LACK OF EFFECT OF A PHYSIOLOGICAL ELEVATION OF PLASMA NON-ESTERIFIED FATTY ACID LEVELS ON INSULIN SECRETION

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SUMMARY - Elevated plasma non-esterified fatty acid (NEFA) levels in obese subjects may contribute to their higher insulin secretory rates by direct effects on the islet β-cells. This may involve short-term metabolic effects, or long-term effects on islet β-cell mass, which is characteristically increased in obesity. We examined the effects of elevating plasma NEFA levels for 5.5 to 7 hours after an overnight fast and during a 90 min 12 mmol/l hyperglycemic clamp in normal women (40.1 ± 9.5 years [mean ± SD]; BMI: 25.2 ± 1.72 kg/m²). Subjects were studied twice. In one study plasma NEFA levels were increased approximately 2-fold by infusion of 20% Intralipid (60 ml/h) and heparin (900 U/h) for 5.5 hours and throughout the glucose clamp. Elevated NEFA levels were associated with a small increase in fasting plasma glucose (5.0 ± 0.1 vs 4.7 ± 0.1 mmol/l, P < 0.05) and C-peptide levels (0.54 ± 0.09 vs 0.41 ± 0.06 mmol/l, P < 0.05). The increase in fasting insulin levels did not, however, reach statistical significance (9.0 ± 2.5 vs 5.3 ± 1.4 μU/l, NS).

During the hyperglycemic clamp, plasma NEFA levels were suppressed to very low levels in the saline control study. Although plasma NEFA levels also fell in the lipid/heparin study, they remained significantly higher than on the control day, and somewhat higher than might be expected postprandially in obese subjects. During the glucose clamp, plasma glucose, insulin, and C-peptide profiles were similar on the two study days. No difference in either first or second phase insulin secretion was observed between the two studies. In conclusion, our findings do not support the hypothesis that the exaggerated insulin secretion in obesity is mediated by islet β-cell metabolism independent of plasma glucose levels.

Key-words: hyperglycemic clamp, insulin, C-peptide, non-esterified fatty acids, insulin secretion.

RÉSUMÉ - Absence d'effet de l'élévation des acides gras non estérifiés à niveau physiologique sur la sécrétion d'insuline.

Le niveau plasmatique élevé des acides gras non-estérifiés (AGNE) chez les sujets obèses peut contribuer à l'augmentation de la sécrétion d'insuline, par des effets directs sur les cellules bêta-pancratiques. Ceci peut impliquer des effets métaboliques à court terme ou à long terme sur la masse des cellules bêta, qui, dans l'obésité est accrue de façon significative. Nous avons examiné les effets produits par l'élévation d'AGNE plasmatiques sur la sécrétion d'insuline pendant 5-7 heures après un jeûne nocturne et au cours d'un clamp hyperglycémique de 12 mmol/l durant 90 minutes chez 9 femmes témoins (40,1 ± 9,5 années ; BMI : 25,2 ± 1,72 kg/m²). Les sujets ont été étudiés deux fois. Dans une première étude, le niveau plasmatique d'AGNE a augmenté environ de deux fois par une infusion d'intralipide 20 % à 60 ml/h et d'héparine à 900 U/h, durant 5,5 heures, avant et pendant le clamp glucidique. Les niveaux élevés d'AGNE ont été associés avec une faible augmentation du niveau plasmatique du glucose à jeun (5,0 ± 0,1 vs 4,7 ± 0,1 mmol/l, P < 0,05) et du C-peptide (0,54 ± 0,09 vs 0,41 ± 0,06 mmol/l, P < 0,05). L'augmentation du niveau à jeun de l'insuline n'a pas été statistiquement significative (9,0 ± 2,5 vs 5,3 ± 1,4 μU/L, NS). Au cours du clamp glucidique, les niveaux plasmatiques d'AGNE ont diminué jusqu'à des valeurs très basses dans le groupe témoin recevant une solution saline. Bien que les niveaux plasmatiques d'AGNE aient aussi diminué dans l'étude lipid/Heparine, ils se sont maintenus à un niveau considérablement supérieur à celui des témoins et quelque peu supérieur à ce qui était attendu chez les sujets obèses au cours de l'état postprandial. Pendant le clamp glucidique les profils plasmatiques de glucose, de l'insuline et du peptide-C ont été similaires au cours des deux études. Aucune différence lors de la première et de la seconde phase de la sécrétion d'insuline n'a été observée. En conclusion, nos résultats ne soutiennent pas l'idée que l'exagération de la sécrétion d'insuline dans l'obésité est le résultat des effets à court terme des AGNE plasmatiques sur le métabolisme des cellules bêta pancréatiques et ce de façon indépendante du niveau plasmatique du glucose.

