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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.

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 × O_2 consumption + 5.2069 × CO_2 production × 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 $\varepsilon_{it}^{\omega}$ 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

the proportion of sweeps when $\beta < 0$ is the posterior probability that variables are negatively correlated. This procedure was repeated for all clinical parameters and acylcarnitines of interest followed by the Bonferroni-Holm method to allow for multiple testing [15].

3. Results

3.1. Whole group clinical data and plasma acylcarnitines at baseline (day 0)

Table 1 shows the clinical characteristics of the subjects at day 0 of the study. More women were included than men, and a substantial number of the subjects showed slightly impaired fasting plasma glucose levels (FPG) [16], and an elevated homeostatic model assessment of insulin resistance (HOMA-IR) index [17]. Table 1 additionally shows the levels of plasma acylcarnitines, of which plasma C16- and C18:1-carnitine levels of our obese subjects were below clinical laboratory reference values.

To detect relationships between plasma acylcarnitines and clinical parameters at baseline, we performed Spearman correlation analyses. Our analysis showed that plasma NEFA correlated strongly with C2, C4OH, C14:1, C16 and C18:1-carnitine (Fig. 1). No other correlations were found (Supplemental Table 1). Multiple regression identified C14:1- and C16-carnitine as contributors to the variation in plasma NEFA (Table 2). C4OH-carnitine is detectable after an overnight fast, but emerges predominantly during starvation [14]. Therefore we repeated the regression analysis without C4OH-carnitine after which only C16-carnitine remained significant.

3.2. Whole group clinical data and plasma acylcarnitines during the weight loss intervention

Fig. 2 shows the effects of the weight loss interventions for the whole group, irrespective of the treatment allocation. Overall, weight decreased between days 0 and 84 (~4.5 kg). As a consequence, BMI decreased between 0 and 28 days, with no additional reduction between days 28 and 84. HOMA-IR, FPG and plasma insulin levels improved between days 0 and 28, with no further improvement hereafter (Fig. 2). Plasma NEFA levels were unaffected by the intervention. Fat mass continued to decrease during the entire weight loss intervention period although this effect was most pronounced during the first 28 days. Lean body mass decreased (~0.6 kg) until 28 days only (Fig. 2). Substrate oxidation measurements showed that both EE and RER were lower after 28 days of treatment, with no further reduction afterwards (Fig. 2). FAO rates did not change upon weight loss. However protein oxidation rates and CHO rates both decreased after 28 days and remained at this level at 84 days (Supplemental figure 1). The decrease in protein oxidation was minor; therefore we assume that the decrease in energy expenditure mainly resulted from lower CHO. Overall clinical improvements due to the weight loss intervention were greatest in the first 28 days, with no further improvement at 84 days.

Whole group plasma acylcarnitine levels for days 0, 28 and 84 are shown in Fig. 3. The weight loss intervention increased carnitine levels after 84 days. C2-carnitine and C4OH-carnitine were higher after 28 days with no further increase at 84 days. C14:1-, C16- and C18:1-carnitine showed a similar pattern over time with an initial increase after 28 days, followed by a decrease at 84 days, although at this point C18:1 levels were still higher compared with baseline (Fig. 3).

We used a Bayesian hierarchical model to investigate whether changes in plasma acylcarnitine levels over time correlated with changes in weight, fat mass, lean mass, HOMA-IR, FPG, NEFA, RER and EE. We found that the increase over time in C4OH-, C16- and C18:1-carnitine correlated significantly with a reduction in both total and lean body mass over time (Supplemental Table 2 and supplemental figure 2). Additionally over time NEFA levels correlated positively with the level of C16-carnitine. We found no significant correlations for the other clinical parameters (data not shown).

3.3. Subgroup clinical data and plasma acylcarnitines during the weight loss intervention

The sibutramine group showed continued weight loss up to day 84 in contrast to the other two groups that only showed weight loss at day 28, but then remained stable up to day 84. The sibutramine

Table 1
Baseline characteristics, clinical parameters and plasma acylcarnitine levels. BMI, body mass index; EE, energy expenditure; RER, respiratory exchange ratio; FPG, fasting plasma glucose; HOMA-IR, homeostatic model assessment of insulin resistance; NEFA, non-esterified fatty acids. Acylcarnitine reference values were acquired and validated by Laboratory Genetic Metabolic Diseases, Academic Medical Center, Amsterdam. Data are represented as mean ± standard deviation.

