The metabolic response to fasting in humans: physiological studies

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6 Equal insulin sensitivity on inhibition of ketogenesis during short term-fasting in lean and obese humans

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Submitted

Abstract

Aims/hypothesis: The ketone bodies D-3-hydroxybutyrate and acetoacetate play a role in starvation and have been associated with insulin resistance: the dose-response relationship between insulin and ketone bodies was demonstrated to be shifted to the right in type 2 diabetes mellitus patients. In contrast ketone body levels have also been reported to be decreased in obesity. To clarify this paradox, we investigated the metabolic adaptation to fasting with respect to glucose and ketone body metabolism in lean and obese men without non insulin dependent diabetes mellitus. We hypothesized ketone body production to be equal under equal plasma insulin levels thereby reflecting absence of insulin resistance on ketogenesis.

Methods: After 38 hours of fasting, glucose and total ketone body fluxes were measured using stable isotopes in the basal state and during a two step pancreatic clamp.

Results: In the basal state, ketone body concentrations and fluxes were higher in lean compared with obese men. During the pancreatic clamp, no differences were found in ketone body fluxes during similar plasma insulin levels while peripheral glucose uptake was lower in obese men.

Conclusions/interpretation: Obese subjects are resistant to insulin's effect on stimulation glucose of glucose uptake, but not its inhibiting effect on ketogenesis, implying differential insulin sensitivity of intermediary metabolism in obesity.

Introduction

Starvation initiates an integrated metabolic response to prevent hypoglycemia and energy depletion (1). The ketone bodies (KBs) D-3-hydroxybutyrate (D-3HB) and acetoacetate (AcAc) play an important role in this adaptation: starvation induces an increase in plasma KB concentration and turnover serving as an important fuel for the central nervous system (2).

Ketogenesis occurs primarily in the liver. During fasting lipolysis rates from white adipose tissue are increased, resulting in release of non esterified fatty acids (NEFA) into plasma (3). These fatty acids are degraded through beta-oxidation within liver mitochondria, resulting in the production of acetyl-CoA. Acetyl-CoA is either incorporated into the tricarboxylic acid cycle or channelled into the ketogenesis pathway (4). The transport of newly synthesized KBs from the hepatocytes to the circulation and the subsequent uptake in extrahepatic tissues occurs via diffusion and the monocarboxylate transporters 1, 2 and 4 (5;6). Ketogenesis is strongly suppressed by insulin and is stimulated in states of insulin deficiency and glucagon excess (4;6).

In addition to their physiological role in fasting, KBs have been associated with insulin resistance: the dose-response relationship between circulating insulin and total KB (TKB) concentration was demonstrated to be shifted to the right in type 2 diabetes mellitus (DM) patients. This was interpreted as evidence of insulin resistance with respect to ketogenesis (7). However, it has been shown that obesity is associated with lower plasma D-3HB levels and a lower D-3HB oxidation compared with lean individuals (8-10). These observations question the relevance of the reported association of KBs with insulin resistance (7).

In this study we investigated the relation between total KB turnover and glucose metabolism in 12 lean healthy and 12 obese non-diabetic men after short-term fasting (38 h), using stable isotopes and the pancreatic clamp technique. We hypothesized total KB turnover rates (TKB Ra) not to be different between lean and obese subjects under equal plasma insulin levels, indicating that insulin resistance has no effect on ketogenesis.

Subjects and methods

Subjects

Twelve healthy lean and 12 healthy obese male subjects were recruited via advertisements in local magazines. Criteria for inclusion were 1) absence of a family history of diabetes

in the lean group but not in the obese group; 2) age 18–60 yr; 3) BMI 20-25 kg/m^2 in the lean group, BMI > 30 kg/m^2 in the obese group; 4) normal oral glucose tolerance test (OGTT) according to American Diabetes Association-criteria (11) in the lean group whereas in the obese group glucose intolerance (but not diabetes) was accepted; 5) no excessive sport activities, i.e. < 3 times per week; and 6) no medication. Subjects were in self reported good health, confirmed by medical history, routine laboratory and physical examination. Written informed consent was obtained from all subjects after explanation of purposes, nature, and potential risks of the study. The study was approved by the Medical Ethical Committee of the Academic Medical Center of the University of Amsterdam.

