Available at www.sciencedirect.com

Metabolism

www.metabolismjournal.com

Combined hypertriglyceridemic and insulin-glucose clamps for the characterization of substrate oxidation and plasma elimination of a long-chain triglyceride emulsion in healthy men

Viveca Åberg[a,b,*], *Anders Thörne*[c,d], *Anders Alvestrand*[d,e], *Jörgen Nordenström*[a,b]

[a] *Department of Breast and Endocrine Surgery, Karolinska University Hospital-Solna, Stockholm, Sweden*
[b] *Department of Molecular Medicine and Surgery, Karolinska Institutet, Stockholm, Sweden*
[c] *Department of Surgery, Södertälje Hospital, Södertälje, Stockholm, Sweden*
[d] *Department of Clinical Science, Intervention and Technology, Karolinska Institutet, Stockholm, Sweden*
[e] *Department of Nephrology, Karolinska University Hospital-Huddinge, Stockholm, Sweden*

ARTICLE INFO

Article history:
Received 3 March 2011
Accepted 17 June 2011

ABSTRACT

This study examines the effect of glucose and insulin on the plasma elimination rate and oxidation of a fat emulsion by using indirect calorimetry in conjunction with lipid and insulin-glucose clamp techniques. Ten healthy subjects were studied on 2 occasions in a randomized, open, crossover study. On one occasion, a hypertriglyceridemic (HTG) clamp was administrated alone; and, on the other, HTG and insulin-glucose (IG) clamps were administrated simultaneously. During HTG clamps, serum triglyceride (TG) concentration was maintained at 4 mmol × L^{-1}. During the IG clamp, insulin was administered at a rate of 20 mU × m^{-2} × min^{-1}; and the glucose level was maintained at 7 mmol × L^{-1}. Continuous indirect calorimetry was carried out throughout the study period. The infusion rate required to maintain stable serum TG concentrations did not differ between the 2 clamps. Mean free fatty acid concentration was lower during the HTG/IG than during the HTG clamp (0.40 ± 0.04 vs 0.82 ± 0.07 mmol × L^{-1}; $P < .001$). However, the increases in β-OH-butyrate levels were significantly lower in the HTG/IG compared with the HTG clamp (0.09 ± 0.04 vs 0.55 ± 0.09 mmol × L^{-1}; $P < .001$). Energy expenditure and the respiratory quotient were significantly higher at steady state in the HTG/IG than in the HTG clamp: 1.47 ± 0.06 vs 1.34 ± 0.04 kcal × min^{-1} ($P < .01$) and 0.85 ± 0.01 vs 0.79 ± 0.01 ($P < .01$), respectively. Insulin and glucose did not significantly change plasma TG disposal rate ($P = .0987$) or total lipid oxidation ($P = .3204$) in this metabolic situation with an abundant supply of both carbohydrates and lipids. β-OH-butyrate increased during both clamps, indicating an ongoing hepatic fatty oxidation despite the administration of glucose/insulin.

Statement of authorship: The specific contribution of each author was as follows: W Åberg performed the indirect calorimetry and the clamp procedures, performed the online measurements, compiled the data, performed the statistical analyses, and wrote the draft of the manuscript. A Thörne, A Alvestrand, and J Nordenström contributed to the study design, the data interpretation, and the final manuscript. All authors read and approved the final manuscript.

* *Corresponding author*. Department of Breast and Endocrine Surgery, Karolinska University Hospital-Solna, SE-171 76 Stockholm, Sweden. Tel.: +46 70 7344192; fax: +46 8 331587.
E-mail address: viveca.aberg@ki.se (V. Åberg).

0026-0495/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2011.06.011

1. Introduction

Fat emulsions are an integral component of total parenteral nutrition (TPN) and are usually administrated together with glucose. The metabolism of fat is determined by the underlying condition such as trauma, infection, or malnutrition and also by substrate interaction when different nutrients are administrated simultaneously [1,2]. It is, therefore, of interest to characterize the metabolic pathways of infused substrates and determine possible substrate competition between carbohydrate and fat, especially as many patients in need of TPN are insulin resistant [3]. Many investigators have demonstrated the existence of glucose/fatty acid fuel competition in humans [4-6], first described by Randle et al [7]. There are many studies showing that elevated plasma free fatty acid (FFA) levels suppress the capacity of insulin to stimulate systematic glucose uptake and oxidation [3,4]. Less attention, however, has been paid to the reverse effect, that is, the effect of glucose on fatty acid oxidation. Studies by Sidossis et al [8,9] have shown that hyperglycemia-hyperinsulinemia may directly inhibit fatty acid oxidation across the leg and the splanchnic regions even when plasma fatty acid availability is maintained constant. These authors have also presented evidence that glucose and insulin determine fatty acid oxidation by controlling the rate of fatty acid entry into the mitochondria [10]. Studies by Groop et al [11], on the other hand, have provided evidence that FFA oxidation is closely correlated to plasma FFA concentrations. These investigators used a euglycemic insulin clamp and kept plasma FFA concentrations stable by infusing heparin. They concluded that the effect of glucose/insulin on FFA metabolism is primarily determined by the rate of FFA mobilization from tissue stores.

