Acute effects of pharmacological modifications of fatty acid metabolism on human satiety

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The role of NEFA in eating behaviour is still poorly known. Our objective was to examine whether etomoxir (ETO), an inhibitor of NEFA oxidation, or (−)-hydroxycitrate (HCA), an inhibitor of lipogenesis which may indirectly stimulate NEFA oxidation, alters satiety. Post-lunch satiety was measured in eight normal-weight male subjects who were deprived of time cues and received on three occasions either ETO (320 mg), HCA (2 g) or placebo (PLA) in random order. Between lunch and dinner, blood was withdrawn continuously and collected every 10 min for measures of plasma concentrations of glucose, insulin, lactate, TAG, NEFA, β-hydroxybutyrate (BHB), leptin and ghrelin. Results showed that HCA began to decrease hunger and desire to eat compared to PLA and ETO 210 min after lunch and increased satiety duration compared to PLA by 70 (SE 23) min ($P < 0.05$), but did not modify energy intake at dinner. ETO did not affect any variable of satiety. HCA increased NEFA concentrations during the pre-dinner period, whereas ETO increased and decreased plasma concentrations of NEFA and BHB, respectively. Mean differences in plasma NEFA concentrations between HCA and PLA were predictive of the differences in satiety duration between treatments (r^2 0·71, $P < 0.01$). Among treatments, plasma leptin concentration at dinner onset was the only blood variable correlated with energy intake at this meal (r − 0·75, $P < 0.0005$). In healthy, normal-weight men, acute HCA increased the intensity and duration of satiety possibly via increased NEFA disposal for oxidation.

(−)-Hydroxycitrate: Eating: Fatty acid oxidation: NEFA: Leptin

Obesity is a major health problem in industrialized countries, and its prevalence continues to increase[1,2]. Diet characteristics that favour passive overeating may contribute to the development of obesity. Consumption of dietary fats above the organism's ability to oxidize them is a primary suspect in this context[3]. Treatments improving this imbalance between fat intake and oxidation may therefore hold some therapeutic potential.

In man, satiety is usually assessed by recording: (1) its intensity through subjective feelings; (2) its duration through the delay until the next meal request occurs, i.e. the inter-meal interval (IMI); and (3) the amount eaten in a meal presented at a fixed or freely chosen time. Simultaneous assessment of all three parameters can be considered to be the most accurate evaluation of satiety in man.

A large body of evidence suggests that metabolic factors contribute to satiety. Thus, a drop in circulating glucose has been repeatedly reported to be associated with meal initiation[4]. Furthermore, fatty acid oxidation inhibitors such as methyl-palmoxirate[5] and mercaptoacetate[6] have been

shown to stimulate eating, and in rats mercaptoacetate stimulated eating by shortening IMI duration without altering meal size[6], suggesting that oxidation of NEFA contributes to satiety. Consistent with this concept, an impaired NEFA oxidation was shown to be a predictive factor of diet-induced obesity in animals[7] and man[8]. Furthermore, the eating-stimulatory effect of mercaptoacetate was found to be mediated by abdominal vagal afferents[9], consistent with the idea that a metabolic hunger signal may originate from the liver.

Studies addressing the role of fat metabolism in man mainly used etomoxir (ETO) and (−)-hydroxycitrate (HCA). ETO reduces NEFA oxidation by inhibiting carnitine-palmytoyl-transferase-1, the rate-limiting enzyme for the transfer of long-chain fatty acids across the mitochondrial membrane. Conversely, HCA, derived from the Indian fruit *Garcinia cambogia*, reduces *de novo* lipogenesis by inhibiting the cytosolic enzyme ATP-citrate-lyase[10]. By limiting the availability of malonyl-CoA, ATP-citrate-lyase inhibition might indirectly increase NEFA oxidation[11]. Very few studies, however, specifically addressed the effects of these agents on satiety.

When given to normal-weight subjects before a high-fat breakfast, ETO (150 mg) enhanced hunger feelings and increased energy intake[12]. For its part, under various experimental conditions, and in different populations, HCA did not affect satiety in all but one study[13].

Therefore, we here specifically examined whether ETO or HCA affect satiety in man and alter metabolism in a way that is consistent with their presumed metabolic mechanisms of action. To do so, we modelled a spontaneous IMI according to a previously described method[14] and continuously recorded eating behaviour and various blood parameters related to fat metabolism or food intake, including the hormones ghrelin and leptin.

Experimental methods

Subjects

Male subjects (20–30 years old) were recruited through advertisements posted at ETH Zurich. Smokers, trained athletes and subjects who had food allergies or aversions to the foods provided during the experiment were excluded. Other exclusion criteria were any personal or family history of diabetes or other metabolic disease, use of medication or a change in body weight >1 kg during the previous year. Moreover, we included only subjects who were not restrained or disinhibited and who did not experience strong hunger feelings between meals. To verify this criterion, subjects completed the FEV questionnaire, the German version of the Three Factor Eating Questionnaire[15] with cut-off scores of 9 and 7 for restrained eating and disinhibition, respectively. Another criterion was to never or very rarely (less than two times per week) eat something between lunch and dinner, and to eat a diet with ≥35 % of energy derived from fat. These two conditions were considered necessary because modulators of fat metabolism do not appear to affect eating at low levels of dietary fat[12] and because fatty acids appear to contribute to satiety only in case of long IMI[16]. To verify these two criteria, a 5 d food diary and a FFQ were completed by the subjects and were analysed with the EBISpro software (Dr J. Erhardt, University of Hohenheim, 2002). Twelve subjects were initially selected, but one decided on short notice not to participate and another one was excluded because he experienced a severe vagal malaise when the intravenous catheter was introduced on the first day. Two other subjects completed only two of the three conditions and were therefore excluded from final analyses. Characteristics of the remaining eight subjects who completed all three treatments and whose data were analysed are shown in Table 1. Fat mass was estimated by bioelectrical impedance analysis using a body fat scale (BF 555; Tanita Corp., Tokyo, Japan) after an overnight fast and with the bladder voided. Subjects gave their written informed consent and were financially compensated for completing the study. The procedure was approved by the Ethics Committee of the Swiss Federal Institute of Technology Zurich (ETH Zurich).

