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Decreased acylcarnitine content improves insulin sensitivity in experimental mice models of insulin resistance

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ABSTRACT

The important pathological consequences of insulin resistance arise from the detrimental effects of accumulated long-chain fatty acids and their respective acylcarnitines. The aim of this study was to test whether exercise combined with decreasing the content of long-chain acylcarnitines represents an effective strategy to improve insulin sensitivity in diabetes.

We used a novel compound, 4-[ethyl(dimethyl)ammonio]butanoate (methyl-GBB), treatment and exercise to decrease acylcarnitine contents in the plasma and muscles in the insulin resistance models of high fat diet (HFD) fed C57BL/6 mice and *db/db* mice.

The methyl-GBB treatment induced a substantial decrease in all acylcarnitine concentrations in both fed and fasted states as well as when it was combined with exercise. In the HFD fed mice methyl-GBB treatment improved both glucose and insulin tolerance. Methyl-GBB administration, exercise and the combination of both improved insulin sensitivity and reduced blood glucose levels in *db/db* mice. Methyl-GBB administration and the combination of the drug and exercise activated the PPARα/PGC1α signaling pathway and stimulated the corresponding target gene expression. Insulin insensitivity in *db/db* mice was not induced by significantly increased fatty acid metabolism, while increased insulin sensitivity by both treatments was not related to decreased fatty acid metabolism in muscles.

The pharmacologically reduced long-chain acylcarnitine content represents an effective strategy to improve insulin sensitivity. The methyl-GBB treatment and lifestyle changes via increased physical activity for one hour a day have additive insulin sensitizing effects in *db/db* mice.

1. Introduction

The primary pathophysiology of type 2 diabetes is associated with the inappropriate action of insulin [1]. The inability of insulin to stimulate glucose utilization in skeletal muscle and storage in adipose tissue results in increasing concentrations of blood glucose and advanced glycation end-products in the blood [2]. A growing body of evidence suggests that the important pathological consequences of insulin resistance arise from the detrimental effects of the accumulation of long-chain fatty acids (LCFA) and their respective acylcarnitines [3,4]. Long-chain (LC) acylcarnitines inhibit pyruvate and lactate oxidation in mitochondria and could induce insulin resistance [5]. Therefore, pharmacological interventions that target acylcarnitine accumulation are a possibility for the development of novel treatment strategies to improve the clinical outcomes of patients with diabetes.

LCFA supply a major part of the energy that is required for muscle function. Nevertheless, in diabetes, the pharmacologically reduced oxidation of fatty acids leads to better glucose utilization [6]. Carnitine palmitoyltransferase-1 (CPT1) is an important step in LCFA uptake and oxidation by mitochondria [7]. Treatment of mice with the CPT1 inhibitor, oxfenicine, resulted in improved whole-body glucose tolerance and insulin sensitivity in a diet-induced insulin resistance model [8]. Taking into account that LC acylcarnitines are produced from LCFAs and L-carnitine, an alternative

Abbreviations: ACOX1, acyl-CoA oxidase 1; ACSL, long-chain fatty acid CoA synthetase; CPT1, carnitine palmitoyltransferase-1; FABP3, fatty acid binding protein 3; HFD, high fat diet; LCFA, long-chain fatty acids; Methyl-GBB, 4-[ethyl(dimethyl)ammonio]butanoate; OCTN2, organic cation transporter 2; PGC1α, peroxisome proliferator-activated receptor-γ coactivator 1α; PPARα, peroxisome proliferator-activated receptor alpha.

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Table 1
The design of the experiments.

No	Group	Mice with impaired insulin sensitivity	Mice with diabetes	Treatment
1	Non-diabetic control	Normal chow	db/Lean	water
2	Control with diabetes	HFD	*db/db*	water
3	Methyl-GBB	HFD	*db/db*	Methyl-GBB[a]
4	Exercise	—	*db/db*	Ex[b]
5	Methyl-GBB + exercise	—	*db/db*	Methyl-GBB[a] + Ex[b]

[a] Methyl-GBB once a day, *p.o.* 5 mg/kg.
[b] Ex—forced walking five days a week, 60 min/day at a speed of 5 m/min.

strategy is to decrease the content of L-carnitine. Indeed, facilitated glucose oxidation and reduced circulating glucose and insulin concentrations were observed in response to limited L-carnitine availability [9,10]. However, neither study measured the content of acylcarnitines to verify their relationship to the progression of insulin resistance and the possible therapeutic effects of this treatment.

ADA and EASD guidelines state that changes in lifestyle including increased physical activity are an integral part of type 2 diabetes therapy [11]. Indeed, exercise training results not only in improved insulin sensitivity and decreased glucose levels in patients with type 2 diabetes or obesity/insulin resistance [12,13] but also reduces coronary heart disease risk and improves exercise capacity [14–16]. However, for various reasons, exercise training often does not reach diabetes treatment endpoints [17]. In addition, results of a combination of lifestyle changes with antidiabetic drug treatments have been inconsistent, and current strategies to combine physical activity with medication have not led to the expected outcomes in clinical practice [18–20]. Therefore, novel strategies to improve physical activity induced effects by drug treatment are necessary.