Experimental protocol

For three days before the fasting period, all volunteers consumed a weight-maintaining diet containing at least 250 g of carbohydrates per day. Then, the subjects started fasting at 2000 h two days before the study day until the end of the study.

Volunteers were admitted to the metabolic unit of the Academic Medical Center of the University of Amsterdam at 0730 h. Subjects were studied in the supine position and were allowed to drink water only. A catheter was inserted into an antecubital vein for infusion of stable isotope tracers, insulin, somatostatin, glucagon and glucose. Another catheter was inserted retrogradely into a contralateral hand vein and kept in a thermo-regulated (60ºC) plexiglas box for sampling of arterialized venous blood.

In all studies, saline was infused as NaCl 0.9% at a rate of 50 mL/h to keep the catheters patent. [6,6-$^2H_2$]glucose (>99% enriched; Cambridge Isotopes, Andover, USA) was used to study glucose kinetics. D[2,4-$^{13}C_2$]-3HB (>99% enriched; Cambridge Isotopes, Andover, USA) was used to study KB turnover.

At T = 0 h (0800 h), blood samples were drawn for determination of background enrichments and a primed continuous infusion the isotopes was started: [6,6-$^2H_2$]glucose (prime, 8.8 µmol/kg; continuous, 0.11 µmol/kg·min) and D[2,4-$^{13}C_2$]-3HB (prime, 1.0 µmol/kg; continuous, 0.10 µmol/kg·min) and continued until the end of the study. After an equilibration period of two hours (38 h of fasting), 3 blood samples were drawn for glucose and KB enrichments and concentrations and 1 for glucoregulatory hormones and NEFA. Thereafter (T = 2.5 h) a two-step pancreatic clamp was started with low dose insulin infusions to suppress ketogenesis: step 1 included an infusion of insulin at a rate of 4.5mU/m^2·min (Actrapid 100 IU/ml; Novo Nordisk Farma B.V., Alphen aan den Rijn, the Netherlands). Glucose 5% was started when necessary to maintain a plasma glucose level of 5 mmol/L. [6,6-$^2H_2$]glucose was added to the 5% glucose solution to achieve

glucose enrichments of 1% to approximate the values for enrichment reached in plasma and thereby minimizing changes in isotopic enrichment due to changes in the infusion rate of exogenous glucose. At the same time (T=2.5 h) infusion of somatostatin (250 µg/h; Somatostatine-ucb; UCB Pharma, Breda, the Netherlands) was started to suppress endogenous insulin and glucagon secretion, as well as a glucagon infusion (1 ng/kg·min); Glucagen; Novo Nordisk, Alphen aan den Rijn, the Netherlands) to replace endogenous glucagon concentrations. Somatostatin and glucagon infusions ran throughout step 1 and 2 of the clamp.

Plasma glucose levels were measured every 5 minutes at the bedside. After 2 h (T = 4.5 h), 5 blood samples were drawn at 5 minute intervals for determination of glucose and D-3HB enrichments and concentrations. Another blood sample was drawn for determination of glucoregulatory hormones and NEFA. Hereafter insulin infusion was increased to a rate of 7.5mU/m^2·min (step 2). Plasma glucose levels were continued to be measured every 5 minutes and the final 5 blood samples were drawn at 5 minute intervals for determination of glucose and D-3HB enrichments and concentrations (T = 6.5 h).

Body composition and indirect calorimetry

Body composition was measured with bioelectrical impedance analysis (Maltron BF906, Rayleigh, UK). Respiratory energy expenditure was measured continuously during the final 20 min of both the basal state and step 2 of the clamp by indirect calorimetry using a ventilated hood system (Sensormedics model 2900; Sensormedics, Anaheim, USA).