Study design

A single-blind within-subject design was used, with each subject participating on three test days for placebo (PLA), ETO

Table 1. Subject characteristics (*n* 8)
(mean values with their standard errors)

	Mean	SE
Age (years)	22·9	2·4
Height (m)	1·84	0·05
Weight (kg)	78·8	7·1
BMI (kg/m^2)	23·2	3·3
Fat mass (kg)	10·4	3·3
Fat mass (%)	13·0	3·3
F1 (cognitive restraint)	4·7	3·0
F2 (desinhibition)	3·8	3·3
F3 (hunger)	4·9	2·5
Mean daily energy intake (MJ)	13·7	2·0
Mean fat intake (%)	46	2

F1–F3, factors 1–3 of the FEV questionnaire, German version of the Three Factor Eating Questionnaire[15].

and HCA separated by 1 week. In each subject we determined: (1) the temporal pattern of hunger ratings from lunch until the spontaneous dinner request, (2) the time between the end of lunch and the dinner request, (3) the amount of food eaten during the *ad libitum* dinner, and (4) the temporal pattern of the plasma glucose, NEFA, β-hydroxybutyrate (BHB), lactate, TAG, insulin, leptin and ghrelin concentrations from 30 min before lunch to the dinner request.

Pharmacological agents

Timing of administration and doses of agents were chosen according to their kinetic profiles, their optimal bioavailability and the doses used in previous studies. Thus, the ETO dose was in the range of doses showing an effect on fatty acid oxidation and hunger in previous studies. For example, hunger and RQ were increased 200 min after only a single 150 mg dose in Kahler *et al.*[12]. An ETO dose of 600 mg given over 36 h has also been shown to increase RQ in healthy subjects[17], whereas 100 mg given daily decreased fatty acid oxidation in subjects with type 2 diabetes[18]. For HCA, we chose the highest dose that was provided in 12 h in previous studies, i.e. 2 g[19–21]. Although the bioavailability of oral HCA is low, the plasma concentration achieved in human subjects after oral administration of 2 g HCA has been found to be higher than after the administration of an HCA dose in rats that had clear effects on fat metabolism (0·022 mmol/kg *v.* 0·017 mmol/kg)[22,23]. Subjects received ETO (Dr. Horst P.O. Wolf GmbH, Allensbach, Germany), HCA (Citrin^R 500; Power Health Products Ltd, York, UK) or PLA at three time-points, i.e. just before breakfast, 10 min before and 240 min after lunch. The ETO treatment consisted of PLA before breakfast and 160 mg ETO before and after lunch (320 mg ETO in total). The HCA treatment consisted of 500 mg HCA before breakfast and 750 mg HCA before and after lunch (2 g in total). In the PLA condition, placebo was given on all three occasions. Substances were taken with a glass of water, facing the experimenter.

Foods

For breakfast, a choice of food items based on those usually consumed by the subjects for breakfast was provided.

The amount was free in the first session. This breakfast was identical in the two further sessions.

Based on our previous studies showing that manipulations mainly affect consumption of the first item of a meal, especially if this is the main dish[24], lunch and dinner comprised only two items each. Lunch consisted of lasagna bolognaise (5·1 kJ/g, 17 % protein, 37 % fat, 46 % carbohydrate, 1 kg served at the first session) and chocolate cake 'black forest' (5·8 kJ/g, 14 % protein, 39 % fat, 47 % carbohydrate, 350 g served at the first session). As for breakfast, lunch was consumed *ad libitum* in the first session, and the energy intake was then determined and fixed for the subsequent sessions. Dinner consisted of rice, chicken and curry (4·8 kJ/g, 20 % protein, 35 % fat, 45 % carbohydrate, 900 g served) and chocolate-flavoured dessert cream (5·4 kJ/g, 12 % protein, 18 % fat, 70 % carbohydrate, 500 g served). All dinner items were served in large dishes. All food items served at lunch and dinner were rated highly palatable by each subject in a prior test using hedonic scales. Water was provided *ad libitum*, but water consumption was not measured. No other beverage was served or allowed.

Subjective ratings of satiety

Motivation to eat was assessed using 100 mm visual analogue scales for hunger, desire to eat and gastric fullness, each anchored with 'not at all' and 'extremely' at the left and right ends, respectively. The scales were rated every 30 min from before lunch until after dinner, but some ratings were randomly interspersed to prevent them from providing any time cue to the subjects.

Blood sampling and plasma assays

Blood samples were obtained as previously described, with a specially designed double-lumen catheter (MTB Medizintechnik, Amstetten, Germany)[25]. Briefly, this system fits into an 18-gauge indwelling cannula inserted into an antecubital vein, and blood is continuously withdrawn throughout the session, at a flow rate of 300 µl/min via a peristaltic pump. Blood is heparinized (7 IU/ml) without any back-infusion of heparin into the vein. The dilution is accounted for when calculating final concentration of the variables. Blood samples were collected automatically in a machine programmed to change tubes every 10 min. It takes about 10 min for the blood to flow from the vein to the sample tube. This lag time is accounted for in the data analysis. Blood samples

were immediately centrifuged (3400 *g*, 15 min, 8°C), and the plasma was aliquoted into Eppendorf tubes and stored at −80°C for further analyses.

Glucose, lactate, NEFA, TAG and BHB were determined by standard colorimetric and enzymatic methods adapted for the Cobas Mira autoanalyser (Hoffman la Roche, Basel, Switzerland). Insulin was determined by RIA using the SB-INSI-5 kit (CEA, Gif-sur-Yvette, France; 7 % accuracy) with a sensitivity of 2 µU/ml. Plasma total ghrelin and leptin were determined by RIA using the commercial Sensitive Human Leptin kit and the Human Ghrelin kit (Linco Research, St Charles, MO, USA). Mean intra- and inter-assay CV were 3·5 and 5·3 % for leptin and 5·9 and 10·3 % for ghrelin.