Interestingly, physical activity leads to a significant increase in medium- to long-chain acylcarnitines [21], while a similar tendency towards short- to medium-chain acylcarnitine accumulation has been observed in obese patients on a high fat diet [22]. This implicates the acylcarnitines in the regulation of metabolic responses to physical activities and diet depending on changes in the levels of acylcarnitines of various chain lengths. Thus, we studied whether the pharmacological reduction of various chain length acylcarnitines would be beneficial in patients with diabetes, particularly when combined with physical intervention.

In this study, to reduce acylcarnitine content and decrease LCFA oxidation, we used a novel compound, 4-[ethyl(dimethyl)ammonio]butanoate (methyl-GBB), which is a very potent inhibitor of L-carnitine transport by the organic cation transporter 2 (OCTN2) and thus significantly decreases the L-carnitine and acylcarnitine content in the plasma and tissues [23–25]. To determine the role of acylcarnitines in the development of insulin resistance, we used high fat diet (HFD) and *db/db* mice models of insulin resistance. The purpose of the current study was to test whether decreasing acylcarnitine content alone or in combination with exercise intervention represents an effective strategy to induce antidiabetic effects.

2. Materials and methods

2.1. Animals and treatment

Thirty C57BL/6 male mice, forty male *db/db* mice (10 weeks old, Harlan Laboratories BV, Venray, Netherlands) and ten age-matched non-diabetic db/Lean male mice were housed under standard conditions (21–23 °C, 12 h shifted light–dark cycle) with unlimited access to food (R70 diet, Lantmännen, Sweden) and water. The experimental procedures were carried out in accordance with the guidelines of the European Community, local laws and policies and were approved by the Latvian Animal Protection Ethical Committee, Food and Veterinary Service, Riga, Latvia. All studies involving animals are reported in accordance with the ARRIVE guidelines [26,27].

Mice were adapted to local conditions for two weeks before the start of treatment. Twenty C57BL/6 mice were treated with a HFD (Special Diets Services, UK) for 8 weeks to induce insulin resistance. As seen in Table 1, C57BL/6 mice were divided into 3 groups and *db/db* mice were divided into 4 groups. In pilot studies methyl-GBB was tested at doses of 5, 10 and 20 mg/kg and a dose of 5 mg/kg was selected for this study. Methyl-GBB phosphate (equivalent to 5 mg/kg of methyl-GBB) was administered with drinking water for 8 weeks. During the same time period, forced exercise was performed for *db/db* mice. For the exercise experiment, a 21 wheels forced exercise/walking wheel apparatus (PsymCon Model 35500, Lafayette Instrument, Lafayette, USA) was used. Before the experiment, mice were adapted to exercise for one week: on the first and second day mice walked 30 min at a speed of 3 m/min, on the third day they walked 40 min at a speed of 3.5 m/min, on the fourth day they walked 50 min at a speed of 4 m/min and on the fifth day they walked 60 min at a speed of 5 m/min. For further experiment, mice walked five days a week, 60 min a day, at a speed of 5 m/min for 8 weeks in total. The mean total number of steps performed in a 60 min test period was 3600 steps (60 steps/min).

Animals were weighed 2 times per week. The blood samples from fed and fasted animals were collected from the tail vein prior the start of insulin and glucose tolerance tests. After euthanasia by cervical dislocation, the organ samples were collected. The obtained plasma and tissue samples were stored at −80 °C until analysis.

2.2. Measurement of acylcarnitine levels by UPLC/MS/MS

Determination of the acylcarnitines in the plasma and muscle tissue samples was performed by the UPLC/MS/MS method. The sample preparation was as previously described [5,28]. The concentrations of acylcarnitines were measured against a seven-point standard curve of palmitoylcarnitine ranging from 10 ng/ml to 1000 ng/ml.

2.3. Determination of biochemical measures and tolerance tests

Glucose and lactate in plasma samples were measured by Instrumentation Laboratory (Milan, Italy) and Roche Diagnostics (Mannheim, Germany) enzymatic kits. Blood glucose was measured using a MediSense Optium (Abbott Diabetes Care, Maidenhead, UK) blood glucose meter and strips. Plasma insulin concentrations were determined with a RIA kit (Millipore, Billerica, USA).

Glucose and insulin tolerance tests were performed on the last 3 days of the 8 week treatment. To perform the glucose tolerance test, the mice were fasted overnight. Then, the glucose solution (0.5 g/kg of body weight) was administered intraperitoneally, and blood samples were drawn from the tail vein at 0 (fasting), 15, 30,