1.1. Hypothesis

Our previous studies have documented marked increases in serum FFA and plasma β-OH-butyrate levels as well as lipid oxidation during administration of a hypertriglyceridemic (HTG) clamp in healthy volunteers [12]. Our hypothesis was that the moderately high insulin levels comparable to the metabolic situation observed in patients receiving TPN would prevent the expected increments of serum FFA and lipid oxidation. In this study, we used the insulin/glucose (IG) and HTG clamp techniques to obtain a stable and standardized metabolic condition for the characterization of the interaction between glucose/insulin and lipid oxidation.

2. Materials and methods

2.1. Subjects

Ten healthy nonsmoking male volunteers participated in the study. Their average age was 30 (range, 24-44) years, weight was 80 (range, 68-95) kg, and body mass index was 24 (range, 22-27) kg/m^2. All subjects were weight stable; none had diabetes mellitus, lung or thyroid dysfunctions, or any other metabolic disorder; and none of them were taking any medication. The nature, purpose, and possible risks of the study were explained to all the individuals before they consented to participate. Approval was obtained from the local ethics committee.

2.2. Study design

The design was an open, crossover, randomized study. The randomization was performed with consecutively sealed envelopes. Subjects were studied on 2 occasions with an interval of at least 1 month, a period considered sufficiently long enough to stabilize their metabolisms. On one of these occasions, an HTG clamp was administrated alone; and, on the other, HTG and IG clamps were administrated in parallel. All subjects were studied after an overnight fast. Before each study, all the volunteers ingested their habitual diet containing at least 150 g of carbohydrates per day.

An intravenous catheter was placed in an antecubital vein for infusion of a long-chain triglyceride (LCT) emulsion, Intralipid 200 mg/mL (Fresenius Kabi, Uppsala, Sweden). When insulin and glucose were infused simultaneously with LCT, a second catheter was placed in a contralateral antecubital vein. In addition, a thin catheter was inserted into the radial artery for blood sampling; and the artery was kept with an infusion of isotonic saline. During the investigations, the subjects rested in a recumbent position and at an ambient temperature of 22°C. After placing the subject's head in a ventilated plastic hood, respiratory gas exchange was continuously measured for 1 hour in the basal resting state and then during clamps.

The HTG clamp was carried out as previously described [13]. The LCT emulsion was administered using a volumetric pump (IVAC, Stockholm, Sweden). A priming dose (13 mg TG × kg body weight^{-1} × min^{-1}) was infused to rapidly increase serum TG concentration to 4 mmol × L^{-1}, the priming period being the time from the start of the infusion until reaching a steady-state TG level of 4 mmol × L^{-1}. The infusion rate was thereafter adjusted to maintain a stable serum TG level of 4 mmol × L^{-1} during a period of 180 minutes, and serum TG concentrations were determined enzymatically every 10 minutes with a Reflotron (Boehringer, Mannheim, Germany) chemistry analyzer. The IG clamp was administrated simultaneously with the HTG clamp. The IG clamp was performed as described by DeFronzo et al [14], with minor modifications to mimic the situation during administration of TPN. Insulin (Actrapid Human 100 IU × mL^{-1}; Novo A/S, Bagsvaerd, Denmark) was dissolved in 96 mL 0.9% sodium chloride (NaCl 154 mmol × L^{-1}), and 4 mL of the subject's blood (400 mU × m^{-2}) was infused as a bolus followed by 20 mU × m^{-2} × min^{-1}. For the determination of blood glucose concentration, arterial blood samples were drawn every 5 minutes using an automated glucose oxidase method. The glucose level was maintained at 7 mmol × L^{-1} during the steady-state period by adjusting the infusion rate with a 20% glucose solution. Blood samples were taken from the arterial catheter in the basal state and at timed intervals throughout the study period for the analysis of substrates and hormones.

2.3. Analytical procedures

A commercial apparatus (Deltatrac, Datex Instrumentarium, Helsinki, Finland) was used for indirect calorimetry. This

device measures oxygen and carbon dioxide concentrations in the gas from the ventilated hood by a paramagnetic and an infrared analyzer, respectively. The analyzers were calibrated before each study with air and precisely known gas concentrations (95.0% oxygen and 5.0% carbon dioxide). Plasma glucose concentration was analyzed enzymatically, and serum insulin was analyzed by radioimmunoassay [15]. Plasma glucose concentrations were also determined by the glucose oxidase method for immediate results (HemoCue, Ängelholm, Sweden). Serum concentrations of TG were determined enzymatically using Reflotron, which provides a value within 190 seconds. The TG concentration in serum was also measured by the routine method at the hospital, using an L-α-glycerol-phosphate oxidase (GPO) method [16]. In a separate experiment, we found an excellent correlation between the methods ($TG_{Reflotron}$ = 1.09, TG_{GPO} = -0.331, n = 30, r^2 = 0.959) [13]. Free fatty acid in serum was determined by enzymatic colorimetric methodology (Wako Chemicals, Nuess, Germany). Plasma glycerol was analyzed using ultrasensitive bioluminescence [17]. Plasma β-OH-butyrate was analyzed enzymatically by spectrophotometer [18]. Plasma adrenaline and noradrenaline were determined by high-performance liquid chromatography and electrochemical detection [19]. Serum lactate was measured fluorometrically [20].