Procedure

Figure 1 shows the daily schedule for each subject. Three subjects were studied on each day. They were asked to arrive at the laboratory at 08.00 hours after an overnight fast. They were then served a breakfast of items corresponding to their usual breakfast as indicated by the 5 d food diary. At 11.00 hours, subjects were confined to individual quiet rooms without windows. They were seated in comfortable armchairs and deprived of time cues by exposing them to artificial light, and by removing all potential sources of visual and auditory time references such as watches, computers or radios. Between 12.30 and 13.00 hours, but for each subject at the same time for all conditions, a catheter was inserted into an antecubital vein for blood sampling. Lunch was served 30 min after the onset of blood sampling. Meal duration was not fixed but measured. Subjects were instructed to spontaneously ask for dinner as soon as they felt hungry and certainly not to delay their request in order to not bother experimenters. When a subject requested dinner, he was asked to guess the current time. The catheter was then withdrawn and the subject was served dinner. A microwave oven was used to quickly heat the main dish of the dinner and to serve it only minutes after the request. Before and after consumption, food plates were accurately and covertly weighed to determine actual intakes. Independent of the time of dinner requests, subjects were not allowed to leave the laboratory before 22.00 hours, to avoid any premature dinner request.

Statistical analysis

Unless otherwise indicated, all results are presented as means and standard errors of the eight subjects who completed all
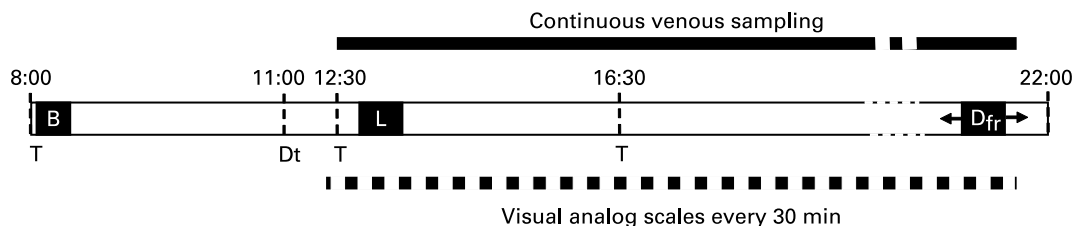
Fig. 1. Schedule of experimental proceedings for a subject. Breakfast (B) was based on items usually consumed by the subjects. Amount eaten was free in the first session, but was identical in the two subsequent sessions. Continuous blood collection began between 12.30 and 13.00 hours across subjects, but exactly at the same time across treatments. Lunch (L) was served 30 min after the catheter was inserted. Treatment (T; placebo, etomoxir or hydroxycitrate) was taken at 8.00, 11.00 and 16.30 hours. Visual analog scales for hunger, desire to eat and gastric fullness were rated every 30 min with some ratings randomly interspersed to obscure any time cues. The dinner had to be freely requested by subjects (D_fr). Dt, deprivation of time cues.

three treatments. Statistical analyses were performed using Systat software version 10.2 (SPSS Inc., Chicago, IL, USA).

The biological parameters were subjected to a stepwise analysis for serial measurements according to the recommendation of Matthews *et al.*[26]. Thus, for each blood parameter, four variables of interest were determined: (1) a post-lunch profile from the start of lunch to the time the first dinner was requested across all subjects and all conditions, i.e. the minimum time prior to any dinner request, which occurred at 260 min; (2) a pre-dinner profile starting 60 min prior to dinner request; (3) an area under the curve (AUC) calculated by the trapezoidal method for the time from lunch onset to the first dinner request of each subject separately across all conditions; (4) a mean plasma concentration over the whole IMI. The first variable allowed the kinetics of the plasma parameters to be studied during a fixed time period including all subjects and conditions. The second variable focused on the important period preceding a meal, where major metabolic changes may occur. In subjects who requested their dinner less than 320 min after lunch in a session (four from twenty-four), the post-lunch and the pre-dinner profiles shortly overlapped. The third variable estimated the maximum difference that could be calculated taking into account all three conditions for each subject. The fourth variable provided one value of the concentrations of each parameter during the whole IMI.

Satiety scores were analysed using the same method as for the first and second (post-lunch profile and AUC) variables of the plasma parameters.

Post-lunch and pre-dinner profiles were analysed by two-way ANOVA for repeated measures with conditions and time as within-subject factors. The level of significance was fixed at $P<0.05$. For significant interactions between conditions and time, subsequent comparisons of means were analysed by Scheffe's contrasts. AUC and mean concentrations were compared between conditions by Student's *t* test adjusted for multiple comparisons by the Holm's procedure[27].

Relationships between plasma parameters and behavioural data were calculated using Pearson correlations. Predictive equations were fitted by multivariate linear regressions using a backward stepping procedure with a level of 0·1 required for a factor to be included. Predictive equations were modelled for three behavioural variables: (1) the difference of IMI duration between treatments (ETO, HCA and PLA), (2) total energy intake at dinner and (3) energy derived from the first item of the dinner meal. For the first variable, tested predictive factors were mean plasma concentrations of each plasma parameter and treatments, whereas for the second and third variables the predictive factors tested were plasma concentrations at dinner request and treatments.

Results

Consistent with the assumption that the time-blinding was effective, there was a significant difference between the real and the guessed clock time for all three treatments (22·5 (SE 10·6), 55·7 (SE 33·5) and 80·7 (SE 22·9) min for PLA, ETO and HCA, respectively, all $P<0.05$). Subjects reported no side-effects during any session.

