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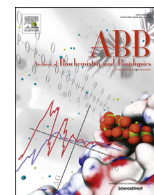

Assessment of plasma acylcarnitines before and after weight loss in obese subjects

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ABSTRACT

Acylcarnitines, fatty acid oxidation (FAO) intermediates, have been implicated in diet-induced insulin resistance and type 2 diabetes mellitus, as increased levels are found in obese insulin resistant humans. Moreover plasma acylcarnitines have been associated with clinical parameters related to glucose metabolism, such as fasting glucose levels and HbA1c. We hypothesized that plasma acylcarnitines would correlate with energy expenditure, insulin sensitivity and other clinical parameters before and during a weight loss intervention. We measured plasma acylcarnitines in 60 obese subjects before and after a 12 week weight loss intervention. These samples originated from three different interventions (diet alone ($n = 20$); diet and exercise ($n = 21$); diet and drug treatment ($n = 19$)). Acylcarnitine profiles were analysed in relation to clinical parameters of glucose metabolism, insulin sensitivity and energy expenditure. Conclusions were drawn from all 60 subjects together. Despite amelioration of HOMA-IR, plasma acylcarnitines levels increased during weight loss. HOMA-IR, energy expenditure and respiratory exchange ratio were not related to plasma acylcarnitines. However non-esterified fatty acids correlated strongly with several acylcarnitines at baseline and during the weight loss intervention ($p < 0.001$). Acylcarnitines did not correlate with clinical parameters of glucose metabolism during weight loss, questioning their role in insulin resistance and type 2 diabetes mellitus.

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1. Introduction

With the increased incidence of obesity and type 2 diabetes mellitus, many studies focus on the interaction between lipid and glucose metabolism, and the relationship to insulin resistance. Within the concept of lipotoxicity increased lipid levels are proposed to interfere with insulin signalling, eventually leading to hyperglycemia. However, the exact mechanisms and the individual lipids that induce insulin resistance have not been characterised definitively. From a cellular point of view, lipotoxicity is thought to occur on a cytosolic level via lipid overload (e.g. ceramides, gangliosides or diacylglycerol) [1–4]. Reduced mitochondrial content or capacity may result in elevated intracellular lipids [3,5]. Alternatively, increased fatty acid oxidation (FAO) rates that are not followed by increased tricarboxylic acid cycle (TCA) activity have been proposed to induce insulin resistance via accumulation of different mitochondrial metabolites such as acylcarnitines [1,6,7].

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This incomplete FAO is suggested to be manifested by altered plasma acylcarnitine profiles, mainly increased levels of long chain acylcarnitines [1,7,8].

Acylcarnitines are fatty acids esterified to carnitine by the outer membrane enzyme carnitine palmitoyl transferase 1 (CPT1) to enable transmitochondrial membrane transport of long-chain acyl-CoAs. Inside the mitochondrion, carnitine is exchanged for CoA via the inner mitochondrial membrane enzyme CPT2. The released acyl-CoA can be further oxidized via beta-oxidation, in which the acyl-CoA is shortened by one acetyl-CoA unit in every round. CPT2 can convert acyl-CoAs into acylcarnitines again, which can be shuttled back into the cytosol and exported to the plasma compartment, ultimately contributing to the typical circulating profile of acylcarnitines [9]. Consequently, acylcarnitines are excellent indicators of altered FAO as demonstrated by conditions in which lipid oxidation rates are elevated or when lipid oxidation is impaired (e.g. short-term fasting and FAO disorders). Metabolomic studies have shown that acylcarnitines may be implicated in insulin resistance [7,8,10], as elevated acylcarnitine levels are found in both rodent models of dietary insulin resistance [7] and in obese, insulin resistant humans [8]. Also several acylcarnitine species correlate moderately with clinical markers such as BMI, plasma glucose levels and insulin sensitivity in obese humans with type 2 diabetes mellitus [8,10].

Here we report on plasma acylcarnitine concentrations before and during weight loss in obese humans. Based on the association of long- and short-chain acylcarnitines with type 2 diabetes mellitus, we hypothesized that these acylcarnitine levels would decrease with concomitant improvements in insulin sensitivity. In contrast, we observed that decreased body weight and improvements in insulin sensitivity were accompanied by increased plasma acylcarnitine levels. Moreover, we found that plasma acylcarnitines correlate strongly with plasma non-esterified fatty acids (NEFA).