2.4. *Calculations*

The amount of administered exogenous fat during the 180-minute steady-state clamp period was considered to reflect the clearance of TG from the plasma compartment. The fractional removal rate was calculated as the TG infusion rate at steady state divided by the pool size, as previously described, by using a regression equation based on height, weight, and hematocrit values [21]. Respiratory gas exchange data were continuously measured; and average values were calculated as mean values for every 1-minute period for oxygen consumption, carbon dioxide production, respiratory quotient (RQ), and energy expenditure (EE) according to previously presented formulas [22]. Baseline EE calculations were based on gas exchange data from the last 30 minutes of the basal fasting period. Carbohydrate and lipid oxidation rates were derived from tables by Lusk [22]. We assumed protein oxidation to be stable in the resting condition and constant throughout the study period, and therefore did not include it in the analyses [23]. The diet-induced thermogenesis (DIT) of fat was calculated as the average increase above basal expended energy in percentage of the energy content of the administered lipids and glucose during the clamp periods (0-180 minutes).

Statistical calculations were performed with Statview version 5.0 (SAS Institute, Cary, NC). The results in the text, tables, and figures are shown as mean values ± standard error of the mean (SEM). The effect of glucose and insulin infusion on the substrate oxidation and plasma removal capacity of intravenous fat emulsion was assessed by analyzes of variance (ANOVA). Differences in between/within HTG and HTG/IG infusion studies were evaluated by repeated measures. Differences in priming volume, total amount of infused TG, and DIT between HTG and HTG/IG clamps were identified using a paired *t* test. Statistical significance was considered present if $P < .05$.

3. Results

The TG concentration was similar in the 2 trials in the basal state as well as during the steady-state periods of the clamps (Fig. 1A). The TG concentrations determined with the Reflotron equipment and the GPO method were almost identical and revealed no differences between the 2 groups either in the basal states or during clamp periods. The average TG concentration (GPO method) during the clamp periods was similar in the 2 clamps (4.0 ± 0.1 vs 3.9 ± 0.1 mmol × L^{-1}; P = .2116). The average infusion rates of fat emulsion, that is, the elimination rate of fat, were no different (P = .5964) with or without the IG clamp (Fig. 1B and Table 1). The plasma fractional elimination rate of fat was not influenced (P = .3097) by the glucose/insulin infusion during the HTG/IG clamp (Table 1). The priming volume required to reach steady-state TG levels was significantly higher with the combined HTG/IG than with the HTG clamp ($P < .05$; Table 1). This corresponds to a priming dose of 43% ± 3% for the HTG/IG and 34% ± 2% for the HTG clamp, respectively ($P < .05$), of the total amount of infused fat during the respective clamps. During the steady-state period, 185 ± 18 mL was infused during the HTG/IG clamp and 200 ± 21 mL was infused with the HTG clamp, respectively (P = .4737). The total amount of energy infused during the HTG clamp was about one half of the energy given during the combined HTG/IG clamp (Table 1).

In the basal state, the concentrations of glucose and insulin did not differ between the 2 clamps. Mean plasma glucose concentration was 7.2 ± 0.1 mmol × L^{-1} during the HTG/IG and 4.9 ± 0.1 mmol × L^{-1} during the HTG clamp ($P < .001$; Fig. 2A). Serum insulin concentrations were, as expected, markedly elevated during the HTG/IG clamp (36.3 ± 3.0 vs 7.0 ± 0.5 mU × L^{-1}; $P < .001$) (Fig. 2B). Serum FFA levels before the start of the HTG and HTG/IG infusions were similar (0.47 ± 0.04 vs 0.48 ± 0.05 mmol × L^{-1}, respectively). The mean FFA concentration was lower during the HTG/IG than during the HTG clamp (0.40 ± 0.04 vs 0.82 ± 0.07 mmol × L^{-1}; $P < .001$), whereas the glycerol concentrations were similar in the 2 clamps (0.18 ± 0.02 vs 0.20 ± 0.02 mmol × L^{-1}; P = .6171) during the clamps (Fig. 3A, B). Plasma β-OH-butyrate concentrations were similar at baseline and increased significantly at the end of the steady-state treatment period in both clamps (Table 2). However, the increases in β-OH-butyrate levels were significantly lower in the HTG/IG compared with the HTG clamp (0.09 ± 0.04 vs 0.55 ± 0.09 mmol × L^{-1}; $P < .001$). Plasma adrenaline and noradrenaline concentrations did not differ significantly between trials at baseline, and these remained stable in both clamps during clamping (Table 2). Lactate concentrations increased significantly from basal levels with the HTG/IG ($P < .05$) but not the HTG clamp (P = .4632; Table 2).