Glucose, KB and NEFA measurements

Plasma glucose concentrations were measured with the glucose oxidase method using a Beckman Glucose Analyzer 2 (Beckman, Palo Alto, USA, intra-assay variation 2-3%). KB samples were drawn in chilled sodium-fluoride tubes and directly deproteinized with ice cold perchloric acid 6% (1:1). AcAc and D-3HB were measured spectrophotometrically using D-3-hydroxy-butyrate dehydrogenase (COBAS-FARA centrifugal analyzer, Roche Diagnostics, Almere, the Netherlands). Plasma NEFA concentrations were determined with an enzymatic colorimetric method (NEFA-C test kit, Wako Chemicals GmbH, Neuss, Germany): intra-assay variation 1%; inter-assay variation: 4-15%; detection limit: 0.02 mmol/L.

[6,6-$^2H_2$]glucose enrichment was measured as described earlier (12). After centrifugation of the acidified KB samples, the supernatants were neutralized with KOH and $HClO_4$. Following centrifugation the supernatants were acidified with HCl and

extracted twice with ethyl acetate. The combined extracts were dried under nitrogen at room temperature. D-3HB and AcAc were converted to their t-butyldimethylsilyl derivatives with MTBSTFA and pyridine. The sample was injected into an Agilent 6890/5973 MSD gaschromatograph/mass spectrometer system (Agilent Technologies, Palo Alto, CA). Separation was achieved on a Varian (Middelburg, The Netherlands) CP-SIL 19CB column (30m x 0.25mm x 0.15µm). Selected ion monitoring (EI), data acquisition and quantitative calculations were performed using the Agilent Chemstation software. Ions were monitored at m/z 275 for unlabeled D-3HB, m/z 277 for D[2,4-$^{13}C_2$]-3HB, m/z 273 for unlabeled AcAc and m/z 275 for [2,4-$^{13}C_2$]-AcAc. The enrichments in D-3HB and AcAc were determined from standard curves of known enrichments injected in the same run.

Glucoregulatory hormones

Insulin was measured with a chemiluminescent immunometric assay (Immulite 2000 system, Diagnostic Products Corporation, Los Angeles, USA), intra-assay variation: 3-6%, inter-assay variation: 4-6%, detection limit: 15 pmol/L. Basal state plasma insulin levels were measured with an ultra sensitive human insulin RIA kit (Linco HI-11K, St. Charles, Missouri, USA; Detection limit 1.5 pmol/L) in order to measure plasma insulin levels below the detection limit of the chemiluminescent immunometric assay. Glucagon was determined with the Linco ^{125}I radioimmunoassay (St. Charles, USA). Intra-assay variation at 71 ng/L 10%, at 147 ng/L 9%; inter-assay variation at 84 ng/L 5%, at 192 ng/L 7%; detection limit 15 ng/L. Cortisol, epinephrine and norepinephrine were determined as described previously (12).

Calculations and statistics

Resting energy expenditure (REE) and glucose and fat oxidation rates were calculated from O_2 consumption and CO_2 production as reported previously (13). Endogenous glucose production (EGP) and peripheral glucose uptake (rate of disappearance/Rd) were calculated using the modified forms of the Steele Equations as described previously (14;15). EGP and Rd were expressed as µmol/kg·min. TKB production (rate of appearance/Ra) was calculated by using formulas for steady state as previously described (16;17):

$$TKB\,Ra = \left(\frac{F}{\left(\frac{\left(\frac{TTR\,A}{TTR\,A+1} \right)[A] + \left(\frac{TTR\,B}{TTR\,B+1} \right)[B]}{[A]+[B]} \right)} \right) - F$$

Where F is the infusion rate (μmol/kg·min), [A] and [B] are the plasma concentrations of AcAc and D-3HB respectively and TTR A and TTR B are the tracer tracee ratios of AcAc and D-3BH respectively. TKB Ra was expressed as μmol/kg·min and as μmol/kcal as proposed earlier for lipolysis (18). The KB ratio was calculated as [D-3HB]/[AcAc] (6). TKB metabolic clearance rate (MCR) was calculated as TKB Ra/[TKB].