2. Research design and methods

2.1. Design of the study

Sixty obese subjects were recruited for an outpatient study on weight loss prediction that has been reported elsewhere [11]. In brief, subjects aged 20–55 years and BMI 30–40 kg/m^{2,} without type 2 diabetes mellitus, history of childhood obesity or previous bariatric surgery, were included. After giving written informed consent, subjects were randomized to one of three 12-week weight loss interventions [1]: diet (−600 kcal/day) alone [2], the same diet with moderate exercise (~10% of daily expenditure), and [3] the same diet with the centrally acting serotonin-norepinephrine reuptake inhibitor, sibutramine, which was approved for weight loss at the time this study was conducted. During the study, subjects visited the clinical unit at 0, 4 and 12 weeks at 07:00 h a.m. after an overnight fast for the measurement of body weight, anthropometry, indirect calorimetry and blood sampling (e.g. plasma acylcarnitine, glucose, NEFA and insulin levels). The study was approved by the protocol review panel of GlaxoSmithKline, the Cambridge Local Research Ethics Committee (08/H0308/10) and the Wellcome Trust Clinical Research Facility Scientific Advisory Board.

2.2. Body composition and energy expenditure

Body weight was measured in light clothing. Body composition was analysed by DXA (GE Lunar Prodigy, software version 12.2 (GE Healthcare, Madison, WI) and quantitative magnetic resonance (Echo MRI-AH; Echo Medical Systems, Houston TX). Indirect calorimetry was performed using a ventilated canopy calorimetry instrument (GEM Nutrition, Daresbury, UK) with the subject lying supine for 20 min before the measurement. Expired gas samples were analysed every 30 s for 20 min. Gas exchanges of O_2 and CO_2 were computed to calculate respiratory exchange ratio (RER) and energy expenditure (EE; kJ/min using the following formula: $15.9131 \times O_2$ consumption $+ 5.2069 \times CO_2$ production $\times 0.9950$) [12].

2.3. Laboratory analyses

Plasma acylcarnitines samples were analysed as described in our previous study [13], and were processed with Masslynx software version 4.1.

Acylcarnitines are depicted as C followed by chain length and degree of saturation. We focused on a subset of acylcarnitine species that we considered quantitatively and qualitatively relevant: Free carnitine (C0), acetylcarnitine (C2; derived from both lipid and carbohydrate oxidation (CHO)), hydroxybutyrylcarnitine (C4OH; the sum of the *L* and *D* stereoisomers derived from FAO and ketone bodies respectively [14]), decanoylcarnitine and tetradecenoylcarnitine (C10 and C14:1 respectively; intermediates that are only produced by FAO and thus indicative of FAO rate) and finally palmitoylcarnitine and oleoylcarnitine (C16 and C18:1 respectively; intermediates that originate from the diet).

Glucose was measured using a hexokinase assay. Insulin was measured using a fluorometric autoDELFIA immunoassay. Plasma NEFA were analysed with a NEFA-HR(2) in vitro enzymatic colorimetric method (Wako Diagnostics, Richmond VA).

2.4. Statistical analysis

Pearson correlation analyses with Bonferroni correction was used to determine if plasma acylcarnitine levels at baseline predicted clinical parameters. In case of significant results, multiple regression analysis was done to establish which acylcarnitine had the greatest effect on a single clinical parameter. Differences between days 0, 28 and 84 for whole group and within subgroup clinical data were analysed using repetitive ANOVA analysis with Bonferroni correction. Not all acylcarnitines were normally distributed according to the Shapiro-Wilk normality test. Therefore acylcarnitines were analysed using the non-parametric Friedman test followed by the Dunn's multiple comparisons test. Statistical analysis was done with SPSS statistical software program version 20.0. Data are depicted as mean and standard deviation.

To analyse if changes in plasma acylcarnitine levels over time coincided with changes in clinical parameters, we used a Bayesian hierarchical model with fixed and random effects. Individual variables were modelled by linear regression over time. For instance, changes in weight were modelled as: $W_i(t) = \alpha_i^\omega + \beta_i^\omega t + \varepsilon_{it}^\omega$ Here $W_i(t)$ is the weight of subject *i* at time *t*, α_i^ω is the mean weight of subject *i*, $\beta_i^\omega t$ is the rate of change of the subject's weight over time *t*, and ε_{it}^ω is a zero mean Gaussian error term: $\varepsilon_{it}^\omega \sim N(0, \sigma^2)$. Similarly, each of the acylcarnitines of interest was modelled for each individual patient by linear regression ($C_i(t) = \alpha_i^c + \beta_i^c t + \varepsilon_{it}^c$, where C is any acylcarnitine of interest). To analyse the correlation over time between individual clinical parameters (for example, weight) and acylcarnitines, we modeled β_i^ω as a linear regression over β_i^c. $\beta_i^\omega = \alpha + \beta\beta_i^c + \varepsilon_i$ where is again a zero mean Gaussian error term. We then applied Gibbs sampling to simulate from the posterior distribution using standard software (Just Another Gibbs Sampler (JAGS, version 3.4.0, http://mcmc-jags.sourceforge.net/)). We used a single Markov chain with a burn-in of 100000 sweeps and then output the values α and β for a further 1000000 sweeps. The proportion of sweeps when β is greater than zero is then the posterior probability that variables are positively correlated, and

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