Available online at www.sciencedirect.com
ScienceDirect
Metabolism Clinical and Experimental 55 (2006) 1658–1663
Metabolism
Clinical and Experimental
www.elsevier.com/locate/metabol

Adipose tissue, hepatic, and skeletal muscle insulin sensitivity in extremely obese subjects with acanthosis nigricans

Dominic N. Reeds, Charles A. Stuart, Obed Perez, Samuel Klein*

Center for Human Nutrition, Washington University School of Medicine, St. Louis, MO 63110, USA
Department of Internal Medicine, University of Texas Medical Branch, Galveston, TX 77550, USA

Received 17 October 2005; accepted 29 August 2006

Abstract

We evaluated insulin action in skeletal muscle (glucose disposal), liver (glucose production), and adipose tissue (lipolysis) in 5 extremely obese women with acanthosis nigricans (AN), who had normal oral glucose tolerance, and 5 healthy lean subjects, by using a 5-stage pancreatic clamp and stable isotopically labeled tracer infusion. Basal plasma insulin concentration was much greater in obese subjects with AN than lean subjects (54.8 ± 4.5 vs 8.0 ± 1.3 μU/mL, $P < .001$), but basal glucose and free fatty acid concentrations were similar in both groups. During stage 1 of the clamp, glucose rate of appearance (R_a) (2.6 ± 0.3 vs 3.7 ± 0.3 μmol · kg FFM^{-1} · min^{-1}, $P = .02$) and palmitate R_a (2.4 ± 0.6 vs 7.0 ± 1.5 μmol · kg FFM^{-1} · min^{-1}, $P < .05$) were greater in obese subjects with AN than lean subjects despite slightly greater plasma insulin concentration in subjects with AN (3.0 ± 0.7 vs 1.1 ± 0.4 μU/mL, $P < .05$). The area under the curve for palmitate R_a (1867 ± 501 vs 663 ± 75 μmol · kg FFM^{-1} · 600 min^{-1}, $P = .03$) and glucose R_a (1920 ± 374 vs 1032 ± 88 μmol · kg FFM^{-1} · 600 min^{-1}, $P = .02$) during the entire clamp procedure was greater in subjects with AN than lean subjects. During intermediate insulin conditions (plasma insulin, ~35 μU/mL), palmitate R_a was 5-fold greater in subjects with AN than in lean subjects (2.6 ± 1.1 vs 0.5 ± 0.2 μmol · kg FFM^{-1} · min^{-1}, $P = .05$). Maximal glucose disposal was markedly lower in obese subjects with AN than in lean subjects (13.0 ± 0.8 vs 23.4 ± 1.8 mg · kg FFM^{-1} · min^{-1}, $P = .01$) despite greater peak plasma insulin concentration (1842 ± 254 vs 598 ± 38 μU/mL, $P < .05$). These data demonstrate obese young adults with AN have marked insulin resistance in multiple tissues. However, marked insulin hypersecretion can compensate for impaired insulin action, resulting in normal glucose and fatty acid metabolism during basal conditions.
© 2006 Published by Elsevier Inc.

1. Introduction

Obesity is associated with an increased risk of insulin resistance and type 2 diabetes mellitus [1]. Moreover, the relative risk of diabetes increases progressively with increasing body mass index (BMI). Men and women with extreme obesity (BMI > 40 kg/m^2) have a 20-fold higher risk of having type 2 diabetes mellitus than those with a BMI of 18.5 to 24.9 kg/m^2 [2,3].

Insulin resistance is particularly pronounced in a subset of obese patients who have acanthosis nigricans (AN) [4]. Acanthosis nigricans is a dermatologic marker of hyperinsulinemia, characterized by thickened, dark skin in the axillae, nape of the neck, and other surfaces exposed to friction [5]. The darkening of the skin is related to an increase in thickness of the superficial epithelium and not to a change in melanin content [6]. Acanthosis nigricans is more common in African Americans and Native Americans than in Hispanics or whites [7,8], and is associated with a marked increase in the risk of developing diabetes [8], and other cardiovascular disease risk factors [9,10].

The purpose of the present study was to carefully characterize adipose tissue, hepatic, and skeletal muscle insulin sensitivity in vivo in extremely obese subjects with AN who had normal oral glucose tolerance. A 5-stage hyperinsulinemic, euglycemic pancreatic clamp procedure, in conjunction with stable isotopically labeled tracer infusions, were used to assess insulin-mediated suppression of lipolysis and glucose production, and stimulation of glucose disposal, in extremely obese subjects with AN and in lean subjects.