All data were analyzed with non parametric tests. Comparisons between groups (T = 2, 4.5 and 6.5 h) were performed using the Mann-Whitney U test. Comparisons within groups (T = 2, 4.5 and 6.5 h) were performed with the Wilcoxon Signed Rank test. Correlations were expressed as Spearman's rank correlation coefficient (ρ). The SPSS statistical software program version 14.0 (SPSS Inc, Chicago, IL) was used for statistical analysis. Data are presented as median [minimum-maximum].

Results

Anthropometric characteristics

Anthropometric data are presented in Table 1. Lean men were younger compared to obese men. As expected from the inclusion criteria there were significant differences in weight, BMI, body fat mass and lean body mass (LBM). Fasting glucose levels were not different between both groups, but glucose concentrations after the OGTT were significantly higher in the obese subjects. Data on routine laboratory examinations prior to inclusion are presented in Table 1.

Table 1 Subject characteristics

	Non-obese (n=12)	Obese (n=12)	P
Age (y)	24 [20-53]	44 [30-54]	<0.01
Length (m)	1.80 [1.71-1.89]	1.80 [1.72-1.92]	0.84
Weight (kg)	73.8 [59.0-83.5]	110.8 [95.0-124.5]	<0.01
BMI (kg/cm2)	22.4 [18.9-24.7]	34.2 [31.0-38.4]	<0.01
Body fat mass (%)	14.6 [7.5-22.9]	35.0 [30.3-42.5]	<0.01
Lean body mass (%)	85.4 [77.1-92.5]	65.0 [57.5-69.7]	<0.01
Fasting plasma glucose(mmol/liter)	4.6 [4.3-5.4][a]	5.1 [4.5-6.4]	0.12
OGTT plasma glucose(mmol/liter)	4.4 [2.8-5.1][a]	5.6 [2.8-9.6]	0.045
Plasma HDL-cholesterol (mmol/liter)	1.16 [0.95-2.09]	1.12 [0.77-1.48]	0.069
Plasma triacylglycerols (mmol/liter)	0.82 [0.20-2.65]	1.20 [0.93-3.31]	0.049
ALT (U/liter)	20 [8-46]	34 [17-100]	0.049

Data are expressed as median [min - max], *a*N = 11. ALT, alanine aminotransferase; OGGT, oral glucose tolerance test.

Table 2 Glucose, ketone body and lipid metabolism measurements.

	basal state		
	lean men (n = 12)	**obese men (n = 12)**	**P**
Glucose (mmol/liter)	4.1 [3.5 - 4.5]	5.2 [4.2 - 5.5]	<0.01
Rd (µmol/kg • min)	-	-	-
Rd (µmol/kg LBM • min)	-	-	-
D-3HB (mmol/liter)	1.24 [0.30 - 2.03]	0.25 [0.09 - 1.40]	<0.01
AcAc (mmol/liter)	0.28 [0.08 - 0.69]	0.16 [0.04 - 0.53]	0.069
TKBc (mmol/liter)	1.54 [0.38 - 2.57]	0.41 [0.16 - 1.93]	<0.01
TKB Ra (µmol/kcal)	1.3 [0.8 - 2.5]	0.6 [0.3 - 1.2]	<0.01
MCR TKB (ml/kg·min)	10.9 [5.5 - 26.6]	11.8 [5.1 - 216.7]	0.91
NEFA (mmol/liter)	1.04 [0.72 - 1.21]	0.75 [0.48 - 1.29]	<0.01

Rd rate of disappearance; MCR metabolic clearance rate; TKBc total ketone body concentration. Data are presented as median [minimum - maximum].

Resting energy expenditure, glucose, KB kinetics and NEFA

Total REE was higher in obese subjects compared to lean subjects in the basal state: 2086 [1623 - 2200] kcal/day vs. 1797 [1428 - 2067] kcal/day respectively, $P < 0.01$; as well as at the end of step 2 of the clamp : 1952 [1226 - 2107] vs. 1682 [1293 - 1958] respectively, $P < 0.01$.