Satiety

Mean energy intake at the free-choice breakfast as determined in the first session was 3877 (SE 393) kJ and mean proportions of macronutrients consumed were 12 (SE 1) % protein, 25 (SE 2) % fat and 63 (SE 1) % carbohydrate. Mean energy intake at the two-item lunch was 4505 (SE 178) kJ and consisted of 14, 38 and 47 % protein, fat and carbohydrate, respectively. At dinner, energy intake was not significantly different between treatments (Fig. 2(A)). The time between the end of lunch and the dinner request was longer after HCA than after PLA (+73 (SE 23) min, $P<0.05$) but not between PLA and ETO (Fig. 2(B)). Mean hedonic scores for lunch items were high and not different between treatments (79 (SE 3), 79 (SE 3) and 81 (SE 4) mm for PLA, ETO and HCA, respectively).

Prior to lunch, hunger and gastric fullness scores were not different among treatments (Fig. 3). Post-lunch hunger profiles until 240 min showed an interaction between time and treatment ($P=0.01$), with hunger scores at 210 and 240 min being lower with HCA than with PLA and ETO treatments (all $P<0.05$). A treatment effect was found for post-lunch
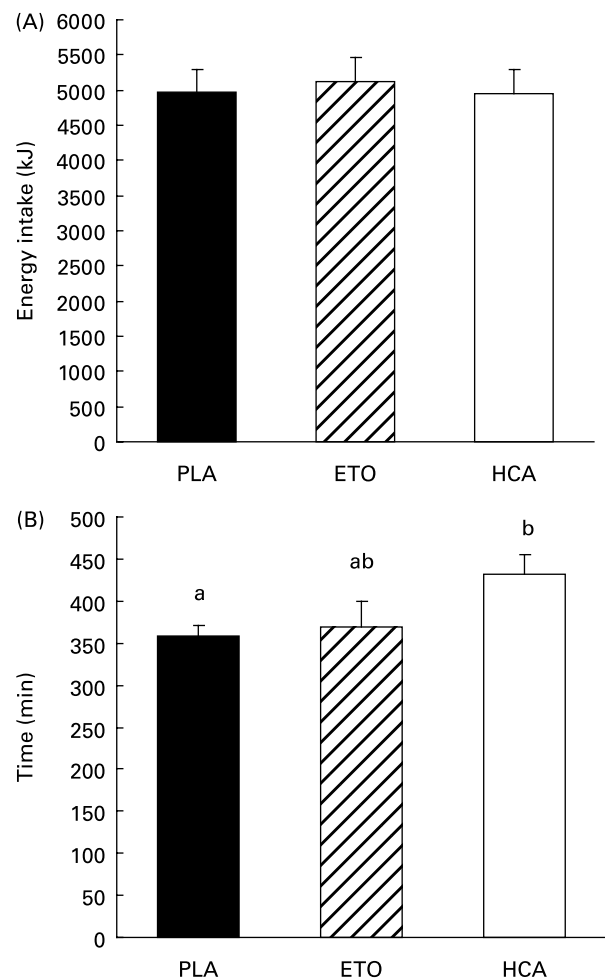
Fig. 2. Energy intake at dinner (A) and mean delay between lunch and spontaneous dinner request (B) after placebo (PLA), etomoxir (ETO) or hydroxycitrate (HCA). Values are means with their standard errors depicted by vertical bars. [a,b] Mean values with unlike superscript letters were significantly different ($P<0.05$).
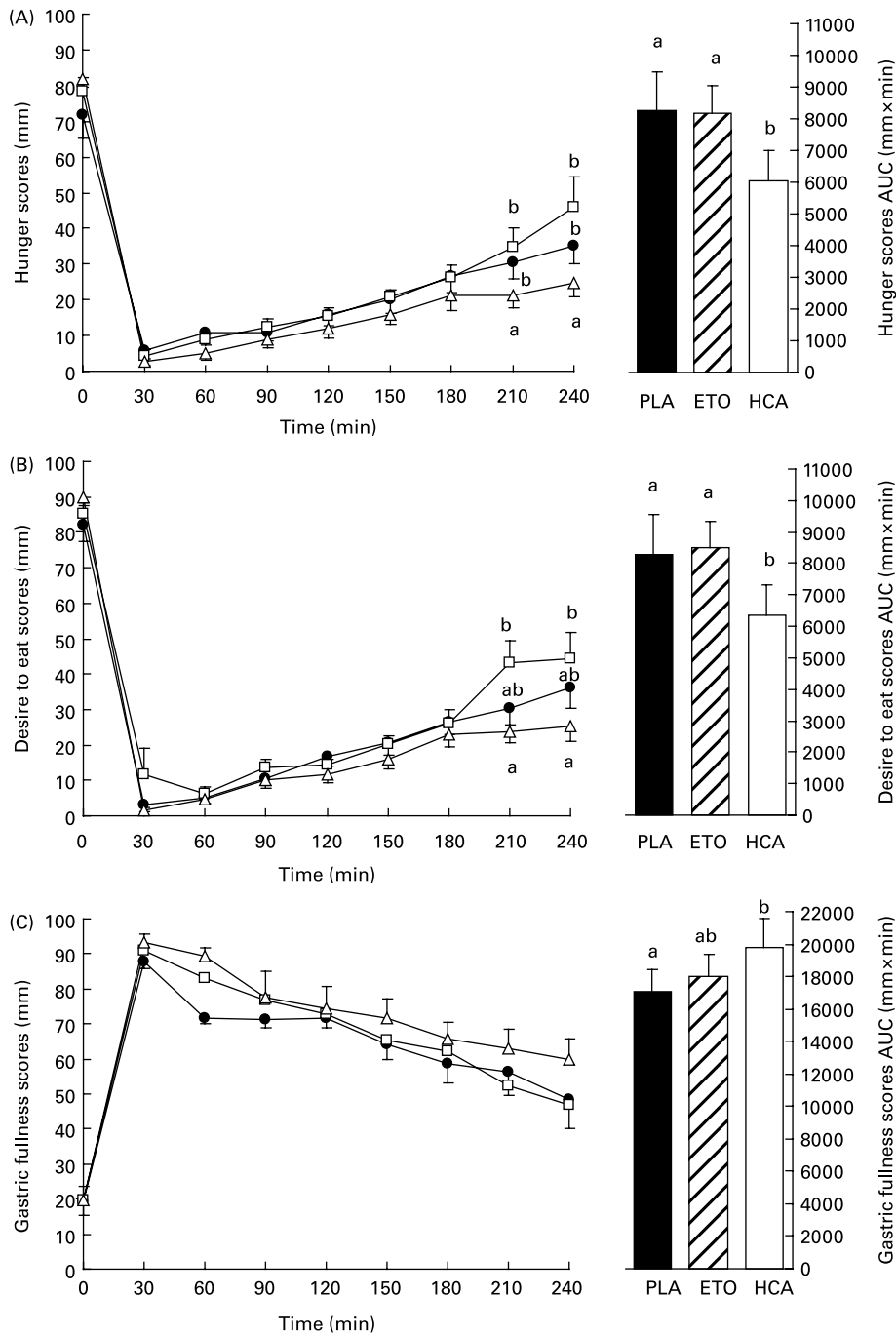
Fig. 3. Time course (left panel) and area under the curve (AUC, right panel) of hunger (A), desire to eat (B) and gastric fullness scores (C) on visual analogue scales between lunch and 240 min, i.e. the delay of the first dinner request across subjects and across conditions (●, placebo (PLA); □, etomoxir (ETO); △, hydroxycitrate (HCA)). Values are means with their standard errors depicted by vertical bars. [a,b] Mean values with unlike superscript letters were significantly different ($P < 0.05$).

