on a decrease of L-carnitine concentration, regulation of energy metabolism and enhancement of preconditioning-like adaptive responses (Dambrova et al., 2002). In the past 10 years, cardioprotective effects of mildronate have been extensively studied in different models of heart infarction and heart failure (Hayashi et al., 2000b; Liepinsh et al., 2006; Sesti et al., 2006; Vilskersts et al., 2009a). It was shown that long-term treatment with mildronate preserved ATP production by optimisation of energy metabolism. Then, a significant reduction of infarct size was shown in an isolated heart infarction model both in vitro (Liepinsh et al., 2006) and in vivo (Sesti et al., 2006). In addition, mildronate was recently demonstrated to be cardioprotective in diabetic Goto-Kakizaki (G-K) rats, where besides the glucose-lowering effect, mildronate treatment at doses of 100 and 200 mg/kg decreased the infarct size by 30% (Liepinsh et al., 2009b). Overall, mildronate treatment effectively reduces myocardial infarction in diabetic GK and non-diabetic Wistar rats.

Treatment with mildronate did not have a significant effect on haemodynamic parameters either before or during ischemia-reperfusion, indicating that the observed effects on infarct size are not related to changes in the cardiac workload. This result gives evidence that mildronate primarily acts as metabolic regulator and provides opportunities to effectively combine mildronate with agents affecting haemodynamic parameters. In animal studies, mildronate is combined with orotic acid, which is also known as an energy metabolism-influencing metabolite (Vilskersts et al., 2009a). Mildronate possesses additive

pharmacological effects with orotic acid, and mildronate orotate might be considered a powerful therapeutic agent facilitating recovery from ischemia-reperfusion injury. In the same study, we found that the administration of mildronate and its orotate salt decreased the duration and incidence of arrhythmias in experimental arrhythmia models.

4.2 Heart failure

The effects of mildronate on haemodynamics, cardiac hypertrophy and metabolic consequences in rats after coronary artery ligation were also studied. Mildronate induces significant attenuation of the development of left ventricular (LV) hypertrophy and of increased LVEDP. These results suggest that mildronate improved LV hypertrophy and LV dysfunction in rats with heart failure induced by myocardial ischemia (Nakano et al., 1999). In a similar study performed by Hayashi et al., the effects of mildronate were examined in rats with congestive heart failure following myocardial infarction (Hayashi et al., 2000b). A survival study was conducted for 6 months, and ventricular remodelling, cardiac function, and myocardial high-energy phosphate levels after treatment for 20 days were measured. Mildronate prolonged survival, prevented expansion of the left ventricular cavity (ventricular remodelling), attenuated the rise in right atrial pressure and augmented cardiac functional adaptability against an increased load. Additionally, an improved myocardial energy state in mildronate-treated rats was observed. A different study by the same authors demonstrated that the mechanisms of mildronate action in heart failure are based on the amelioration of $[Ca^{2+}]_i$ transients through an increase in SR Ca^{2+} uptake activity (Hayashi et al., 2000a).

Several clinical studies evaluated the efficacy of mildronate in the complex therapy of chronic heart failure (CHF). Statsenko, ME et al. evaluated the clinical efficacy of mildronate in addition to basic therapy in patients with CHF and type 2 diabetes mellitus (DM2) during the postinfarction period (Statsenko et al., 2007). The subjects were 60 II to III NYHA CHF patients aged 43 to 70 years who were also suffering from DM2. The patients were observed during the early postinfarction period (weeks 3 to 4 from the onset of myocardial infarction). The use of mildronate in addition to basic therapy was associated with a more evident decrease in CHF functional class, an increase in 6-min walking test distance, a tendency towards normalisation of diastolic heart function and an increase in LVEF. Also, in a recent study, the advantage of treatment with mildronate (1 g/day) as a corrector of metabolism in combination with standard therapy for the exercise tolerance of patients with CHD over treatment with a placebo in combination with standard therapy was noted (Dzerve et al., 2010). The overall conclusion from clinical studies is that the use of mildronate in basic therapy favours the normalisation of vegetative homeostasis and improves the quality of life.

4.3 Metabolic syndrome and diabetes

The effects of mildronate on glucose metabolism and its benefits in the treatment of diabetes have been proven experimentally only during the last decade, even though they had been suggested previously (Dambrova et al., 2002). This delay could be because mildronate at doses up to 400 mg/kg did not affect the blood glucose concentration in non-diabetic rats. Mildronate reduced blood glucose in Wistar rats only at a dose of 800 mg (Degrace et al., 2007). The possible anti-diabetic effects of mildronate were then examined in an experimental model of type 2 diabetes in GK rats (Liepinsh et al., 2009b).

The main finding of this study was that decreased L-carnitine availability leads to fed and fasted blood glucose concentration-lowering effects without increasing the insulin concentration in diabetic GK rats. As mentioned before, mildronate also induced cardioprotective effects in GK rat hearts. At the end of an 8-week treatment, the necrosis zone in mildronate-treated diabetic rat hearts was significantly decreased by 30%. Overall, these findings directly indicate that mildronate treatment could be beneficial in diabetes patients with cardiovascular diseases. Furthermore, mildronate effectiveness was tested in rats with streptozotocin-induced diabetes mellitus (Sokolovska et al., 2011). As expected, long-term mildronate treatment caused a significant decrease in the mean blood glucose and glycated haemoglobin (HbA1c) concentrations in streptozotocin-treated rats. Thus, the glucose-lowering effects were shown experimentally both in type 1 and type 2 diabetes mellitus.