The EE and the RQ were no different (not significant) in the basal state in the 2 clamps (Table 3). During the HTG/IG clamp, the EE increased by 14% ± 2% (+0.19 ± 0.01 kcal × min^{-1}; $P < .001$); and this was a significantly greater increase ($P < .01$) than the 6% ± 2% (+0.08 ± 0.01 kcal × min^{-1}; $P < .05$) observed during the HTG clamp. Respiratory quotient was significantly

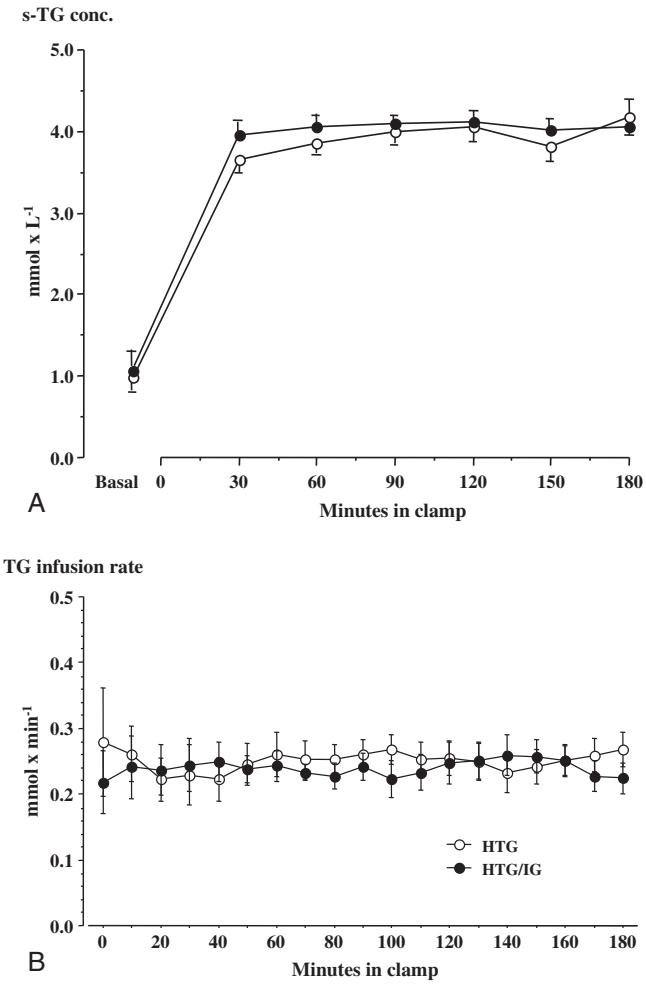

Fig. 1 – A, Serum TG concentration (mmol × L^{-1}) and (B) the TG infusion rate (mmol × min^{-1}) of exogenous fat during HTG (○) and HTG/IG (•) in 10 healthy individuals. Means ± SEM.

higher at steady state in the HTG/IG (0.85 ± 0.01) than in the HTG clamp (0.79 ± 0.01; P < .01; Table 3). Lipid oxidation was similar in both clamps at baseline. During lipid infusion alone, lipid oxidation increased by 26% ± 7% (+0.19 ± 0.01 kcal × min^{-1}; P < .01). In the HTG/IG clamp, lipid oxidation did not change significantly in comparison with baseline: –5% ± 7% (–0.05 ± 0.01 kcal × min^{-1}; P = .3535). Lipid oxidation accounted for 63% ± 3% of EE at baseline in both clamps. Administration of lipids alone resulted in an increase in the proportion of lipid oxidation/total oxidation to 73% ± 2% (P < .05), whereas the corresponding proportion in the HTG/IG clamp was 52% ± 5% (P < .05). There was a positive correlation between total lipid oxidation and serum FFA concentration in the HTG/IG clamp (r^2 = 0.696; P = .0027; Fig. 4). but not in the HTG clamp (r^2 = 0.303; P = .0991). Baseline carbohydrate oxidation was similar in the 2 clamps at baseline. Carbohydrate oxidation increased by 60% ± 16% (+0.23 ± 0.02 kcal × min^{-1}; P < .001) during the HTG/IG clamps, in contrast to the HTG clamp where a decreased carbohydrate oxidation was noted (–19% ± 7%; –0.11 ± 0.02 kcal × min^{-1}; P < .05; Table 3). Carbohydrate oxidation accounted for 37% ± 3% of the EE at baseline in both clamps. It increased to 48% ± 5% (P < .05) during the HTG/IG and decreased to 27% ± 2% (P < .05) during the HTG clamp. The increase in EE above basal EE in relation to administered energy during the clamp (DIT) was virtually of the same magnitude, that is, 4.5% ± 0.5% and 3.4% ± 0.8% (P = .1993) during the HTG/IG and HTG clamps, respectively.

4. Discussion

This investigation was designed to study the effect of moderate hyperglycemia and hyperinsulinemia obtained by an IG clamp technique on plasma elimination and oxidation of a lipid emulsion in healthy men. We found that the infusion rate of lipids required to maintain stable serum TG concentrations did not differ between HTG and combined HTG/IG clamps. Furthermore, the plasma TG fractional elimination rate was similar during the HTG and HTG/IG clamps. Our results show, therefore, that the plasma TG elimination rate is not influenced by moderate increments of plasma glucose and insulin levels. This is at variance with results obtained by Mittendorfer et al [24] who found that glucose and insulin markedly decrease very low-density lipoprotein–TG clearance. These authors, however, used a different method for analyzing TG kinetics and did not infuse lipids and glucose simultaneously, which may explain the observed difference. Serum FFA levels were significantly lower during the HTG/IG clamp in comparison with the situation when the HTG clamp was administrated. The administrated glucose and insulin thus prevented the rise in FFA levels that was observed during the HTG clamp. The design of our study does not allow the characterization of the underlying mechanism, but it is conceivable that infusion of glucose and insulin increased