desire to eat profile until 240 min ($P < 0.05$) and the interaction between time and treatment approached significance ($P = 0.07$). Comparisons showed that at 210 and 240 min, desire to eat scores were lower with HCA than with ETO (both $P < 0.05$). No treatment effect was found for post-lunch gastric fullness profiles.

Hunger and desire to eat AUC were smaller with HCA than with PLA and ETO treatments (all $P < 0.05$). Gastric fullness

AUC was larger with HCA than with PLA treatment ($P < 0.05$), but the difference failed to reach significance with ETO.
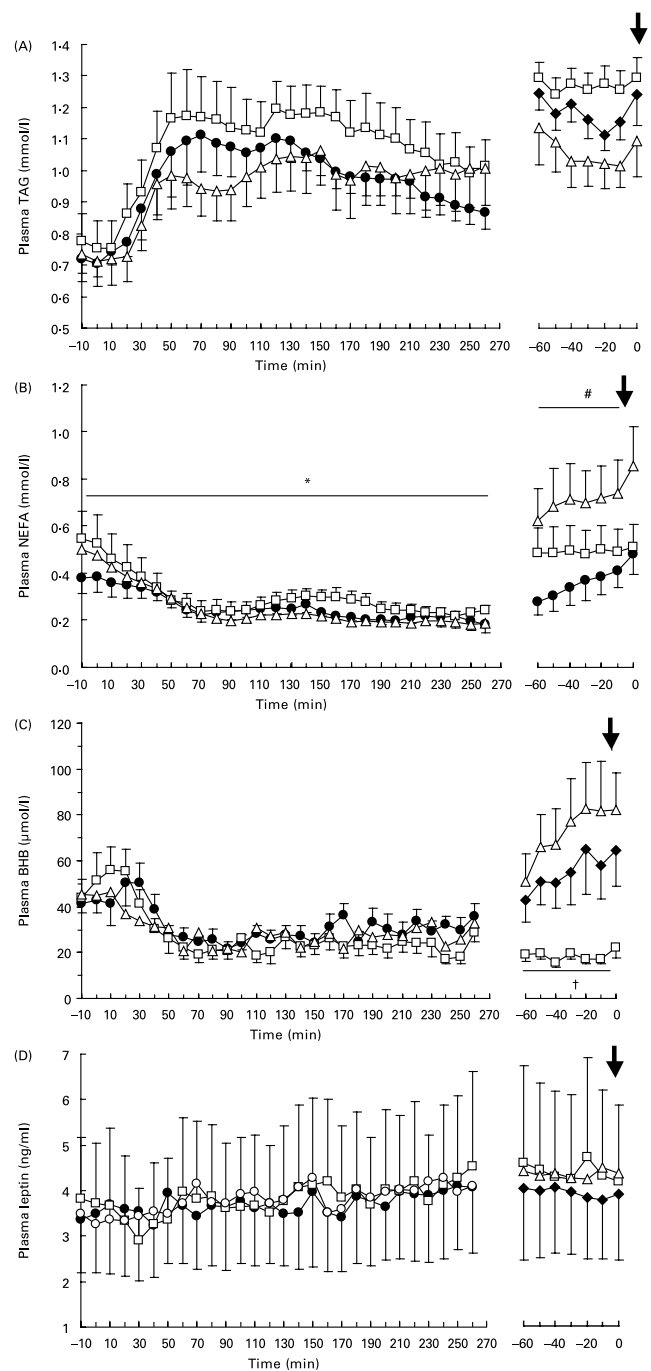
Plasma parameters