	Total	Diet	Exercise	Sibutramine	
Subjects (*N*)	60	20	21	19	
Age (years)	40 (±8.6)	41 (±7)	41 (±8)	40 (±11)	
Sex (*m/f*)	23/37	10/10	7/14	6/13	
Bodyweight (kg)	100.9 (±12.6)	105.0 (±12.7)	98.7 (±11.7)	99.2 (±13.0)	
BMI (kg/m2)	34.8 (±2.7)	35.2 (±2.2)	34.5 (±3.0)	34.8 (±2.8)	
Fat mass (kg)	43.8 (±7.6)	43.0 (±9.1)	41.4 (±11.5)	43.2 (±8.4)	
Lean mass (kg)	52.2 (±10.9)	56.4 (±11.7)	52.2 (±10.9)	51.0 (±9.7)	
EE (kJ/min)	5.2 (±0.8)	5.4 (±0.7)	5.2 (±0.9)	5.1 (±0.8)	
RER	0.81 (±0.05)	0.81 (±0.04)	0.79 (±0.04)	0.84 (±0.06)	
FPG (mmol/L)	5.6 (±0.5)	5.8 (±0.5)	5.4 (±0.3)	5.7 (±0.6)	
Insulin (pmol/L)	14.7 (±10.1)	13.2 (±11.3)	13.2 (±6.2)	17.9 (±11.9)	
HOMA-IR	3.7 (±2.9)	3.5 (±3.4)	3.2 (±1.6)	4.6 (±3.3)	
NEFA (mmol/L)	0.5163 (±0.18)	0.49 (±0.16)	0.50 (±0.18)	0.56 (±0.19)	
Acylcarnitines					*Laboratory reference values*
C0 (μmol/L)	32.9 (±7.8)	33.5 (±7.6)	34.3 (±6.8)	31 (±9.1)	*22.30–54.80*
C2 (μmol/L)	4.86 (±1.68)	4.55 (±0.96)	5.06 (±1.13)	4.96 (±2.56)	*3.40–13.00*
C4OH (μmol/L)	0.026 (±0.021)	0.02 (±0.012)	0.027 (±0.012)	0.03 (±0.031)	*0.00–0.15*
C10 (μmol/L)	0.18 (±0.11)	0.16 (±0.10)	0.020 (±0.13)	0.18 (±0.08)	*0.04–0.30*
C14:1 (μmol/L)	0.079 (±0.035)	0.07 (±0.03)	0.085 (±0.036)	0.08 (±0.036)	*0.02–0.18*
C16 (μmol/L)	0.024 (±0.012)	0.021 (±0.008)	0.026 (±0.014)	0.024 (±0.011)	*0.06–0.24*
C18:1 (μmol/L)	0.028 (±0.009)	0.029 (±0.008)	0.028 (±0.009)	0.028 (±0.012)	*0.06–0.28*