Table 1 – Triglyceride infusion rate, fractional TG elimination rate, and priming and total volumes of infused TG in 10 young men during a HTG clamp and a combined HTG/IG clamp

	HTG	HTG/IG
TG infusion rate (mmol × L × min^{-1})	0.249 ± 0.004	0.239 ± 0.003
Fractional TG elimination rate (mmol × min^{-1})	0.084 ± 0.008	0.075 ± 0.006
Priming volume of TG (mL)	99 ± 6	133 ± 9*
Total amount of TG infused during the clamp (mL)	299 ± 23	319 ± 22
Total amount of energy given during the clamp (kcal)	400 ± 42 (from fat)	737 ± 51† (371 ± 37 from fat; 366 ± 37 from glucose)

Mean values ± SEM. Paired t test: *P < .05; †P < .001; HTG vs HTG/IG clamp.

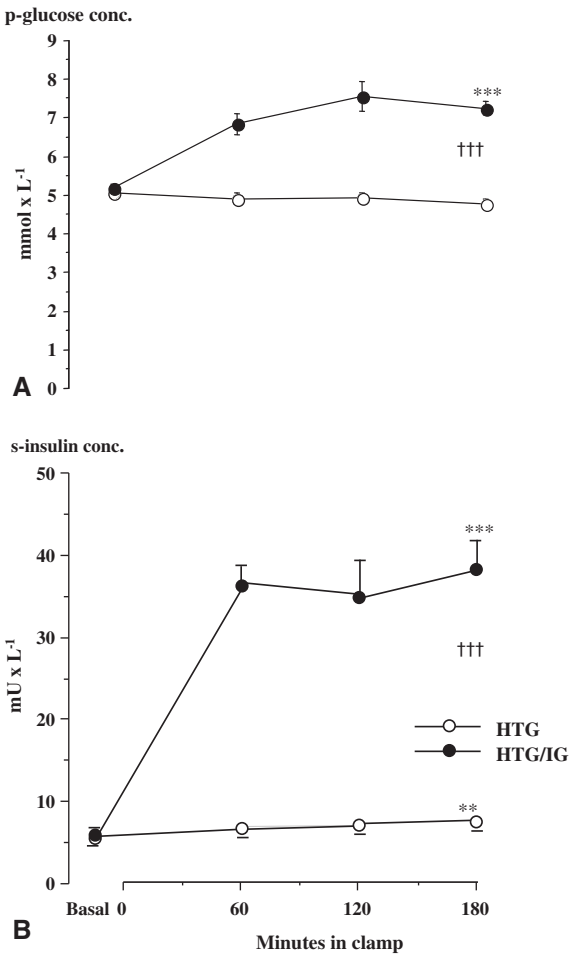

Fig. 2 – A, Plasma concentration of glucose (mmol × L^{-1}) and (B) serum concentration of insulin (mU × mL^{-1}) during HTG (○) and HTG/IG (●) clamps in 10 healthy individuals. Means ± SEM. Differences from baseline values are denoted by asterisks: **P < .01, ***P < .001. Repeated-measures ANOVA for treatment time × intervention: †††P < .001.

the fractional removal rate of FFA in accordance with our previous findings [25]. It has repeatedly been shown that insulin has a marked effect in suppressing FFA release from adipose tissue [26]. Sidossis et al [8,9] have shown that hyperglycemia-hyperinsulinemia may directly inhibit fatty acid oxidation even when plasma fatty acid availability is maintained constant. This mechanism is the reverse process of the so-called glucose–fatty acid cycle hypothesis proposed by Randle et al [7]. In another study by Sidossis et al [10], these investigators concluded that glucose and/or insulin determined fatty acid oxidation by controlling the rate of long-chain fatty acid entrance into the mitochondria. In the present study, the infusion of insulin and glucose during the insulin-glucose clamp resulted in physiological increments in serum insulin concentrations and moderate hyperglycemia, whereby lipid oxidation and serum FFA remained virtually unchanged despite simultaneous infusion of lipids. This suggests an increased channelling of FFA, presumably toward TG synthesis rather than oxidation. Under these circumstances, carbohydrate oxidation increased significantly. The mechanism involved could either be that insulin suppressed lipid oxidation directly by inhibiting the mobilization and/or oxidation of intracellular lipid stores, or that the inhibition of lipid oxidation was secondary to a stimulation of glucose oxidation by insulin (the Randle cycle). The design of the present study does not allow an elaboration on which of these mechanisms plays the greater role, but Groop et al [11] have demonstrated that FFA oxidation is primarily determined by the serum FFA concentration. Our observation of a close correlation between lipid oxidation and serum FFA concentration supports these findings by Groop et al. For any given increment in serum FFA concentration, however, the lipid oxidation rate increased twice when glucose and insulin were administered. This suggests that factors other than the FFA concentration are contributing to lipid oxidation and that differences in FFA concentration are not driving any differences in lipid oxidation between the trials.