Mots-clés : clamp hyperglycémique, insuline, peptide-C, acides gras non estérifiés, sécrétion d'insuline.
I

creased plasma NEFA levels in obese subjects with a central distribution of body fat [1-3] may play a key role in the development of insulin resistance and hyperinsulinemia, and ultimately overt type 2 diabetes mellitus [4, 5]. Elevation of plasma NEFA impairs the ability of insulin to stimulate skeletal muscle glucose uptake and to suppress hepatic glucose output [6, 7]. In non-diabetic obese subjects, normal glucose tolerance is maintained in the face of higher NEFA levels and insulin resistance by increased insulin secretion. It has been suggested that increased NEFA levels may be important in augmenting insulin secretion rates in obesity by a direct effect on the islet β-cells, but that in the long-term, elevated NEFA may lead to β-cell dysfunction [8-11] and eventual β-cell loss [12].

Fatty acids play an important role in stimulus-secretion coupling in the β-cell [13-15]. They acutely augment insulin secretion from cultured islets or the perfused rat pancreas [15-20]. In humans, acute elevation of NEFA levels by a fat meal and heparin infusion was also found to enhance glucose-stimulated insulin secretion [21, 22]. However, findings in these early in vivo studies [21, 22] are difficult to interpret, as ingested fat stimulates secretion of insulinotropic gastrointestinal peptides [23]. More recently, elevation of the plasma NEFA concentration by an intravenous infusion of a triglyceride emulsion and heparin was also found to enhance basal and glucose-stimulated insulin secretion [24-26]. However, this effect appears to be short-lived [19, 24, 26, 27]. Prolonged exposure (12-48 h) of islets to physiological fatty acid concentrations causes a deterioration in glucose-stimulated insulin secretion [27-29]. This biphasic effect of elevated NEFA levels on insulin secretion is also observed in vivo [24, 26, 27]. Thus, in ad libitum fed rats infused for up to 48 h with a triglyceride emulsion and heparin to elevate plasma NEFA levels, basal and glucose stimulated insulin secretion were enhanced during the first 3-6 h, but after 48 h basal insulin secretion returned to normal, while glucose-stimulated insulin secretion was impaired [27]. The waning of the stimulatory effect of NEFA on insulin secretion may be due, at least in part, to a fatty acid mediated inhibition of proinsulin biosynthesis and islet insulin depletion [30-32].

Dobbins et al. [10, 33] showed that after a prolonged fast both basal and glucose-stimulated insulin secretion become dependent on the availability of plasma NEFA. Such a permissive role of circulating NEFA was also found in overnight fasted obese subjects but not in lean subjects [10]. Suppression of NEFA levels in overnight fasted hyperinsulinemic obese subjects reduced both basal and glucose-stimulated insulin secretion [10]. While these studies demonstrate a heightened dependency of insulin secretion in obesity on the availability of circulating fatty acids, they do not necessarily imply that the higher NEFA levels in obese subjects are responsible for their increased insulin secretion rates. Demonstration of enhanced insulin secretion with a physiological elevation of plasma NEFA comparable to that found in obesity beyond the 3 h or so associated with transient stimulation of insulin secretion would however lend support to a role of NEFA in the exaggerated insulin secretion rates of obese subjects.