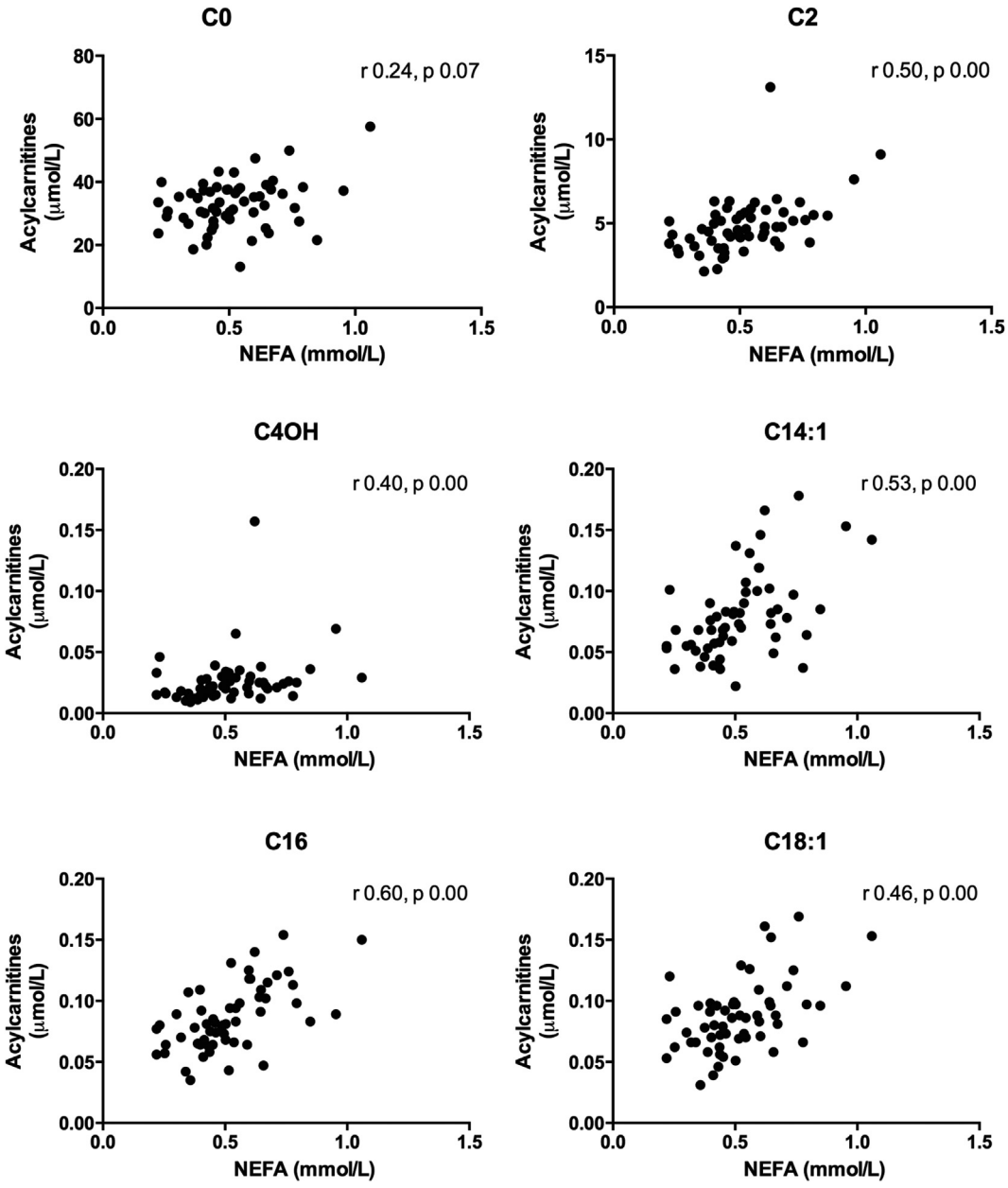

Fig. 1. Spearmans correlations between specific plasma acylcarnitine levels and plasma non esterified fatty acids (NEFA) for the whole group on day 0. The significance level shown is after Bonferroni correction.

Table 2
Multiple regression analysis of plasma NEFA levels and selected plasma acylcarnitines. Multiple regression analysis illustrates that variation in plasma NEFA is statistically driven by C14:1 and C16-carnitine. When C4OH-carnitine was omitted (as it predominantly emerges during starvation), only C16-carnitine remained significant.

Analysis including C4OH-carnitine				Analysis excluding C4OH-carnitine			
Model R^2 0,448	Unstandardized Coefficient B	95% confidence interval for B	P	Model R^2 0,413	Unstandardized Coefficient B	95% confidence interval for B	P
C0	0,00	−0,01 – 0,00	0,19	C0	−0,002	−0,008 – 0,004	0,54
C2	0,04	−0,01 – 0,10	0,12	C2	0,007	−0,030 – 0,044	0,72
C4OH	−3,40	−7,28 – 0,47	0,08				
C10	−0,39	−0,94 – 0,16	0,16	C10	−0,248	−0,788 – 0,293	0,36
C14:1	2,55	0,19 – 4,90	0,03	C14:1	1840	−0,418 – 4097	0,11
C16	2,51	0,16 – 4,85	0,04	C16	3071	0,768 – 5373	0,01
C18:1	−0,13	−2,42 – 2,16	0,91	C18:1	−0,159	−2498 – 2179	0,89

group had a reduction in HOMA-IR at day 28, after which the levels plateaued in contrast to the exercise and placebo groups where HOMA-IR did not change during the study. Plasma NEFA did not change in any of the three groups. In the exercise and placebo