Figs. 4 and 5 show post-lunch and pre-dinner profiles of all plasma parameters. After lunch, treatment only affected
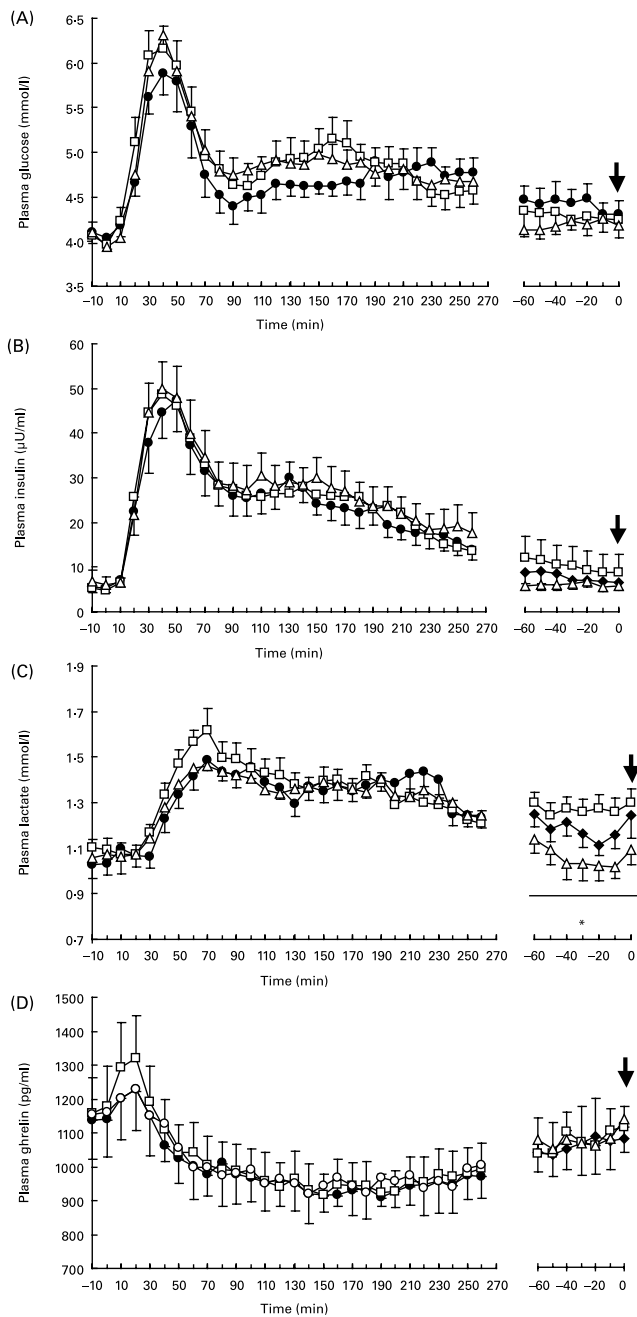
Fig. 4. Postprandial (left panel) and pre-dinner (right panel) plasma glucose (A), insulin (B), lactate (C) and ghrelin (D) concentrations during the interval between lunch and spontaneously requested dinner (↓) (●, placebo (PLA); □, etomoxir (ETO); △, hydroxycitrate (HCA)). Values are means with their standard errors depicted by vertical bars. HCA mean values were significantly different from those of PLA and ETO: *$P < 0.05$.

Fig. 5. Postprandial (left panel) and pre-dinner (right panel) plasma TAG (A), NEFA (B), β-hydroxybutyrate (BHB; C) and leptin (D) concentrations during the interval between lunch and spontaneously requested dinner (↓) (●, placebo (PLA); □, etomoxir (ETO); △, hydroxycitrate (HCA)). Values are means with their standard errors depicted by vertical bars. HCA mean values were significantly different from those of ETO, *$P < 0.05$. HCA mean values were significantly different from those of PLA, #$P < 0.05$. ETO mean values were significantly different from those of HCA and PLA, †$P < 0.05$.

plasma NEFA ($P < 0.05$), without a treatment × time interaction. Post-lunch plasma NEFA concentrations were higher with ETO than with HCA ($P < 0.05$), but the difference failed to reach significance between ETO and PLA ($P = 0.07$).

In the pre-dinner period, treatment affected plasma lactate ($P < 0.05$), NEFA ($P < 0.05$) and BHB ($P < 0.005$) profiles without an interaction with time. With HCA treatment, pre-dinner concentrations of plasma lactate were lower than with PLA ($P < 0.05$) and ETO ($P < 0.05$), whereas NEFA

concentrations were higher than with PLA ($P < 0.05$). The difference in the pre-dinner profile of NEFA between HCA and ETO treatments just failed to reach significance ($P = 0.07$). The pre-dinner concentrations of BHB were lower with ETO than with PLA ($P < 0.05$) and HCA treatments ($P < 0.01$).

AUC and mean IMI concentrations are shown in Table 2. ETO increased the AUC for plasma NEFA compared to PLA ($P<0.01$) or HCA treatment ($P<0.005$) and reduced the AUC for BHB compared to PLA ($P<0.05$). Mean plasma NEFA concentrations were higher with HCA and ETO treatment than with PLA ($P<0.05$ and $P<0.01$, respectively), and mean BHB concentrations were lower with ETO than with PLA and HCA treatments (both $P<0.05$).

Correlations between satiety and plasma parameters

Among plasma parameters, the difference in mean NEFA concentrations between HCA and PLA treatments was the only one correlated with treatment differences in IMI duration (r 0.84, $P<0.01$). The difference in mean plasma NEFA concentrations was also the only predictive factor for the difference in IMI duration between HCA and PLA (r^2 0.71, $P<0.01$) (Table 3).

When all treatments were pooled, plasma leptin concentration at dinner request was highly correlated with energy intake at dinner (r -0.75, $P<0.0005$). When treatments were analysed separately, the correlation was significant with HCA (r -0.79, $P<0.05$) and ETO treatments (r -0.91, $P<0.005$), but failed to reach significance with PLA (r -0.63, $P=0.07$). Furthermore, a correlation between plasma glucose concentrations and energy intake at dinner was found (r -0.55, $P=0.005$). Both leptin and glucose were only correlated with intake of the first meal item (r -0.75, $P<0.0005$ for leptin; r -0.67, $P<0.0005$ for glucose) and not with dessert. The best fitting model for predicting energy intake at dinner from plasma parameters at meal request only included leptin with a negative coefficient (r^2 0.56, $P<0.00005$) (Table 3). When only the first item of the dinner was considered, the best fitting model included leptin, ghrelin and lactate, the latter two with a positive coefficient (r^2 0.67, $P<0.00005$).