Our aim was to test the hypothesis that a physiological elevation of plasma NEFA levels in normal subjects, comparable to that found in subjects with central obesity, would augment basal and glucose-stimulated insulin secretion. Since plasma NEFA levels are elevated during the night in obese subjects, but decrease to very low levels post-prandially [34], we increased NEFA levels for a period of time that simulated this physiologic situation, and then assessed insulin secretion.

RESEARCH DESIGN AND METHODS

Subjects

Nine healthy female subjects (40.1 ± 9.5 years [mean ± SD]; 67.7 ± 3.9 kg; BMI: 25.2 ± 3.72 kg/m²) were studied on two occasions, ~ 2-7 days apart. The study was approved by the Human Subjects Institutional Review Board of the University of California, San Diego, and written informed consent was obtained from each subject. None were on treatment known to affect glucose tolerance. All consumed a diet containing at least 200 g carbohydrate per day.

Protocol

Studies were performed in the morning after a 10-12 h overnight fast. At 0300 h, an 18-gauge cannula was inserted into an antecubital vein for infusion of substances. At 0800 h an 18-gauge cannula was inserted retrograde into a distal forearm vein for blood sampling. This hand was then kept in a hand warmer at 70 °C to arterialize the blood. After each blood sample, this cannula was flushed with 0.154 mol/l NaCl in water. On one occasion, a triglyceride emulsion (Intralipid 20%, Fresenius Kabi Clayton, Clayton, NC) containing 22.5 g/l of glycerol as emulsifier, and heparin were infused to raise plasma NEFA levels. On the other occasion 0.154 mol/l NaCl in water was infused. The Intralipid and heparin infusions were started 5.5 h prior to the start of the clamp and continued until the end of the hyperglycemic clamp. Intralipid was infused at 60 ml/h; heparin was infused at 900 U/h.

After four basal blood samples were taken for measurement of plasma glucose, insulin, C-peptide, triglyceride, and NEFA concentrations, plasma glucose was rapidly raised to 12 mmol/l and clamped at this level for 90 min. The amount of glucose required to
raise the plasma glucose to the desired level was calculated by multiplying the desired increment in plasma glucose concentration by 0.19 x body weight in kg [35]. Sixty percent of this was infused over 2 min as 50% w/v glucose via a Harvard syringe pump; the remainder was given over the subsequent 3 min. Plasma glucose was then clamped at 12.0 mmol/l by adjusting the rate of infusion of glucose (20% w/v) according to plasma glucose results obtained every 2.5-5 min. Blood samples for plasma glucose, insulin and C-peptide concentrations were also taken at 2, 3, 4, 5, 6, 8, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80 and 90 min. Blood (1.0 ml) for determination of plasma NEFA was taken at 20, 40, 60, 80 and 90 min into EDTA coated microfuge tubes, immediately centri-fuged (10 s, 14000 g) in an Eppendorf microcentri-fuge, and the plasma immediately frozen on solid CO2. Plasma samples were then stored at -70° C until assayed.

Analyses

Plasma glucose was measured by a glucose oxidase method immediately after sampling using a Yellow Springs Instruments analyzer (YSI 2700 Plus; Yellow Springs, OH). Serum insulin was measured by a double antibody technique [36]. The intra- and inter-assay coefficients of variation (CV’s) were 6.8 and 7.9% respectively. C-peptide was measured by radio-immunoassay [37] with a lower detection limit of 0.02 nmol/l and intra- and inter-assay CV’s of 6 and 10%, respectively. Serum triglyceride was determined using an acyl-CoA oxidase-based colorimetric kit (WAKO NEFA-C, Richmond, VA) with intra- and interassay CV’s of 2.3 and 3.4%, respectively. Samples obtained from subjects infused with Intralipid and heparin were assayed immediately after thawing to avoid in vitro lipolysis.