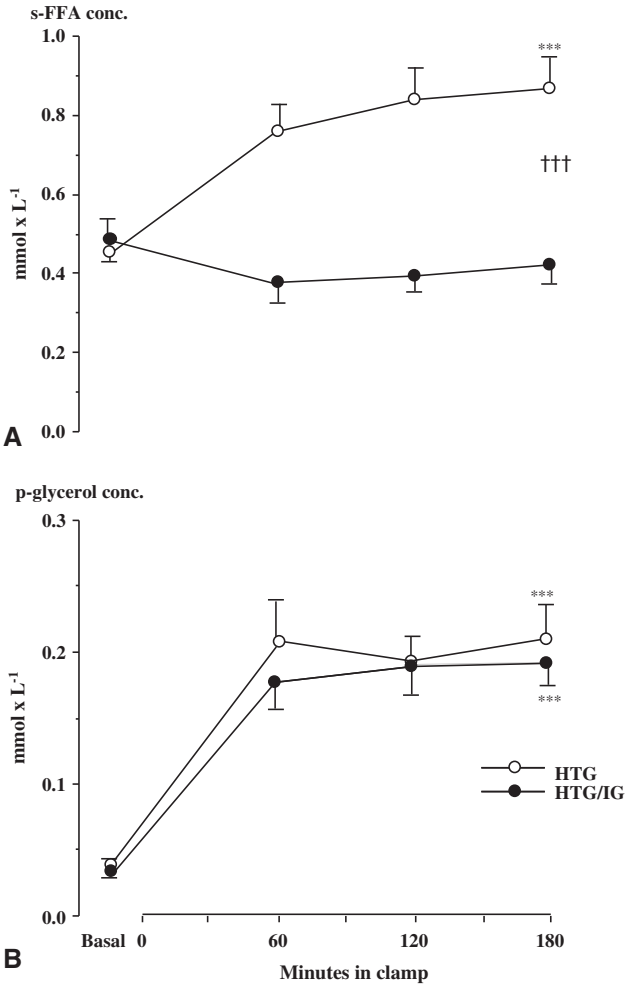

Fig. 3 – A, Serum concentration of FFA (mmol × L^{-1}) and (B) plasma concentration of glycerol (mmol × L^{-1}) during HTG (○) and HTG/IG (●) clamps in 10 healthy individuals. Means ± SEM. Differences from baseline values are denoted by asterisks: ***P < .001. Repeated-measures ANOVA for treatment time × intervention: †††P < .001.

Table 2 – Plasma concentrations of ß-OH-butyrate, adrenalin, noradrenalin, and serum lactate in 10 young men during a HTG clamp and a combined HTG/IG clamp

	Baseline	Minutes in clamp			ANOVA for intervention (differences over time)	ANOVA for treatment time × intervention (difference between clamps)
		60	120	180		
P-β-OH-butyrate (mmol × L^{-1})						
HTG	0.11 ± 0.01			0.66 ± 0.09	$P < .001$	$P < .001$
HTG/IG	0.11 ± 0.01			0.20 ± 0.04	$P < .05$	
P-adrenaline (nmol × L^{-1})						
HTG	0.13 ± 0.02			0.17 ± 0.04	$P = .1970$	$P = .9301$
HTG/IG	0.14 ± 0.02			0.18 ± 0.04	$P = .3972$	
P-noradrenaline (nmol × L^{-1})						
HTG	1.11 ± 0.15			0.97 ± 0.07	$P = .7980$	$P = .5595$
HTG/IG	1.07 ± 0.17			1.07 ± 0.17	$P = .5783$	
S-lactate (mmol × L^{-1})						
HTG	0.56 ± 0.06	0.51 ± 0.02	0.50 ± 0.04	0.49 ± 0.04	$P = .4632$	$P < .01$
HTG/IG	0.66 ± 0.08	0.84 ± 0.06	0.85 ± 0.06	0.84 ± 0.04	$P < .05$	

Mean values ± SEM.

The basal measurements of EE and RQ were performed after a 12-hour fast. Respiratory quotient values of 0.82 to 0.81 indicated that, at baseline, the subjects had a metabolic situation in which fat, rather than carbohydrates, was the prominent oxidative fuel. As expected, administration of fat during the HTG clamp did not change this situation. During the combined administration of insulin/glucose together with fat, RQ increased. It is well established that increased glucose and insulin concentrations inhibit peripheral lipolysis and fatty acid release, thereby decreasing FFA concentration in plasma [11]. Kelley et al [27] found that maintenance of plasma FFA concentrations by a lipid infusion during hyperinsulinemia maintains leg muscle FFA uptake and oxidation.

