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Original article

Palmitoyl-carnitine production by blood cells associates with the concentration of circulating acyl-carnitines in healthy overweight women

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SUMMARY

Background: Circulating acyl-carnitines (acyl-CNTs) are associated with insulin resistance (IR) and type 2 diabetes (T2D) in both rodents and humans. However, the mechanisms whereby circulating acyl-CNTs are increased in these conditions and their role in whole-body metabolism remains unknown. The purpose of this study was to determine if, in humans, blood cells contribute in production of circulating acyl-CNTs and associate with whole-body fat metabolism.

Methods and results: Eight non-diabetic healthy women (age: 47 ± 19 y; BMI: 26 ± 1 $kg \cdot m^{-2}$) underwent stable isotope tracer infusion and hyperinsulinemic-euglycemic clamp study to determine *in vivo* whole-body fatty acid flux and insulin sensitivity. Blood samples collected at baseline (0 min) and after 3 h of clamp were used to determine the synthesis rate of palmitoyl-carnitine (palmitoyl-CNT) *in vitro*. The fractional synthesis rate of palmitoyl-CNT was significantly higher during hyperinsulinemia (0.788 ± 0.084 *vs.* 0.318 ± 0.012% $\cdot hr^{-1}$, $p = 0.001$); however, the absolute synthesis rate (ASR) did not differ between the periods ($p = 0.809$) due to ~30% decrease in blood palmitoyl-CNT concentration ($p = 0.189$) during hyperinsulinemia. The ASR of palmitoyl-CNT significantly correlated with the concentration of acyl-CNTs in basal ($r = 0.992$, $p < 0.001$) and insulin ($r = 0.919$, $p = 0.001$) periods; and the basal ASR significantly correlated with plasma palmitate oxidation ($r = 0.764$, $p = 0.027$).

Conclusion: In women, blood cells contribute to plasma acyl-CNT levels and the acyl-CNT production is linked to plasma palmitate oxidation, a marker of whole-body fat metabolism. Future studies are needed to confirm the role of blood cells in acyl-CNT and lipid metabolism under different physiological (i.e., in response to meal) and pathological (i.e., hyperlipidemia, IR and T2D) conditions.

1. Introduction

Type 2 diabetes and atherosclerotic CVDs (aCVDs) are significant contributors to morbidity and mortality in the US [5,6,11]. Obesity and insulin resistance (IR) predispose one to the development of T2D and aCVDs. IR is accompanied by excessive accumulation of lipid metabolites, e.g., acyl-carnitine (acyl-CNT) in skeletal muscle [2,10,13,19,27]. Several studies have shown associations between excessive accumulation of intramuscular lipids and the development of IR in muscle [2,7,10,12,13,15,18,27]. Recently, elevated plasma concentrations of acyl-CNTs have been reported in insulin-resistant individuals and patients with T2D when compared to healthy adults [1,17]. However, the source of these acyl-CNTs remains unknown. To address this question, Schooneman et al. [21]

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measured plasma and tissue acyl-CNT profiles in mice and showed that, in general, the plasma acyl-CNT profile did not reflect the acyl-CNT profile of any specific tissues, including skeletal muscle or the liver. However, these authors did not determine the contribution (if any) of blood cells (i.e., peripheral blood mononuclear cells; PBMC, and platelets) to plasma acyl-CNTs [21]. Acyl-CNTs are produced by the outer mitochondrial membrane enzyme carnitine palmitoyl transferase 1 (CPT-1), meaning that any cells that contain mitochondria, such as PBMCs and platelets, may contribute to plasma acyl-CNT levels [3,8,23,27]. Since the plasma acyl-CNT profile is used as a marker of inborn metabolic disorders, most studies to date have focused on the pediatric population, with few data available on adults [3,8,23]. The purpose of this study was to determine the potential contribution of blood cells (i.e., PBMC and platelets) to fatty acid oxidation (FAO) and insulin sensitivity in healthy women. We combined indirect calorimetry, stable isotope tracer and hyperinsulinemic-euglycemic clamp approaches to measure palmitate oxidation and systemic insulin sensitivity *in vivo* with *in vitro* assessment of the synthesis rate of palmitoyl-carnitine (palmitoyl-CNT) by blood cells. We hypothesized that the production of palmitoyl-CNT by blood cells contributes to circulating acyl-CNT concentration. Further, we theorize that the production of palmitoyl-CNT by blood cells correlates with whole body fatty acid metabolism.