Plasma glucose levels were significantly higher in the obese subjects compared to the lean subjects in the basal state and step 1 of the clamp (Table 2). During step 2, plasma glucose levels were not different. EGP was significantly lower in obese subjects in the basal state after 38 h of fasting (Figure 1). No change in EGP was observed between step 1 and the basal state in obese subjects ($P = 0.24$), but a significant decrease was observed for step 2 compared to step 1 ($P = 0.015$). EGP decreased in the lean subjects during the clamp; step 1 vs. basal state and step 2 vs. step 1: $P < 0.01$ and $P < 0.01$ respectively. Rd of glucose was lower during all time points in obese men compared to lean controls (Table 2).

Data on KB are presented in Table 2 and Figure 1. Plasma D-3HB was lower in obese men in the basal state, but no differences were found during the clamp; plasma AcAc tended to be lower in obese subjects in the basal state but no differences were observed between groups during step 1 of the clamp. During step 2, plasma AcAc tended to be higher in obese subjects. TKB Ra (in either µmol/kg·min or µmol/kcal) was significantly lower in obese subjects in the basal state but during the clamp no differences were observed (Figure 1). TKB Ra decreased within lean subjects during the clamp; step 1 vs.

clamp (step 1)			clamp (step 2)		
lean men (n = 12)	obese men (n = 12)	P	lean men (n = 12)	obese men (n = 12)	P
4.8 [4.2 - 5.5]	6.3 [4.7 - 8.2]	<0.01	5.0 [4.5 - 5.2]	5.5 [4.5 - 7.3]	0.119
12.8 [10.3 - 22.7]	8.0 [4.6 - 11.7]	<0.01	12.4 [10.3 - 18.6]	7.1 [3.6 - 10.2]	<0.01
16.1 [12.3 - 25.6]	12.5 [7.0 - 17.3]	0.017	14.7 [11.9 - 22.7]	10.8 [5.2 - 15.0]	<0.01
0.35 [0.02 - 1.00]	0.30 [0.14 - 0.90]	0.93	0.05 [0 - 0.40]	0.09 [0.03 - 0.60]	0.11
0.11 [0.01 - 0.43]	0.15 [0.04 - 0.40]	0.62	0.04 [0 - 0.17]	0.08 [0.01 - 0.34]	0.073
0.45 [0.03 - 1.43]	0.43 [0.18 - 1.30]	0.84	0.10 [0 - 0.54]	0.20 [0.04 - 0.94]	0.088
-	-	-	0.3 [0 - 0.7]	0.3 [0.1 - 1.6]	0.33
13.2 [6.9 - 55.1]	11.5 [6.8 - 15.0]	0.33	24.3 [0 - 329.3]	13.6 [8.3 - 25.3]	0.039
0.39 [0.18 - 0.49]	0.52 [0.39 - 0.79]	<0.01	0.22 [0.06 - 0.48]	0.31 [0.23 - 0.63]	0.012