A number of studies have also reported that increasing the FFA concentrations by a combined lipid and heparin infusion during hyperinsulinemic-euglycemic clamping decreases glucose oxidation [27, 28]. It seems likely that, under these conditions with hyperinsulinemia and high plasma FFA levels, this effect is mediated by an inhibition of glucose transport rather than by an intracellular inhibition of glucose oxidation [27,28]. Wolfe et al [6] have shown that increased fatty acid concentrations do not affect glucose oxidation when glucose uptake is maintained constant. This suggests that the intracellular availability of glucose, rather than fatty acids, is the prime determinant of the substrate (ie, glucose or fat) that is oxidized. Our data also showed that a markedly increased energy intake of fat above basal EE (approximately 1.3 kcal/min) during the HTG (+2.2 kcal/min) and the combined HTG/IG clamp (+2.1 kcal/min from fat and +2.0 kcal/min from glucose) only marginally influenced the ratio of lipid and carbohydrate oxidation. At baseline, lipid and carbohydrate oxidation provided 63% and 37%, respectively, of total EE. At the end of the clamps, lipid oxidation provided for 73% of total EE during the HTG clamp and for 52% of total EE during the HTG/IG clamp. This indicates that, despite a markedly increased carbohydrate and lipid intake, a high degree of lipid oxidation was maintained.

Table 3 – Energy expenditure, RQ, and lipid and carbohydrate oxidation in 10 young men during a HTG clamp and a combined HTG/IG clamp

	Baseline	Minutes in clamp			ANOVA for intervention (differences over time)	ANOVA for treatment time × Intervention (difference between clamps)
		60	120	180		
EE (kcal × min^{-1})						
HTG	1.26 ± 0.03	1.32 ± 0.04	1.33 ± 0.04	1.35 ± 0.04	$P < .01$	$P < .01$
HTG/IG	1.28 ± 0.05	1.43 ± 0.05	1.46 ± 0.05	1.51 ± 0.07	$P < .001$	
RQ						
HTG	0.82 ± 0.01	0.80 ± 0.01	0.78 ± 0.01	0.78 ± 0.01	$P < .001$	$P < .001$
HTG/IG	0.81 ± 0.01	0.84 ± 0.01	0.85 ± 0.01	0.85 ± 0.01	$P < .001$	
Lipid oxidation (kcal × min^{-1})						
HTG	0.79 ± 0.04	0.92 ± 0.05	0.99 ± 0.05	1.02 ± 0.05	$P < .001$	$P < .001$
HTG/IG	0.81 ± 0.07	0.76 ± 0.08	0.74 ± 0.08	0.79 ± 0.08	$P = .3204$	
Carbohydrate oxidation (kcal × min^{-1})						
HTG	0.47 ± 0.04	0.40 ± 0.03	0.34 ± 0.02	0.33 ± 0.04	$P < .01$	$P < .001$
HTG/IG	0.47 ± 0.05	0.66 ± 0.07	0.73 ± 0.07	0.72 ± 0.08	$P < .001$	

Mean values ± SEM.

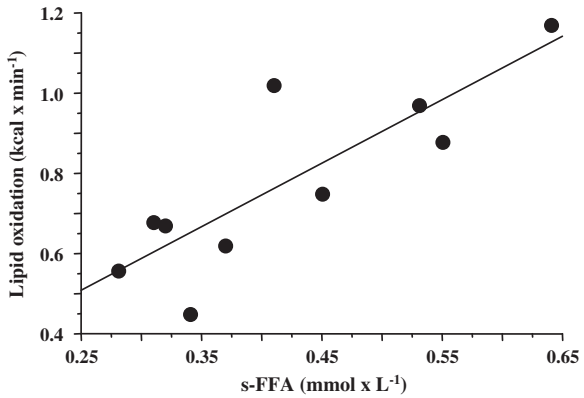

Fig. 4 – The correlation between lipid oxidation (kcal × min^{-1}) and concentration of serum FFA (mmol × L^{-1}) during the HTG/IG clamp ($y = 0.114 + 1.578x$, $r^2 = 0.696$; $P = .0027$) in 10 healthy individuals (n = 10; mean values of 4 measurements in 10 subjects).

The significant increase in serum β-OH-butyrate concentration during the combined infusion HTG/IG clamp suggests that increased glucose and insulin availability does not shut off hepatic fatty acid oxidation, although the elevated plasma insulin concentrations would, to a certain degree, be expected to limit FFA-induced ketogenesis [29]. This is in line with our findings showing that elevated insulin concentrations in the HTG/IG clamp are associated with smaller increases in plasma β-OH-butyrate concentrations than in the HTG clamp. Plasma adrenaline and noradrenaline did not change from baseline and during the clamps in both trials. This suggests that the clamps did not exert any metabolic stress.

5. Conclusion

In conclusion, the administration of glucose and insulin provided as an IG clamp in healthy volunteers did not influence the plasma elimination rate of a fat emulsion determined by an HTG clamp. Moreover, the modestly decreased FFA levels and augmented glucose oxidation noted during the combined HTG/IG clamp were accompanied by a high rate of lipid oxidation. Furthermore, the increment in plasma β-OH-butyrate during both clamp occasions indicates that there was an ongoing hepatic fatty oxidation despite the administration of insulin/glucose.

Acknowledgment

This work was supported by grants from the Karolinska Institute, the Swedish Society of Medicine, and the Swedish Research Council (grant 03X-727).

Conflict of Interest

There is no conflict of interest to declare.