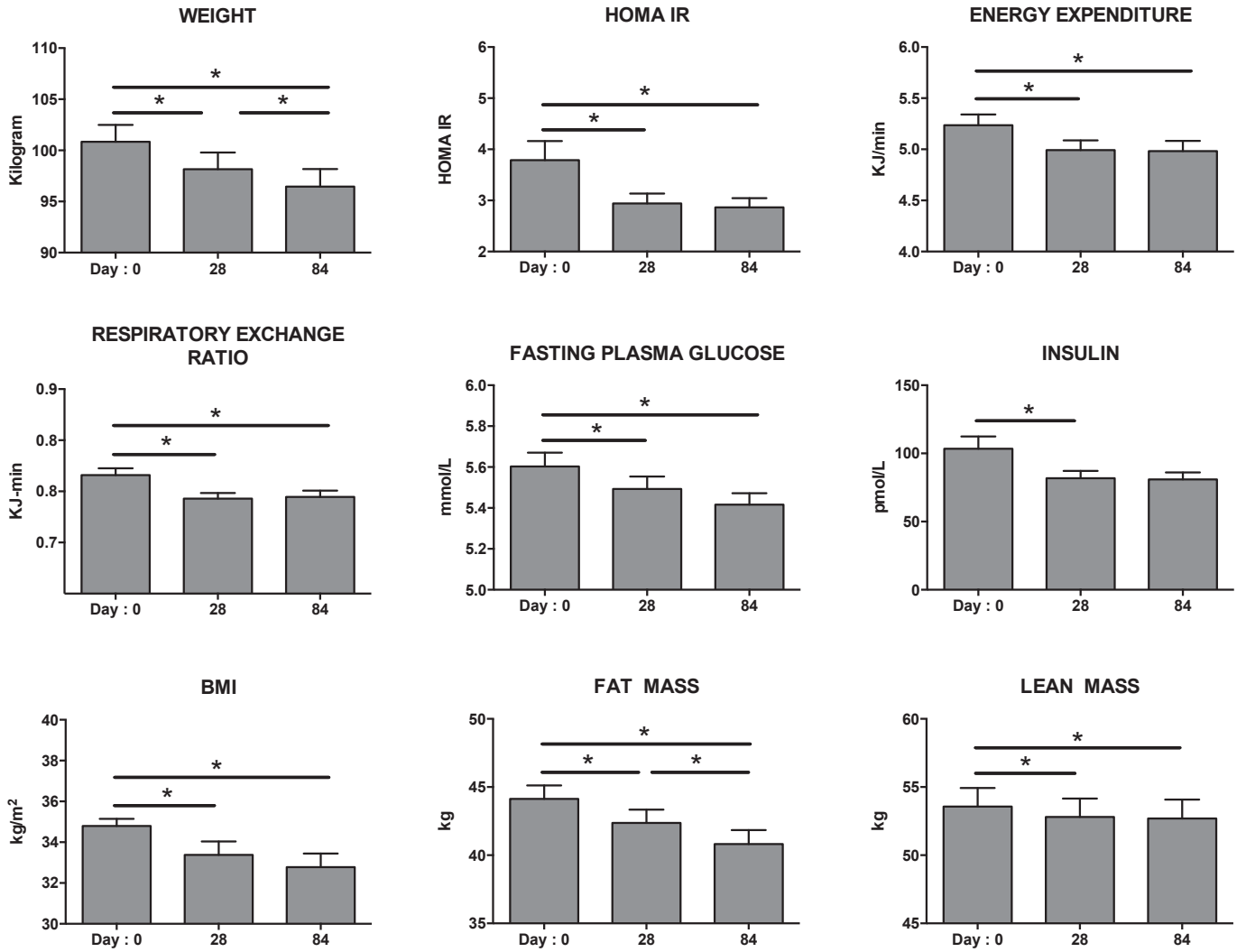

Fig. 2. Clinical parameters for the whole group on day 0, 28 and 84. HOMA-IR, homeostatic model assessment of insulin resistance; BMI, body mass index. Bars and whiskers represent mean and SEM. * = $P < 0.05$ after Bonferroni correction.

groups, RER and FAO were not affected by the weight loss intervention, but the sibutramine group had a lower RER and higher FAO after 28 days that remained stable thereafter.