Discussion

The aim of the present study was to investigate the role of NEFA in satiety by administering to young and healthy male subjects two modulators of NEFA metabolism, ETO and HCA, which were expected to decrease and increase satiety, respectively. The present results confirmed only the latter prediction, i.e. HCA increased the subjective post-lunch satiety scores and the mean duration of satiety until the dinner request in our group of subjects by more than 70 min, although energy intake at dinner was unaffected. This increased delay is unlikely to be caused by nausea or other digestive malaise. Because of the continuous blood withdrawal, we regularly asked subjects if they experienced any abnormal sensation or manifestation, but they reported none. The fact that their energy intake at dinner was not different among HCA, PLA and ETO conditions also argues for the absence of any digestive malaise.

The satiety-enhancing effect of HCA did not appear to be mediated by a change in food palatability because no differences in hedonic scores were found. This is consistent with findings in rats, in which HCA did not induce a conditioned taste aversion to saccharin[28]. The fact that the length of

Table 2. Area under the curve (AUC) and plasma concentrations of hormones and metabolites*
(Mean values with their standard errors)

	PLA		ETO		HCA	
	Mean	SE	Mean	SE	Mean	SE
Glucose						
AUC (mmol/l × min)	1577	113	1592	110	1599	98
Plasma (mmol/l)	4.63	0.09	4.69	0.12	4.67	0.12
Insulin						
AUC (μU/ml × min)	7133	851	7347	922	7826	1099
Plasma (μU/ml)	18.38	2.08	20.07	2.79	18.64	2.33
Lactate						
AUC (mmol/l × min)	430	23	440	29	428	25
Plasma (mmol/l)	1.27	0.03	1.34	0.04	1.23	0.05
Ghrelin						
AUC (ng/ml × min)	327	48	331	38	338	49
Plasma (ng/ml)	1.11	0.13	1.11	0.12	1.11	0.11
TAG						
AUC (mmol/l × min)	326	33	371	38	333	52
Plasma (mmol/l)	0.97	0.06	1.17	0.10	0.97	0.09
NEFA						
AUC (mmol/l × min)	86[a]	11	104[b]	13	80[a]	10
Plasma (mmol/l)	0.26[a]	0.02	0.35[b]	0.03	0.31[b]	0.04
BHB						
AUC (μmol/l × min)	11 774[a]	1964	7609[b]	1077	9330[ab]	1112
Plasma (μmol/l)	37.36[a]	4.51	22.13[b]	2.24	37.23[a]	3.34
Leptin						
AUC (ng/ml × min)	1289	515	1342	614	1341	519
Plasma (ng/ml)	3.24	1.20	3.72	1.57	3.72	1.36

BHB, β-hydroxybutyrate; ETO, etomoxir; HCA, ($-$)-hydroxycitrate; PLA, placebo.
[a,b] Mean values within a row with unlike superscript letters were significantly different ($P<0.05$).
* For details of subjects and procedures, see Experimental methods.

Table 3. Regression models for behavioural variables*

Dependent variable	β	SE	P
ΔIMI (HCA − PLA)			
Constant	−42·23	15·51	0·03
NEFA (mmol/l)	675·30	176·869	<0·01
EI$_{dinner}$			
Constant	5754·21	192·55	<0·0001
Leptin (ng/ml)	−178·78	33·52	<0·0005
EI$_{item1}$			
Constant	459·65	230·98	0·06
Leptin (ng/ml)	−35·34	6·59	<0·0001
Ghrelin (pg/ml)	0·14	0·06	<0·05
Lactate (mmol/l)	345·56	156·04	<0·05

β, standard regression coefficient; HCA, (−)-hydroxycitrate; IMI, inter-meal interval; PLA, placebo.
* Tested behavioural variables were the difference in IMI between HCA and PLA conditions (ΔIMI), total energy intake at dinner (Ei$_{dinner}$) and energy intake derived from the main dish of the dinner meal presented as first item (EI$_{item1}$). Predictive variables were plasma glucose, insulin, lactate, ghrelin, TAG, NEFA, β-hydroxybutyrate and leptin concentrations (mean concentrations and concentrations at dinner onset for ΔIMI and energy intake, respectively), and treatments (PLA, etomoxir, HCA). These equations were the best fitting model for each dependent variable. The r^2 and significance of each equation are indicated in the Results. P significant at <0·1.

satiety was sensitive to HCA and that subjects could not guess the real time at debriefing argues for the effectiveness of the time blinding. Thus, in young male subjects in energy equilibrium and on a naturalistic diet pattern, acute HCA administration was effective in prolonging satiety. In previous studies under varying conditions, we and others have repeatedly found that when meals can be freely requested, subjects adapt to experimental conditions primarily by varying the IMI rather than meal size[29–32]. This is in agreement with results obtained in rats[33]. Moreover, the conceptual basis of the present study was the putative role of NEFA as an additional source of energy for the metabolic flux during the IMI, thus prolonging satiety and delaying hunger[30]. Meal onset presumably results from the interaction of metabolic, neuroendocrine and sensory factors. This of course limits the relevance of the present results for everyday life, where times of meals are often fixed by environmental factors.

The observed changes in plasma parameters did not provide a clear explanation for the effect of HCA on satiety. The increases in circulating NEFA and BHB (approaching significance) during the pre-dinner period are consistent with an increase in NEFA oxidation by HCA, possibly through an increased release of NEFA from adipose tissue and their subsequent oxidation. However, substrate oxidation was not directly measured, which would be necessary to confirm such an assumption. Some recent data in fact suggest an enhancing effect of HCA on lipolysis in human adipocytes[34], but the existence of such an effect in naturalistic conditions *in vivo* needs to be demonstrated. On the other hand, plasma BHB and NEFA concentrations suggest that ETO inhibited NEFA oxidation, but did not stimulate eating, showing that an inhibition of NEFA oxidation does not always translate into a stimulation of eating, providing a contrasted picture of the relation between NEFA oxidation and satiety.