REFERENCES

[1] Kinney JM. Metabolic responses of the critically ill patient. Crit Care Clin 1995;11:569-85.
[2] Boden G, Jadali F, White J, et al. Effects of fat on insulin-stimulated carbohydrate metabolism in normal men. J Clin Invest 1991;88:960-6.
[3] Boden G, Chen X. Effects of fat on glucose uptake and utilization in patients with non-insulin dependent diabetes. J Clin Invest 1995;96:1261-8.
[4] Thiébaud D, DeFronzo R, Jacot E, et al. Effect of long chain triglyceride infusion on glucose metabolism in man. Metabolism 1982;31:1128-36.
[5] Ferrannini E, Barett E, Bevilaquas S, DeFronzo R. Effect of fatty acids on glucose production and utilization in man. J Clin Invest 1983;72:1737-47.
[6] Wolf B, Klein S, Peters E, et al. Effect of elevated free fatty acids on glucose oxidation in normal humans. Metabolism 1988;37:323-9.
[7] Randle P, Garland P, Hales C, et al. The glucose fatty acid cycle. Its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. Lancet 1963;1:785-9.
[8] Sidossis L, Wolfe R. Glucose and insulin-induced inhibition of fatty acid oxidation: the glucose–fatty acid cycle reversed. Am J Physiol 1996;270:E733-8.
[9] Sidossis L, Mittendorfer B, Chinkes D, et al. Effect of hyperglycemia-hyperinsulinemia on whole body and regional fatty acid metabolism. Am J Physiol 1999;276: E427-34.
[10] Sidossis L, Stuart C, Shulman G, et al. Glucose plus insulin regulate fat oxidation by controlling the rate of fatty acid entry into the mitochondria. J Clin Invest 1996;98:2244-50.
[11] Groop L, Bonadonna R, Shank M, et al. Role of free fatty acids and insulin in determining free fatty acid and lipid oxidation in man. J Clin Invest 1991;87:83-9.
[12] Åberg W, Thörne A, Olivecrona T, et al. Fat oxidation and plasma removal capacity of an intravenous fat emulsion in elderly and young men. Nutrition 2006;22:738-43.
[13] Nordenström J, Thörne A, Åberg W, et al. The hypertriglyceridemic clamp technique. Studies using long-chain and structured triglyceride emulsions in healthy subjects. Metabolism 2006;55:1443-50.
[14] DeFronzo R, Tobin J, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. Am J Physiol 1979;237:E214-23.
[15] Livessy J, Hodgkinson S, Roud H, et al. Effect of time, temperature and freezing on the stability of immunoreactive LH, FSH, TSH, growth hormone, prolactin and insulin in plasma. Clin Biochem 1980;13:151-5.
[16] Wahlefeld AW. Triglycerides. Determination after enzymatic hydrolysis. In: Bergmeyer HV, editor. Methods of enzymatic analysis, 2nd ed, 4. New York: Verlag Chemie Weinheim. Academic Press, Inc; 1974. p. 1831-5.
[17] Hellmér J, Arner P, Lundin A. Automatic luminometric kinetic assay of glycerol for lipolysis studies. Anal Biochem 1989;177: 132-7.
[18] Wildenhoff K. A micro-method for the enzymatic determination of acetoacetate and 3-hydroxybutyrate in blood and urine. Scan J Clin Lab Invest 1970;25:171-9.
[19] Hallman H, Farnebo LO, Hamberger B, et al. A sensitive method for the determination of plasma catecholamines using liquid chromatography with electrochemical detection. Life Sci 1978;23:1049-52.
[20] Passonneau Jv. Fluorimetric method. Determination after enzymatic hydrolysis. In: Bergmeyer HV, editor. Methods of enzymatic analysis, 2nd ed, 3. New York: Verlag Chemie Weinheim. Academic Press, Inc; 1974. p. 1468-72.
[21] Nadler SB, Hildago JU, Bloch T. Prediction of blood volume in normal human adults. Surgery 1962;51:224-32.

[22] Bursztein S, Elwyn D, Askanazi J, et al. Energy metabolism, indirect calorimetry and nutrition. Baltimore (MD): Williams& Wilkins; 1989.
[23] Goodpaster B, Wolfe R, Kelley D. Effects of obesity on substrate utilization during exercise. Obesity Res 2002;10:575-84.
[24] Mittendorfer B, Patterson B, Klein S, et al. VLDL-triglyceride kinetics during hyperglycemia-hyperinsulinemia: effects of sex and obesity. Am J Physiol Endocrinol Metab 2003;284:E708-15.
[25] Nordenström J, Carpentier Y, Askanazi J, et al. Free fatty acid mobilization and oxidation during total parenteral nutrition in trauma and sepsis. Ann Surg 1983;198:725-35.
[26] Frayn KN, Arner P, Yki-Järvinen H. Fatty acid metabolism in adipose tissue, muscle and liver in healthy and disease. Essays Biochem 2006;42:89-103.
[27] Kelley D, Mokan M, Simoneau JA, et al. Interaction between glucose and free fatty acid metabolism in human skeletal muscle. J Clin Invest 1993;92:91-8.
[28] Wolfe RR, Peters EJ. Lipolytic response to glucose infusion in human subjects. Am J Physiol 1987;252:E218-23.
[29] Keller U, Gerber P, Stauffacher W. Fatty acid-independent inhibition of hepatic ketone body production by insulin in humans. Am J Physiol 1988;254:E694-9.