Plasma acylcarnitine levels showed differential changes during the weight loss intervention. Overall the greatest effect was seen in the sibutramine group and a modest effect was seen for the exercise group. In the placebo group, carnitine did not change, whereas the exercise group showed a modest increase at day 84 compared to day 28. In the sibutramine group, carnitine increased steadily during the intervention becoming significant at day 84. In contrast to the placebo and exercise group, C2- and C4OH-carnitine levels in the sibutramine group showed an increase at 28 days and remained high at 84 days. In the placebo and sibutramine group, C10- and C14:1-carnitine initially increased at day 28 but not in the exercise group. C16- and C18:1-carnitine were higher at day 28 compared to day 0 in all groups, with the exception of C16-carnitine in the exercise group. At day 84, C10- and C18:1-carnitine were decreased again in the placebo group compared to day 28.

We repeated the Bayesian hierarchical modelling to determine if changes in plasma acylcarnitine levels over time correlated with changes in clinical parameters and found that the change in plasma C4OH- and C18:1-carnitine remained significantly correlated with weight change in the sibutramine group, but not in the other groups (data not shown).

4. Discussion

In this study we have shown that plasma acylcarnitines increase in obese humans upon weight loss while insulin sensitivity improved. Additionally, acylcarnitines correlated positively with NEFA at baseline and over time. Several studies have reported that increased plasma acylcarnitine levels associate with obesity and insulin resistance [7,8,10,18]. In our study, plasma NEFA correlated positively with plasma C16-carnitine and other species, namely C2-, C14:1- and C18:1-carnitine. In the context of this strong correlation of acylcarnitines with plasma NEFA, which are known to induce insulin resistance, the overall absence of correlations between acylcarnitines and markers of insulin sensitivity is remarkable [19,20]. Since plasma NEFA are indicative of lipolysis, acylcarnitines in plasma may reflect white adipose tissue (WAT) breakdown [21]. As a result, NEFA released from WAT could drive FAO rates generating acylcarnitines.

The origin of the different acylcarnitines that correlate with plasma NEFA is intriguing. C2-carnitine is derived from both lipid