Data analysis

Data are presented as mean ± SEM. Fasting triglyceride levels were log transformed prior to statistical analysis to normalize the distribution of these values, and are presented as mean ×/÷ SEM. The areas under curves for each parameter were calculated using the trapezoidal rule. The acute first phase insulin secretory response was calculated as the area under the plasma insulin and C-peptide curves from 0-10 min after the start of the glucose infusion. Second phase insulin secretory response was calculated as the area under the plasma insulin and C-peptide curves between 30 and 90 min. The significance of differences was tested by Student’s paired t test. A P value of < 0.05 was considered statistically significant.

RESULTS

Fasting plasma glucose, lipid, insulin and C-peptide levels (Table I)

The Intralipid and heparin infusion led to an approximate doubling of fasting plasma NEFA levels and an increase in fasting plasma triglyceride concentrations by comparison with the control study (both P < 0.001). This was associated with a small but significant increase in fasting glucose levels (P < 0.05). Fasting plasma C-peptide levels were also significantly higher when plasma NEFA were elevated (P < 0.05). The increase in fasting insulin levels did not, however, reach statistical significance. This may represent a type 2 statistical error given the small number of subjects studied.

Hyperglycemic clamps

During the glucose clamp, plasma NEFA levels in the control study fell to very low levels. Although plasma NEFA concentrations also fell in the Intralipid study, they remained much higher than in the saline control study (Fig. 1). Plasma glucose concentrations attained during the 12 mmol/l glucose clamps are shown in Figure 1. The plasma glucose concentration from 10 to 90 min of the clamp was 12.0 ± 0.1 mmol/l in the saline control study, and 12.1 ± 0.1 mmol/l during the Intralipid study. The mean CV’s of plasma glucose, calculated for each subject, were 4.2 ± 0.5% and 4.8 ± 0.8%, respectively. The glucose requirement to maintain the hyperglycemic clamp from 30 to 90 min did not differ between the two studies (control, 7.86 ± 1.65; Intralipid, 7.00 ± 1.03 mg/kg/min).

Table I. Fasting plasma glucose, NEFA, triglyceride, insulin and C-peptide concentrations in nine normal women in the absence or presence of an infusion of Intralipid and heparin. The values represent the mean of four measurements taken during the 20 min period prior to the glucose clamp.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Intralipid</th>
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<tbody>
<tr>
<td>Plasma glucose (mmol/l)</td>
<td>4.7 ± 0.1</td>
<td>5.0 ± 0.1*</td>
</tr>
<tr>
<td>NEFA (μmol/l)</td>
<td>475 ± 51</td>
<td>974 ± 53**</td>
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<tr>
<td>Triglyceride (mg/dl)</td>
<td>80.4 ± 1.3</td>
<td>342.6 ± 1.3**</td>
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<tr>
<td>Plasma insulin (mU/l)</td>
<td>5.3 ± 1.4</td>
<td>9.0 ± 2.5</td>
</tr>
<tr>
<td>Plasma C-peptide (nmol/l)</td>
<td>0.41 ± 0.06</td>
<td>0.54 ± 0.09*</td>
</tr>
</tbody>
</table>

Mean ± SEM for plasma glucose, NEFA, insulin, and C-peptide levels. Mean ×/÷ SEM for plasma triglyceride levels which were log transformed prior to statistical analysis. *: P < 0.05; **: P < 0.001, compared to the control study.
Plasma insulin and C-peptide concentrations showed the expected biphasic pattern in both studies (Fig. 1). Neither first nor second phase insulin levels differed between the two studies. The area under the 0-10 min plasma C-peptide concentration curve was also similar on the two study days (control, 12.50 ± 2.13; Intralipid, 11.87 ± 2.28 nmol.l⁻¹.min). The area under the plasma C-peptide concentration curve between 30 and 90 min of the hyperglycemic clamp was also not significantly different between the two studies (control, 114.94 ± 18.57; Intralipid, 107.14 ± 13.55 nmol.l⁻¹.min).