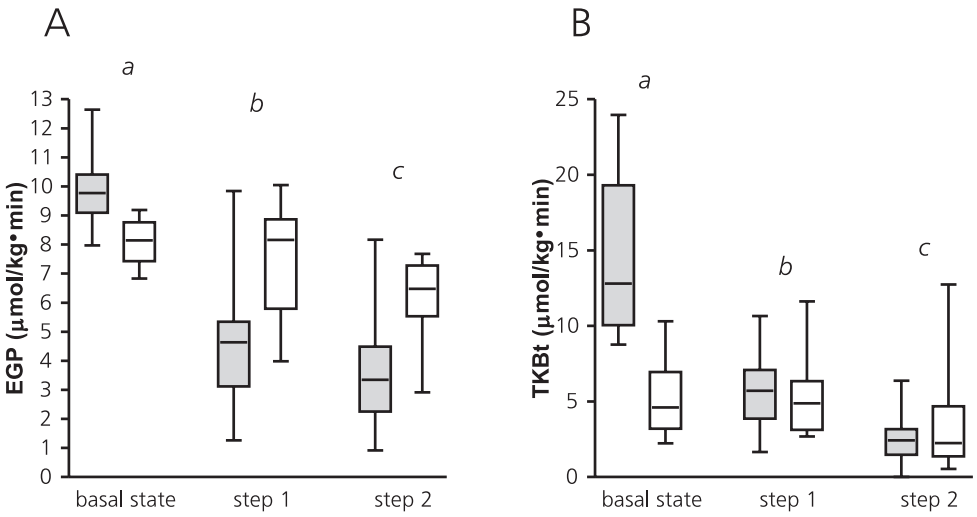

FIGURE 1 Panel A: Differences between obese (open box plots) and lean (grey box plots) subjects in endogenous glucose production (EGP, µmol/kg•min) during the basal state and during step 1 and step 2 of the clamp; [a]p < 0.01, [b]p <0.01 and [c]p = 0.01 respectively.
Panel B: Differences between obese (open box plots) and lean (grey box plots) subjects in total ketone body turnover (TKB Ra, µmol/kg•min) during the basal state and during step 1 and step 2 of the clamp; [a]p < 0.01, [b]p = 0.64 and [c]p = 0.77 respectively.

basal state and step 2 vs. step 1: $P < 0.01$ and $P < 0.01$ respectively. No change in TKB Ra was observed between step 1 and the basal state in obese subjects ($P = 0.48$), but a significant decrease was observed for step 2 compared to step 1 ($P < 0.01$). When TKB Ra was expressed as µmol/kg LBM ·min comparable results were obtained (data not shown).

Table 3 Glucoregulatory hormones

	basal state		
	lean men (n = 12)	**obese men (n = 12)**	**P**
Insulin (pmol/liter)	8 [8-25]	65 [8-102]	<0.01
Glucagon (ng/liter)	75 [66-123]	86 [66-107][a]	0.26
Cortisol (nmol/liter)	277 [138 - 422]	339 [98 - 726]	0.51
Epinephrine (nmol/liter)	0.28 [0.06 - 0.45]	0.11 [0.05 - 0.58]	0.056
Norepinephrine (nmol/liter)	0.70 [0.19 - 1.15]	1.11 [0.41 - 1.41]	<0.01

Data are presented as median [minimum - maximum]. [a]N = 11.

The MCR_{TKB} showed no differences in the basal state and step 1 of the clamp (Table 2). During step 2, obese subjects had lower MCR_{TKB} compared to lean subjects. The KB ratio in the basal state was lower in obese subjects compared to lean subjects: 2.1 [1.3 – 3.8] vs. 3.8 [1.9 - 7.0] respectively, $P < 0.01$. The KB ratio's during the clamp were not different between obese and lean subjects (step 1: 2.1 [1.1 – 3.8] vs. 2.2 [1.0 - 4.3] respectively, $P = 0.54$; step 2: 1.7 [0.6 – 4.0] vs. 1.5 [0 - 3.5] respectively, $P = 0.31$).

Obese men had lower plasma NEFA levels in the basal state, but higher plasma NEFA levels during the clamp compared to lean men (Table 2). In the basal state, plasma NEFA correlated with TKB Ra in lean men; a trend towards a correlation was found in obese men (Figure 2).