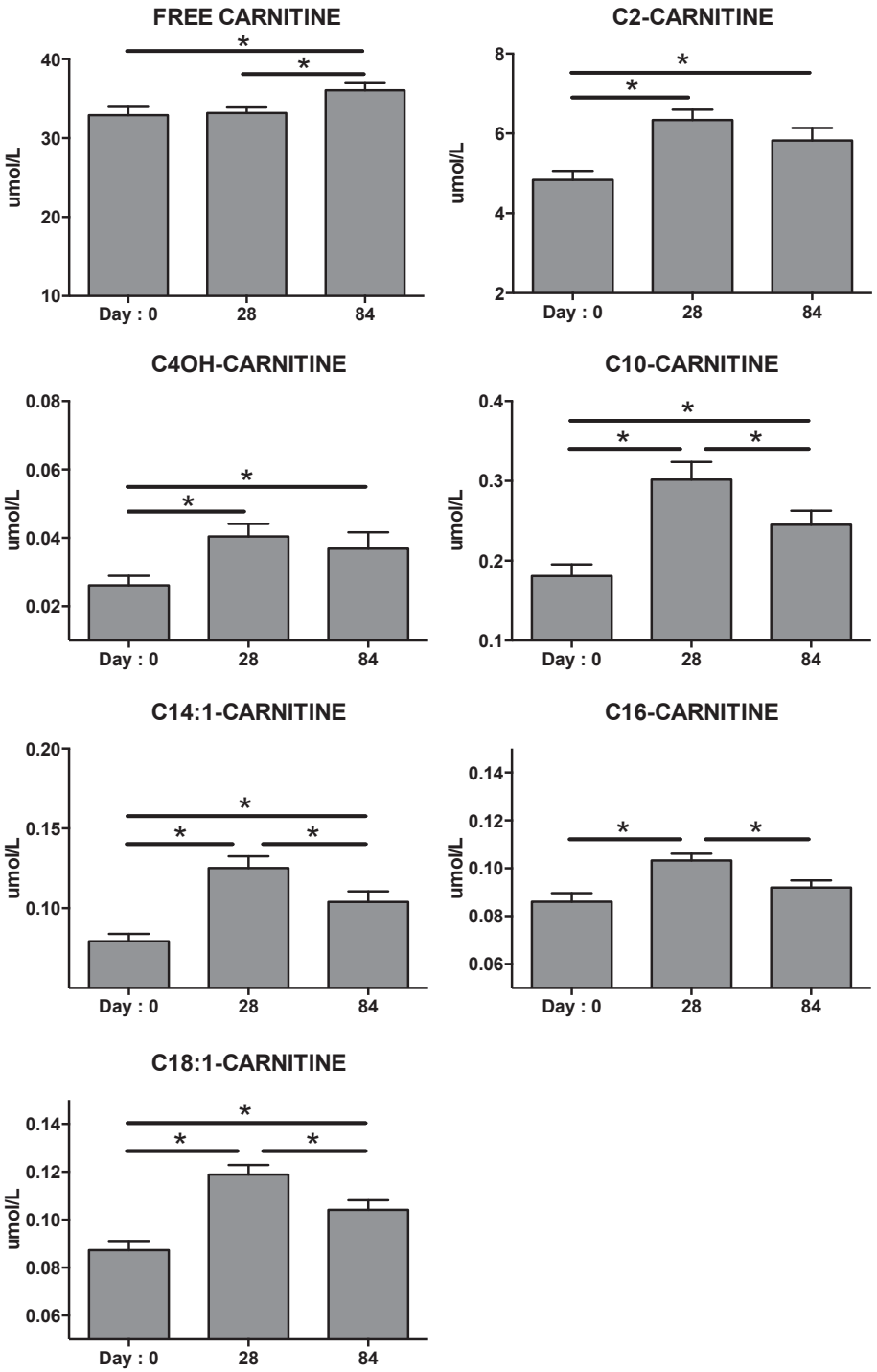

Fig. 3. Plasma acylcarnitine levels for the whole group on day 0, 28 and 84. Bars and whiskers represent mean and SEM. * = $P < 0.05$ after Dunn's multiple comparison correction.

and carbohydrate oxidation and our observed correlation between C2-carnitine and plasma NEFA suggests lipid as main source in our subjects [22,23]. C16- and C18:1-carnitine are derived from palmitate and oleate, which are the main human dietary fatty acids that are stored in WAT; hence their correlation with NEFA may support lipolysis as a responsible mechanism. High plasma insulin levels in our obese insulin resistant subjects may explain why C16- and C18:1-carnitine were below reference values. Finally, C14:1-carnitine is an interesting acylcarnitine since it is only produced after two cycles of beta-oxidation of C18:1-CoA [24]. Therefore C14:1-carnitine is a good marker of FAO. It remains unclear what the tissue origin of this acylcarnitine is, but as FAO also takes place in WAT, C14:1-carnitine could still be derived from WAT [13,25]. Alternatively, plasma NEFA may reflect the load of fatty acids in general, thereby correlating with the most metabolically relevant acylcarnitines.

Effects of weight loss on acylcarnitine profiles have been described in only two studies, both of which studied lean subjects [26,27]. Redman et al. compared caloric restriction with and without exercise and showed no changes in acylcarnitines in the

exercise group, but increased acylcarnitines in the group without exercise, accompanied by a greater improvement in insulin sensitivity analogous to our study [27]. Here, caloric restriction combined with exercise possibly improves the coupling of FAO and TCA flux, preventing acylcarnitines from accumulating. In contrast to our results, Falk-Petersen et al. demonstrated in lean sedentary insulin resistant offspring of parents with type 2 diabetes [26] that plasma acylcarnitine levels do not change after a 9 week hypocaloric diet, despite weight reduction and improved insulin sensitivity. For both studies, it should be emphasized that plasma acylcarnitines do not reliably reflect individual tissue metabolite levels as we have shown recently [13,28].