In a recent study, the effects of mildronate treatment were compared to the effects of metformin treatment in obese Zucker rats (Liepinsh et al., 2011). In this study, mildronate treatment, to the same extent as metformin, significantly reduced the blood glucose concentration in the fed and fasted states and increased the insulin concentration in obese Zucker rats. In addition, several effects caused by the combination of mildronate and metformin treatment were additive or even synergistic. An additive effect in an oral glucose tolerance test and in the reduction of insulin concentration was observed. Moreover, treatment with the combination of both drugs increased liver glycogen content, suggesting a significant improvement in liver insulin sensitivity. Thus, the additional increase in body insulin sensitivity is a benefit of treatment with this combination. These changes may be explained by an increase in the expression of glucose metabolism genes, such as Glut-1, HK-2 and, in particular, GLUT 4. Furthermore, treatment with mildronate or metformin alone did not significantly change the weight gain of obese Zucker rats. In contrast to monotherapy, treatment with the combination of mildronate and metformin induced a significant reduction in weight gain and especially fat mass, which was decreased by 47% in combination-treated rats. Thus, the combination therapy of mildronate with metformin could have a potential therapeutic value in the treatment of obese patients with type 2 diabetes. Additionally, one of the well-known side effects of metformin is related to an increased lactate level. In this study, mildronate treatment separately or in combination with metformin reduced the lactate concentration in obese Zucker rat plasma, and therefore, mildronate used in combination could reduce the risk of metformin-induced lactate acidosis.

4.4 Diabetes complications

Mildronate treatment induces protective effects not only in the heart but also in other tissues. Vilskersts et al. demonstrated that chronic treatment with mildronate at a dose of 100 mg/kg significantly reduced the size of atherosclerotic plaques in the aortic roots and in the whole aorta in apoE/LDLR(-/-) mice (Vilskersts et al., 2009b). The effect of long-term treatment with mildronate on the functional manifestation of metabolic disturbances and diabetes-induced complications in diabetic animals was also studied (Liepinsh et al., 2009b). The contractile responsiveness to phenylephrine was significantly enhanced in the aortas of GK rats, and this hypersensitivity was normalised by mildronate at a dose of 200 mg/kg. The functional impairment of coronary arteries characterised by significantly lower coronary flow was observed in isolated GK rat hearts compared to those of Wistar rats.

After treatment by mildronate, coronary flow was significantly increased, indicating that long-term treatment with mildronate prevents endothelial dysfunction in animals with type 2 diabetes.

Peripheral neuropathy characterised by abnormality in the thermal pain threshold of diabetic GK rats was associated with the development of diabetes (Ueta et al., 2005). The loss of thermal response is dependent on the severity and duration of hyperglycaemia and is also a characteristic diabetic complication in the clinic. Long-term mildronate administration both controlled hyperglycaemia and prevented diabetic neuropathy. It is very likely that by influencing energy metabolism, mildronate reduces neuropathy in type 2 diabetes animals.

An open randomised trial in diabetic patients investigated 70 matched patients with type 2 diabetes mellitus and sensomotor neuropathy; the effectiveness of mildronate was evaluated for 3 months in addition to basic therapy (Statsenko et al., 2008). Mildronate administration did not influence glucose and Hb1Ac concentration, but it significantly improved the clinical condition of the study group patients vs. the controls by neuropathy and symptoms count scales, electrophysiological properties of the nerve fibres and optimisation of oxygen tissue balance. Overall, mildronate is suggested for the treatment of diabetes at a dose of 1 g/day as an addition to standard therapy.

5. Conclusion

In conclusion, a mildronate-induced decrease in L-carnitine concentration leads to a partial reduction in FA oxidation and shifts energy metabolism from free FA towards predominantly glucose utilisation by the myocardium to increase the effectiveness of ATP generation. Long-term pharmacologically decreased availability of L-carnitine induces adaptive effects by causing changes in FA and glucose metabolism-related gene expression, which are beneficial in the treatment of heart diseases and diabetes. The L-carnitine homeostasis is a valuable target for the regulation of energy metabolism pathways and treatment of metabolic diseases.

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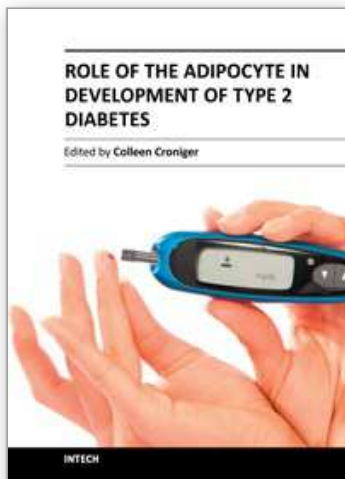
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Role of the Adipocyte in Development of Type 2 Diabetes

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Adipocytes are important in the body for maintaining proper energy balance by storing excess energy as triglycerides. However, efforts of the last decade have identified several molecules that are secreted from adipocytes, such as leptin, which are involved in signaling between tissues and organs. These adipokines are important in overall regulation of energy metabolism and can regulate body composition as well as glucose homeostasis. Excess lipid storage in tissues other than adipose can result in development of diabetes and nonalcoholic fatty liver disease (NAFLD). In this book we review the role of adipocytes in development of insulin resistance, type 2 diabetes and NAFLD. Because type 2 diabetes has been suggested to be a disease of inflammation we included several chapters on the mechanism of inflammation modulating organ injury. Finally, we conclude with a review on exercise and nutrient regulation for the treatment of type 2 diabetes and its co-morbidities.

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