* Corresponding author. Washington University School of Medicine, Box 8031, St. Louis, MO 63110-1093, USA. Tel.: +1 314 362 8708; fax: +1 314 362 8230.
E-mail address: sklein@im.wustl.edu (S. Klein).

0026-0495/$ – see front matter © 2006 Published by Elsevier Inc.
doi:10.1016/j.metabol.2006.08.006

2. Subjects

Five extremely obese (BMI >40 kg/m^2) women with AN and 5 lean subjects (3 men, 2 women) participated in this study, which was approved by the institutional review board and the General Clinical Research Center Scientific Advisory Committee of the University of Texas Medical Branch at Galveston. Written informed consent was obtained from all subjects before their participation. Subjects with AN had 3 to 4+ (moderate to severe) acanthosis on the neck according to the Stahn scoring criteria [8]. Mean plasma testosterone was 70 ± 20 ng/dL (reference range, 10-90 ng/dL), but 2 subjects had slightly elevated levels (103 and 129 ng/dL). Plasma concentrations of dehydroepiandrosterone-sulfate and androstenedione concentrations were normal in all subjects (data not shown). Obese subjects with AN were younger than lean subjects (18 ± 2 and 31 ± 2 years, $P < .05$). Subjects were screened with a careful medical examination, including a history, physical examination, resting electrocardiogram, routine blood tests, and an oral glucose tolerance test. Subjects with impaired oral glucose tolerance, diabetes, significant organ system dysfunction, or who were taking medications known to affect glucose or lipid metabolism were excluded from participating in this study. All subjects were sedentary (<1 hour of exercise per week).

3. Materials and methods

3.1. Body composition assessment

Total body fat and fat-free masses were determined by dual-energy x-ray absorptiometry (Hologic QDR 1000/w, Waltham, MA) [11].

3.2. Pancreatic clamp procedure

Subjects were admitted to the General Clinical Research Center at the University of Texas Medical Branch the day before the clamp procedure. All female subjects were studied during the follicular phase of their menstrual cycle. Subjects were required to maintain a 3-day food diary documenting consumption of a diet containing at least 250 g/d of carbohydrate. At 6:00 PM, subjects consumed a standardized meal, which contained 50 kJ/kg (12 kcal/kg) adjusted body weight (ideal body weight + [(actual body weight − ideal body weight) × 0.25]) per day, composed of 55% carbohydrate, 15% protein, and 30% fat. At 6:00 AM the following morning, after subjects fasted overnight, one intravenous catheter was inserted into a dorsal hand or wrist vein, which was heated to 55°C by using a thermostatically controlled box to obtain arterialized blood samples [12,13], and the second intravenous catheter was inserted into an antecubital vein in the contralateral arm to infuse insulin and stable isotopically labeled tracers.

After baseline blood samples were obtained, a primed (6 μg/kg), constant (100 ng · kg^{-1} · min^{-1}) infusion of somatostatin and constant infusion (0.6 ng · kg^{-1} · min^{-1}) of glucagon was started and continued throughout the clamp. A primed (4 μmol/kg) constant (0.04 μmol · kg^{-1} · min^{-1}) infusion of [$^2H_2$]glucose and a constant infusion (0.05 μmol · kg^{-1} · min^{-1}) of [1-^{13}C] palmitate were also started. After a baseline period (0-120 minutes, stage 1), a multistage, hyperinsulinemic, euglycemic clamp procedure was started and continued for 10 hours (Fig. 1). Insulin was infused at rates of 2, 5, 15, and 240 mU · m^{-2} · min^{-1} in lean subjects and 5, 15, 40, and 480 mU · m^{-2} · min^{-1} in obese subjects with AN during each 120-minute stage of the clamp procedure. Insulin was infused at higher rates in subjects with AN because of their known insulin resistance. The infusion rates of [$^2H_2$]glucose and [^{13}C]palmitate were progressively decreased as insulin infusion rates increased to account for the expected decline in endogenous glucose production and whole-body lipolytic rate. Whole blood glucose concentration was maintained at 95 mg/dL (5.3 mmol/L) by an infusing 10% dextrose containing 26.5 mmol/L of [$^2H_2$]glucose.