**DISCUSSION**

Obese subjects with a central distribution of body fat are characterized by insulin resistance and hyperinsulinemia. Since fatty acids play an important role in islet β-cell physiology [13-15], and acutely augment insulin secretion [15-19, 26, 27], it has been suggested that the increased plasma NEFA levels in obesity may augment basal and glucose-stimulated insulin secretion and contribute to their high circulating insulin levels. However, in vitro data and studies in animals indicate that the stimulatory effect of NEFA on insulin secretion is short-lived [19, 27]. After ~6 h, increased NEFA levels have been shown to impair insulin secretion [26-29]. In obesity, plasma NEFA levels are higher than in lean control subjects during the night, but decline to very low levels postprandially, comparable to those found in lean subjects [34]. Thus, if the elevated plasma NEFA levels in obesity play a role in augmenting insulin secretion independent of any long-term effects on islet β-cell mass, such an effect should still be apparent after at least 5-7 h of elevated NEFA levels, since this would approximate the minimal duration of NEFA level elevation during an overnight fast. To test this hypothesis we used an infusion of Intralipid and heparin to increase plasma NEFA to levels found in many obese subjects for 7 h. Basal insulin secretion was assessed after 5 h of elevated NEFA levels, and glucose-stimulated insulin secretion was assessed during the last 90 min of the lipid/heparin infusion.

The lipid/heparin infusion increased fasting plasma NEFA levels approximately 2-fold to 974 μmol/L. During the glucose clamp, plasma NEFA levels were suppressed to very low levels in the saline control study. Although plasma NEFA levels also fell in the lipid/heparin study, they remained significantly higher than on the control study day, and somewhat higher than might be expected post-prandially in obese subjects (Fig. 1).

Basal C-peptide and insulin levels were increased during the study with Intralipid. However, a small increase in plasma glucose was also noted which could have been responsible for the enhancement of basal insulin secretion. The increase in plasma glucose
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concentrations is consistent with the finding that elevated NEFA levels in the basal state enhance hepatic glucose production and impair glucose clearance [38]. Boden et al. showed that if the compensatory increase in insulin secretion seen with elevated NEFA levels is prevented by co-infusion of somatostatin and a basal insulin infusion, there is a marked rise in fasting glucose levels [38]. Our findings concur with those of Paolisso et al. [24] who found an increase in basal insulin levels in association with a small increase in fasting plasma glucose levels after infusion of Intralipid and heparin for 6 h, but a subsequent decline in basal insulin secretion after 24 h, despite a further rise in plasma glucose levels.

During the glucose clamps, the plasma glucose, insulin, and C-peptide profiles were similar on the two study days (Fig. 1). Our findings differ from those of Chalkley et al. [25] who found that acute elevation of plasma NEFA in normal subjects, and subjects at high risk of developing type 2 diabetes mellitus, increased second phase insulin secretion during a hyperglycemic glucose clamp, but did not influence basal or first phase insulin secretion. In their study NEFA levels were elevated for less than 2 h. Given the biphasic effect of increased NEFA availability, this might be expected to favor an acute stimulatory effect [19, 24, 27]. Another possible reason for the difference in their findings is the supraphysiologic NEFA levels achieved in their study. NEFA elevation on insulin secretion, or led to some other interaction accounting for the difference in experimental results. Another factor may have been the 48 h fasted state of the subjects [10].

In summary, a 7 h elevation of plasma NEFA to levels observed in centrally obese subjects did not augment glucose-stimulated insulin secretion. A small increase in basal insulin secretion was observed, but this may have been secondary to an increase in fasting glucose levels. While obese subjects may show increased dependency of their insulin secretion on circulating fatty acids [10], our findings do not support the idea that their exaggerated insulin secretion and hyperinsulinemia are mediated by direct effects of plasma NEFA levels on islet β-cell metabolism. However, since islet β-cell mass is increased in obesity [45], chronically elevated plasma NEFA could contribute to the hyperinsulinemia of obesity by inducing β-cell hyperplasia [8].

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