Here, there and everywhere. A multi organ approach to acylcarnitine metabolism

Schooneman, M.G.

Citation for published version (APA):
C1

A Short Introduction
We eat, therefore we are. The human body obtains energy via the breakdown of macronutrients, carbohydrates, lipids and proteins. The resulting sugars, fatty acids and amino acids can be oxidized via several metabolic pathways under different metabolic circumstances. In a postprandial state, we mainly oxidize carbohydrates because these macronutrients are predominant in most diets. But when carbohydrate oxidation (CHO) rates fall, either because most glucose is oxidized, or because glucose reserves are not replenished, we switch to lipids as main oxidative substrate (1). And in these days, a substantial percentage of all human beings carry along large reserves of lipids.

Lipids are stored mainly as triglycerides, and when needed for oxidation they are hydrolyzed into glycerol and free fatty acids (FFA). FFAs are converted into acyl-CoAs, which are oxidized inside the mitochondrion via beta-oxidation. Because the mitochondrial membrane is impermeable to acyl-CoAs, carnitine is essential for the transport of acyl-CoAs into mitochondria (2, 3). Here, acyl-CoAs are transesterified to carnitine by the enzyme carnitine palmitoyltransferase-1 (CPT1) on the outer leaflet of the mitochondrial membrane. The resulting acylcarnitine is then shuttled over the membrane by carnitine aylcarnitine translocase (CACT) towards the inner leaflet of the mitochondrial membrane, where CPT2 can release the acyl-CoA for further beta-oxidation (4). Due to its mitochondrial function, carnitine and acylcarnitines mainly reside within tissues (5). But as they can cross the cell membrane, they can be found in the plasma compartment as well. Here they form a characteristic acylcarnitine profile, which is considered to reflect the intracellular acyl-CoA pool. This profile has been studied extensively in the last few decades in relation to inherited metabolic diseases (6) and more recently for their possible involvement in diet-induced metabolic derangements and insulin resistance (7-10).

As stated earlier, FAO rates increase when CHO rates decline, for example during fasting. Therefore, acylcarnitine levels, being FAO-intermediates, increase in a fasted state (5, 11, 12). This shift from CHO towards FAO is accompanied by a physiological resistance to the insulin signal, mainly in skeletal muscle tissue, as the remaining glucose in plasma needs to be spared to fuel the central nervous system. Apart from fasting-induced insulin resistance, also obesity-associated insulin resistance is accompanied by a rise in acylcarnitine levels. Under circumstances of overfeeding and obesity, insulin resistance occurs as well and FAO rates remain high, even under fed conditions where glucose is readily available. This leads to high lipid levels in insulin sensitive tissues, which could potentially interfere directly with insulin signalling inside the cytosol, a theory referred to as lipotoxicity. Several lipid intermediates accumulate and impair insulin sensitivity, such as ceramides, gangliosides and diacylglycerol (7, 13-15). In addition, acylcarnitines accumulate due to high or incomplete FAO (8-10).

Several studies have shown associations between acylcarnitines and insulin resistance. Here, insulin resistant states such as (prolonged) fasting and diet-induced obesity and type 2 diabetes mellitus were accompanied by elevated plasma acylcarnitines (9, 11, 12). Additionally several individual species showed correlations with markers of insulin resistance and glucose tolerance in both rodents and humans (99, 16-19). An important limitation of the proposed association between acylcarnitines and insulin resistance is that acylcarnitine profiles are often measured in plasma. Since acylcarnitine metabolism
and FAO are cellular processes in insulin sensitive tissues, it remains to be determined what plasma acylcarnitines actually reflect. Moreover, much knowledge on acylcarnitine kinetics is lacking. Therefore a deeper understanding of acylcarnitine metabolism is crucial in the interpretation of the proposed associations with FAO derangements and insulin resistance. This thesis aims to elucidate the kinetic properties of acylcarnitines of different chain lengths in the plasma compartment. Furthermore we studied the interaction of acylcarnitine metabolism between plasma and different insulin sensitive tissues. In all our studies we compared acylcarnitine metabolism in fasted, fed and a HFD-induced, insulin resistant state.

**Thesis outline**

**Chapter 2** is an introductory review on the existing literature on acylcarnitine metabolism in relation to insulin resistance. We included in vitro, animal and human studies. After this inventory of associations between acylcarnitines and insulin resistance, we tried to subsequently clarify what the alterations in acylcarnitine metabolism actually reflect. In order to study the relation between the plasma acylcarnitine profile and acylcarnitine metabolism on tissue level, **chapter 3** focussed on correlations between the profiles in the different compartments in fed and fasted mice. Here we expected to find a relation between plasma acylcarnitines and muscle or liver acylcarnitines, as they are suggested to play an important role in acylcarnitine metabolism. Following chapter 3, the aim of **chapter 4** was to determine the role of different organs in acylcarnitine metabolism. Therefore we measured fasted and postprandial trans organ fluxes of acylcarnitines in a catheterized and conscious pig model. The basic kinetics of acylcarnitines were studied in **chapter 5**, using stable C2- and C16-carnitine isotopes in mice with various degrees of insulin resistance. Here we tried to elucidate if acylcarnitine kinetics such as rates of appearance or elimination rates are different in various insulin resistant states.

**In chapter 6**, effects of weight loss on acylcarnitine levels were studied in relation to insulin sensitivity and energy expenditure in 60 obese human subjects. Based on the assumption that elevated acylcarnitine levels accompany insulin resistance, we expected that acylcarnitine levels would decrease along with improvements in glucose tolerance. **Chapter 7** describes the effects of increasing carnitine levels in an obese model on FAO rates, energy expenditure and insulin sensitivity. Here we report the effects of increased carnitine availability by administration of the carnitine precursor gamma-butyrobetaine in lean and obese mice. Finally, **chapter 8** discusses the results of the separate projects in relation to the current knowledge of acylcarnitine metabolism and if our results support the hypothesis on the role of acylcarnitines in the etiology of insulin resistance. The importance of acylcarnitine tissue specificity, kinetics and substrates in different metabolic circumstances is put in perspective.
References