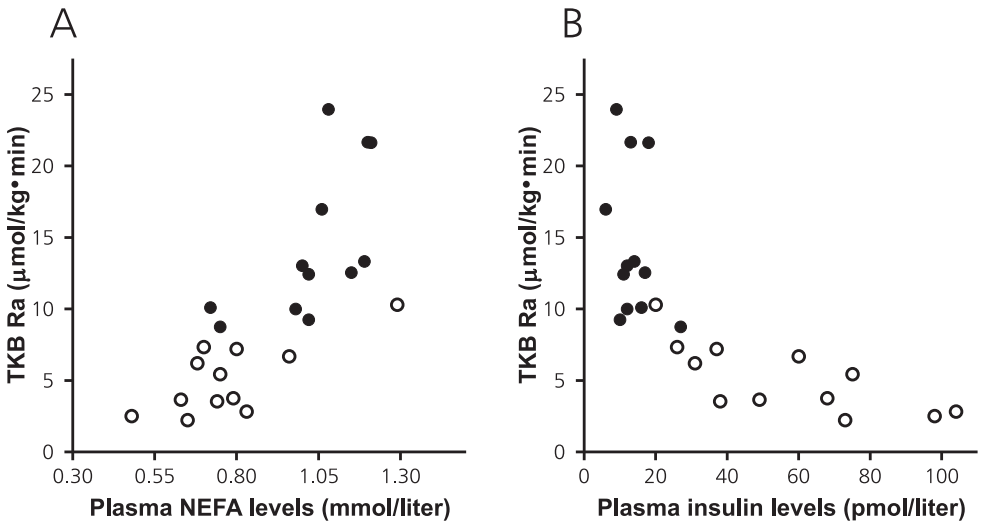

FIGURE 2 Panel A: Correlations of plasma NEFA levels with TKB Ra in the basal state for obese subjects (○):ρ = 0.55, and p = 0,063; and lean subjects (●):ρ = 0.76, and p < 0.01.
Panel B: Correlations of plasma insulin levels with TKB Ra in the basal state for obese subjects (○):ρ = -0.78, and p < 0.01; and lean subjects (●):ρ = -0.24, and p = 0.46.

	clamp (step 1)			clamp (step 2)		
	lean men (n = 12)	obese men (n = 12)	P	lean men (n = 12)	obese men (n = 12)	P
	33 [24-40]	40 [15-50]	0.06	49 [26-69]	58 [41-79]	0.31
	73 [58-84]	91 [60-125]	<0.01	67 [48-88]	90 [53-122][a]	<0.01
	206 [89 - 505]	191 [65 - 773]	1.0	182 [125 - 494]	286 [151 - 629]	0.033
	0.095 [0.05 - 0.31]	0.05 [0.05 - 0.92]	0.16	0.105 [0.05 - 0.37]	0.08 [0.05 - 1.15]	0.63
	0.56 [0.1 - 1.07]	1.04 [0.64 - 1.38]	<0.01	0.46 [0.06 - 1.48]	0.88 [0.31 - 1.35]	0.06

Glucoregulatory hormones

Insulin levels were higher in obese subjects compared to lean subjects in the basal state (Table 3). During step 1, a trend towards higher insulin levels in obese compared to lean subjects was found, however there was no difference during step 2 (Table 3). In the basal state there was a significant negative correlation between plasma insulin levels and TKB Ra in obese but not in lean men (Figure 2).

Plasma glucagon levels were not different in the basal state, but were significantly higher in obese subjects during the clamp. Cortisol levels were significantly higher in obese subjects during step 2 of the clamp but not at other time points (Table 3). The epinephrine concentration tended to be lower in obese men in the basal state; no differences were found during step 1 and 2 of the clamp. Norepinephrine levels were higher in obese subjects in the basal state and step 1 of the clamp and tended to be higher during step 2 of the clamp.

Discussion

In patients with type 2 DM, a right shifted dose response curve for insulin versus ketogenesis has been reported, suggesting insulin resistance on ketogenesis (7). The present study was designed to investigate ketone body metabolism in obese insulin resistant subjects after 38 h of fasting in relation to glucose metabolism. We found that, after short-term fasting, rates of ketogenesis are higher in lean subjects but this difference is no longer present after reaching similar plasma insulin levels. This implies that there is no insulin resistance on ketogenesis in obese subjects during short-term fasting

We confirmed earlier data on lower EGP in obese subjects in the basal state after 38 h of fasting due to higher plasma insulin levels (9). We did not express EGP as µmol/kg

LBM ·min since glucose utilization during short-term fasting by skeletal muscle is typically low in contrast to glucose utilization by the central nervous system (19).