Importantly, we have previously shown that short-term fasting elevated plasma acylcarnitine levels, which was accompanied by profound fatty acid oxidation and insulin resistance [30]. However, in the current study, HOMA-IR did not correlate with acylcarnitine levels and weight loss resulted in both increased acylcarnitine levels and improved HOMA-IR. Together with the data on substrate oxidation and lipolysis this suggests that changes in acylcarnitines primarily reflect changes in substrate oxidation and availability and to a lesser degree insulin sensitivity.

Carnitine availability depends on dietary intake and endogenous synthesis. Both carnitine uptake via OCTN2 and carnitine synthesis are regulated by PPAR-alpha and are stimulated under hypocaloric conditions [29]. The absence of increased carnitine after 28 days of intervention may reflect increased esterification to fatty acids and efflux of toxic lipid metabolites. This effect was probably lessened after 84 days when weight loss decreased and carnitine levels increased. Indeed, the increase in lipid derived acylcarnitines, including C2-carnitine, was most pronounced after the first 4 weeks. Here the hypocaloric condition and subsequent weight loss could cause improved CrAT activity and increased C2-carnitine production, and long chain acylcarnitines that are released from the mitochondria and into the plasma compartment [30]. When this diet induced CrAT activity declines again over time, more carnitine is released, explaining the delay in this increase of carnitine levels. These changes over time were not seen for the amino acid derived acylcarnitines, reflecting that under hypocaloric conditions lean body mass is more protected than fat mass [31].

The effects on acylcarnitine levels and the clinical parameters such as weight loss were greatest at 28 days and weakened at 84 days. It is often seen in weight loss studies that the initial effects outweigh the later effects, which may not only be due to changes in treatment compliance over time [32–34]. Under hypocaloric conditions, whole body energy expenditure decreases hampering further weight loss once a lower body weight is attained [35–37]. The increase in acylcarnitines over time also showed a relation with weight loss.

We have analysed and reported on whole group changes over time because of the limited number of subjects and the primary interest in changes in acylcarnitines. However, the weight loss intervention consisted of three arms (diet, sibutramine and exercise) [11]. Over time the sibutramine group lost most weight and had the highest acylcarnitine levels. This may confirm the idea that lipolysis, which is apparently greater when weight loss is greater, is reflected as higher levels of plasma acylcarnitines. Although the different interventions were extremely instructive for our understanding of acylcarnitine metabolism, the subgroups do have limitations, as the groups were relatively small, the exercise intervention was only modest and the marketing application for sibutramine has been withdrawn due to side effects of the drug [38]. Additionally, another limitation of our study is that we used the HOMA-IR which is a rough estimate of insulin resistance in contrast to the more elaborate clamp [39]. Finally the relative limited sample size, and the fact that we did not account for dietary habits may have influenced our results.

In conclusion, we have found an increase in several acylcarnitine species in association with weight loss in obese human subjects, despite improvements in insulin sensitivity. It is likely that the level of plasma acylcarnitines is driven by the rate of lipolysis as well as improved efflux from cells, potentially resulting from improved CrAT activity and not by deranged mitochondrial FAO. However, when plasma acylcarnitines are used as markers for insulin resistance, they need to be interpreted in relation to clinical aspects such as diet and changes in weight. The relevance of changes in these lipid intermediates in relation to insulin sensitivity remains challenging.

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Author contributions M.G.S. performed plasma acylcarnitine analyses, analysed data and wrote the paper. A.N. designed and performed the research, analysed data and wrote the paper. S.M.H. analysed data and wrote the paper. G.K.A. performed statistical analyses and wrote the paper. P.R.M. designed the research and analysed data. S.R.M. designed the research, analysed data and wrote the paper. C.Y.T. performed the research, analysed data and wrote the paper. S.V. wrote the paper. A.V.-P. designed the research and wrote the paper. D.J.N. designed the research and wrote the paper. M.R.S. designed the research, analysed data and wrote the paper.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.abb.2016.07.013.

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The authors have nothing to disclose.

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