A crude interpretation of the present results would be that with HCA a longer period with low glucose and high fat utilization is tolerated before eating is initiated. HCA has been reported to decrease cumulative energy intake in animal studies, mainly in conditions of enhanced fat synthesis, such as in animals fed a high carbohydrate diet[35,36], or while refeeding after fasting and body weight loss[37–40]. In man, HCA doses (1·5–3 g) similar to the one we used were tested under a variety of conditions (subjects with overweight or obesity, in positive energy balance, or with normal weight) and were reported to cause greater weight reduction than control treatments in many[19,20,41] but not all[42] of these studies. However, effects of HCA on eating behaviour or subjective parameters of satiety[13,41–44] were scarcely reported, and none of these studies was specifically designed to examine the effects of HCA on satiety in normal-weight subjects eating their regular diet, as in the present experiment. Interestingly, in the only study conducted in weight-stable obese subjects, a reduction in spontaneous intake was found during an acute experimental session[13].

One possible mechanism of the satiating effect of HCA is an indirect stimulation of fatty acid oxidation, in particular in the liver. Hepatic fatty acid oxidation has been proposed to trigger a vagally mediated satiety signal[6]. However, we have previously shown that subdiaphragmatical vagal deafferentation, a surgical procedure that eliminates all vagal afferents, did not prevent HCA from reducing food intake in rats[39], suggesting that other mechanisms are involved. HCA has also been shown to promote whole-body fat oxidation and to spare glucose utilization in mice[45], although this could not be demonstrated in man[43,44,46,47], except in conditions of forced *de novo* lipogenesis such as prolonged passive overfeeding with a high-carbohydrate diet[48], or during endurance exercise[49–51]. While we did not measure whole-body substrate oxidation in the present study, there was some evidence of an indirect stimulation of hepatic NEFA oxidation by HCA. Thus, BHB tracked circulating NEFA and tended to be higher, and lactate was lower after HCA than after PLA throughout the pre-dinner period. This suggests that after HCA administration subjects requested dinner in a metabolic state characterized by a higher rate of NEFA oxidation and lower rate of glycolysis according to the glucose–fatty acid cycle[52]. However, an alternative explanation is that this metabolic profile only reflects the longer duration of satiety after HCA, with plasma concentrations and oxidation of NEFA increasing substantially in the late part of long IMI[14,16,29,30].

The main limitation of the present study is the absence of direct measurement of substrate oxidation. It is quite delicate to assess spontaneous IMI when it is regularly interrupted by gas-exchange measurements and a respiratory chamber would be required to examine if, in the present condition, HCA actually increased whole-body NEFA oxidation. All interpretations concerning fat oxidation are therefore primarily based on plasma BHB and NEFA concentrations and must be taken with caution until substrate oxidation during the IMI is assessed.

We did not observe an effect of HCA on plasma leptin concentrations between lunch and dinner. The reported findings in man of a decrease in circulating leptin or adipose tissue leptin expression in response to HCA presumably reflect an indirect effect related to a decrease in fat mass by HCA rather than a direct effect on leptin production[19–21]. As we have shown, even small variations in fat mass can be associated with a sustained decrease in circulating leptin[53]. In the present study,

leptin was again a potent negative predictor of energy intake in the spontaneous requested meal. We repeatedly reported this relation when subjects were allowed to decide on the timing of meals and on the amount they ate[14,16]. That this relation is strongest when the first and main item of the meal is considered is also a constant finding in our studies. Interestingly, when the first meal item was used for the modelling, ghrelin became also a significant predictor of intake with a positive coefficient. To our knowledge, a possible effect of HCA on ghrelin had not been explored yet. Ghrelin has been implicated in meal initiation in man because its administration increases food intake[54] and because its plasma concentration increases before fixed[55] or spontaneously requested[56] meals. Here, ghrelin increased before dinner request to similar concentrations with all treatments.

HCA might also affect satiety by reducing the rate of postprandial intestinal glucose absorption[57]. This would prolong glucose delivery from the intestine and, hence, delay a glucopaenia-related meal initiation signal. However, no differences in postprandial blood glucose appearance were found in the present study, although the frequent blood sampling was appropriate to observe small kinetic differences. Also, postprandial peaks of circulating glucose were reached at approximately 45 min after lunch with all treatments, which corresponds to our previous findings with this blood-sampling method[58].

Finally, HCA has been shown to reduce serotonin reuptake in brain, thus increasing serotonin availability and action[59], to up-regulate genes encoding serotonin receptors[60] and to substantially increase circulating serotonin concentrations[20]. Although all these effects were demonstrated with a new HCA extract (SX), an increase in serotoninergic neurotransmission could be a common property of this compound family, but the present results do not allow us to judge on a possible contribution of serotonin to the observed HCA effects.

Contrary to a previous study conducted in young male subjects[12], we failed to show an effect of ETO on eating, and the metabolic effect of ETO was weaker. In the previous study, however, ETO increased food intake only in subjects who were accustomed to high dietary fat intake and when ETO was given before breakfast after consumption of a 65·8 % (energy) fat dinner on the previous evening. When ETO was given after a 60 % fat lunch, it did not increase subsequent intake, which is similar to the present findings. The macronutrient composition of meals in the present study was chosen to explore the relevance of the role of NEFA and fat metabolism in everyday life rather than extreme dietary conditions. ETO supposedly stimulates eating by inhibiting hepatic NEFA oxidation. This mechanism seems not effective or at least of minor importance under normal eating conditions in man.

Although chosen with caution, according to previous studies reporting significant effects of ETO and HCA on eating behaviour, and considering the kinetics of these two agents, we do not know whether these doses and modalities of administration were optimal. This applies in particular to ETO for which we did not find an effect.

In conclusion, the present results show for the first time that HCA can enhance the intensity of satiety sensation and delay the spontaneous request of the subsequent meal in normal-weight human subjects during a spontaneous eating pattern. The mechanism of this effect is still uncertain, but could be

somehow related to an increased availability of NEFA for the intermeal metabolism.

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