Plasma D-3HB and TKB levels as well as TKB Ra were higher in lean subjects compared to obese subjects in the basal state as shown earlier (8-10). There are multiple ways to explain this difference. First, higher insulin levels can inhibit the activity of 3-hydroxy-3-methylglutaryl-CoA synthase (mHS) in the liver (4;6;20). Obesity-induced insulin resistance is characterized by higher plasma NEFA levels that induce hepatic and peripheral insulin resistance and subsequent hyperinsulinemia (21). Indeed plasma insulin levels correlated negatively with TKB Ra in the obese subjects in the basal state, whereas the lack of this correlation in lean subjects may be explained by the suppressed plasma insulin levels after 38 h of fasting. Secondly, it has been demonstrated that plasma glucose levels inhibit ketogenesis independently from insulin (22). It is unlikely, however, that the higher plasma glucose levels in the obese volunteers induced lower plasma KB levels, since the suppressive effect of glucose on KBs was observed during profound hyperglycemia (i.e. 12 mmol/L) (22). Finally, and more importantly, the lower TKB Ra in obese subjects in the basal state may be explained by lower NEFA levels for these are reported to increase ketone body production independently of insulin (17). Besides decreasing hepatic ketogenesis, insulin also inhibits lipolysis by inhibiting hormone sensitive lipase (HSL) in adipose tissue (4;6). Indeed we found a convincing correlation of NEFA and TKB Ra in the basal state in lean subjects and a trend towards a correlation in obese subjects.

Initially, MCR_{TKB} did not differ between lean and obese men which emphasizes the notion that the rate of utilization of KBs is proportional to their circulating levels (4). However during step 2, MCR_{TKB} was lower in obese subjects, explaining the trend towards higher plasma TKB concentrations at that time point.

Our study does not support the data by Singh et al (7) who demonstrated a significant right shift of the dose-response relationship between circulating insulin and TKB concentrations in type 2 diabetes mellitus patients with DM2. This can be explained by differences in study design since we studied obese insulin resistant subjects with approximately four-fold higher insulin levels in the basal state, whereas the plasma insulin levels in the patients of Singh were similar to lean controls. Furthermore our volunteers had been fasting for 38 h opposed to the overnight fast in their study (7).

In this study we used a single isotope method to analyze TKB Ra (16). Earlier, the use of a single isotope model in measuring TKB Ra was debated because of the rapid interconversion of AcAc and D-3HB (25). Later, it was discussed by Beylot et al that the single isotope method provided a reasonable estimate of TKB Ra even in the post-absorptive state when interconversion between AcAc and D-3HB is relatively high (16;17).

Our volunteers had plasma KB levels well above post-absorptive levels indicating less interconversion of AcAc and D-3HB thereby permitting a single isotope analysis (16). Moreover during the clamp KB ratios were not different. On theoretical grounds our results in the basal state could be overestimated in the lean group by 15%. However the enormous difference in TKB Ra between the lean and obese subjects in the basal state can not be due to such overestimation.

The studied groups were not matched for age. It has been shown that aging in healthy men is associated with increased KB levels during prolonged fasting, but not in the post-absorptive state (26). Therefore, our findings are not likely to be attributed to age differences in our groups.

Plasma glucagon levels were not different in the basal state, but this does not preclude glucagon from being a regulator of ketosis in lean subjects. Although the exact role of glucagon in ketogenesis is unclear to date (17;27), Miles et al showed that glucagon increases KB production when NEFA concentrations are increased in the setting of isolated insulin deficiency (27). This may explain why the higher plasma glucagon levels during the clamp did not result in higher TKB Ra in obese men compared with lean men. Furthermore we witnessed a trend towards higher plasma epinephrine levels in lean subjects in the basal state and higher plasma norepinephrine levels throughout the day. Epinephrine increases ketogenesis via lipolysis (4;28), but norepinephrine may induce ketogenesis directly (29). TKB Ra was not significantly different during the clamps, suggesting that norepinephrine in the presence of higher insulin levels is not a major drive of ketogenesis in this setting.

In conclusion, we show in this study that ketogenesis rates are equally sensitive for insulin in obese and lean subjects. Meanwhile obese subjects display a lower peripheral insulin sensitivity of insulin mediated glucose uptake supporting differential insulin hepatic sensitivity of KB metabolism and glucose metabolism in obese non diabetic men.

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