2. Materials and methods

Healthy women were eligible for the study. Exclusion criteria included any evidence of acute illnesses, diabetes mellitus [defined as fasting plasma glucose > 126 $mg \cdot dl^{-1}$ or taking any hypoglycemic agents], taking medications that affect lipid metabolism, pregnancy or lactation, a history of substance abuse, and the inability to provide informed consent. Upon enrollment the subjects underwent a history and physical. All study procedures were approved by the Institutional Review Board at the University of Texas Medical Branch (UTMB), Galveston, TX, and all participants provided signed informed consent and the studies were conducted at Clinical Research Center (CRC), UTMB.

2.1. In vivo infusion study

Volunteers participated in a 6 h infusion study, as described in Fig. 1. The infusion study consisted of two periods, each 3 h long. The first period of the study was designed to determine the basal fatty acid turnover. Indirect Calorimetry was performed at 120 min to determine whole-body fat oxidation, using Vmax Encore (CareFusion Corporation, San Diego, CA), as described previously [28]. Plasma palmitate oxidation was measured using an infusion of stable isotope-labeled palmitate tracer, as described below. After the collection of baseline blood and breath samples to determine background enrichments, a bolus of $NaH^{13}CO_3$ (55 mmol/kg, dissolved in 0.9% NaCl) was given to prime the bicarbonate pool. Thereafter, a constant infusion of $U^{13}C_{16}$-palmitate (Cambridge Isotopes, Andover, MA) in 5% human albumin (Albuminar-5, CSL Behring LLC., Kankakee, IL) was administered (infusion rate: 0.06 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) to assess plasma palmitate oxidation [28]. Blood samples were obtained every 30 min for the first 150 min, and then every 10 min until 180 min. Breath samples were obtained at 150, 160, 165 and 170 min after the start of stable isotope tracer infusion. At 180 min, a hyperinsulinemic-euglycemic clamp was conducted for the last 3 h of the infusion study, as previously described [14]. Briefly, insulin (Lilly, Indianapolis, IN), dissolved in sterile NaCl 0.9%, was administered at a rate of 1 $mU \cdot kg^{-1}$ of fat free mass (FFM)$\cdot min^{-1}$. The glucose infusion rate (GIR) during the steady-state period of the clamp was used as a marker for whole-body insulin sensitivity.

2.2. Clinical chemistry measurements

Fasting levels of plasma very low density lipoprotein cholesterol (VLDL-C), triglycerides (TG), total cholesterol (TC), high density lipoprotein cholesterol(HDL-C) and low density lipoprotein cholesterol (LDL-C) were measured using a Vitros 5600 analyzer (Ortho Clinical Diagnostic, Rochester, NY) in the Clinical Pathology Laboratory at UTMB. Plasma glucose concentrations were determined using an automated glucose analyzer (Stat 2300; Yellow Spring Instruments). Serum insulin concentrations were determined using an Immulite 2000 Insulin (Siemens Medical Solutions USA, Inc., Norwood MA).

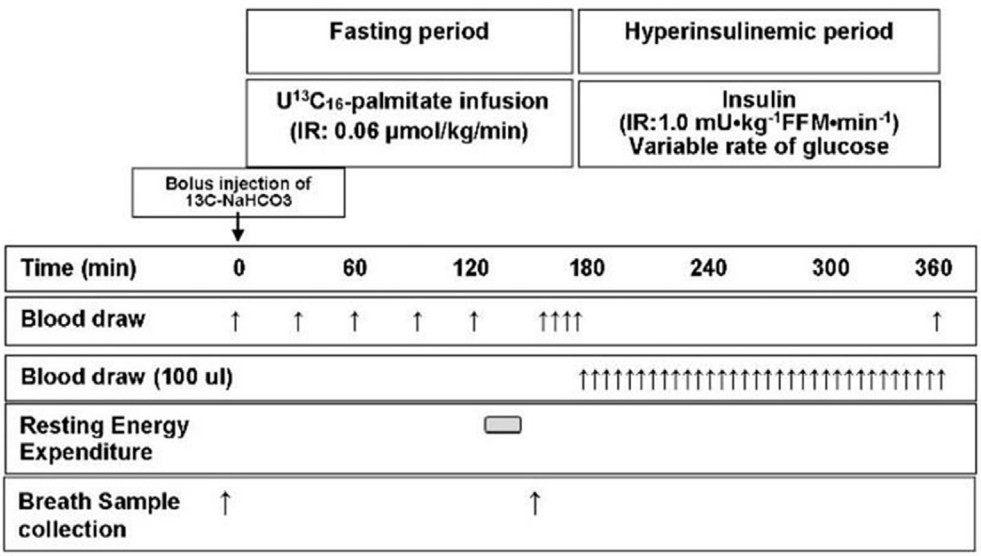

Fig. 1. A schematic presentation of the *in vivo* infusion study.

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