Blood samples were obtained before beginning the tracer and hormone infusions to determine baseline plasma substrate and hormone concentrations and background isotopic enrichments. Blood samples were obtained every 10 minutes during the last 30 minutes of each stage of the pancreatic clamp to determine plasma substrate and hormone concentrations, and glucose and lipid kinetics. Blood samples were also obtained every 5 minutes throughout the pancreatic clamp to determine whole blood glucose concentrations.

3.3. Analyses of samples

Blood glucose concentrations were determined by using an automated glucose analyzer (Yellow Spring Instruments, Yellow Springs, OH). Plasma insulin concentrations were measured by radioimmunoassay [14].

Tracer-to-tracee ratios (TTRs) of palmitate and glucose in plasma were determined by gas chromatography-mass

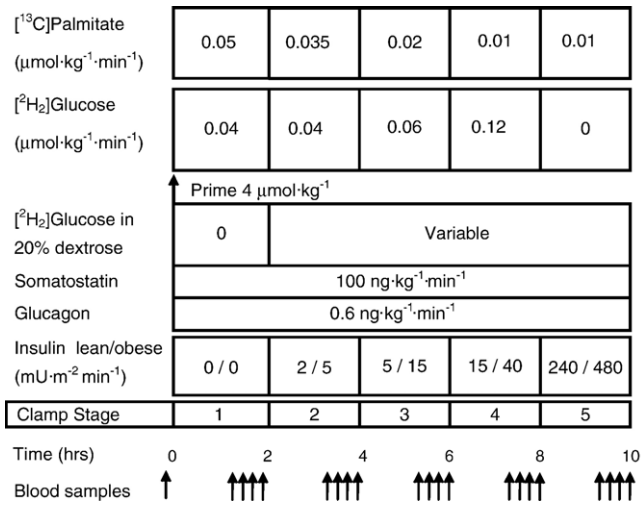

Fig. 1. Schematic diagram of isotope infusion study/hyperinsulinemic-euglycemic pancreatic clamp. Insulin infusion rate (μU · m^{-2} · min^{-1}) listed for healthy lean subjects then subjects with AN.

Table 1
Baseline characteristics of the study subjects

	Lean subjects	Obese subjects with AN	P
BMI (kg/m^2)	22.0 ± 1.1	49.2 ± 3.2	<.001
Weight (kg)	65.0 ± 3.0	135.3 ± 9.7	.001
Fat mass (kg)	12.4 ± 2.6	74.1 ± 7.0	<.001
Fat mass (% body weight)	18.6 ± 3.4	54.4 ± 1.7	<.001
Lean body mass (kg)	52.5 ± 1.5	61.2 ± 3.3	.08
Plasma insulin (μU/mL)	8.0 ± 1.3	54.8 ± 4.5	<.001
Plasma glucose (mg/dL)	82.2 ± 1.4	87.4 ± 4.6	.3
Plasma FFA (μmol/L)	407.8 ± 72.5	383.1 ± 46.4	.8

Values are expressed as means ± SE.

spectrometry by using an MSD 5971 system (Hewlett-Packard, Palo Alto, CA) with capillary column. Plasma samples were analyzed for [^{13}C]palmitate TTR as described previously [15]. Free fatty acids (FFAs) were isolated from plasma and converted to their methyl esters. Ions formed by electron impact ionization and ions at mass-to-charge ratio of 270.2 and 271.2, representing the molecular ions of unlabeled and labeled methyl esters, respectively, were selectively monitored. Instrument response was calibrated by using standards of known isotopic enrichment.

3.4. Calculations

Steele's [17] equation for steady-state conditions [16] was used to calculate substrate (palmitate and glucose) rate of appearance (R_a) in plasma and glucose rate of disappearance (R_d) from plasma during the last 30 minutes of the basal period and each stage of the hyperinsulinemic euglycemic clamp.

Palmitate and glucose R_a were calculated by dividing each tracer infusion rate by the average arterial TTR obtained during the last 30 minutes of each stage of the clamp [17,18]. Glucose R_d was calculated as the sum of endogenous glucose R_a plus infused glucose.

Because of technical issues in sample processing, palmitate R_a could not be calculated for all stages for 2 of the lean subjects; these subjects were omitted from area under the curve (AUC) calculations.

3.5. Statistical analysis

Baseline differences in body composition, and plasma substrate and hormone concentrations between the 2 groups of subjects were assessed by using a 2-tailed Student t test for independent samples. The significance of differences between palmitate R_a and glucose disposal between groups were evaluated by using a Student t test for independent samples, and by using the Mann-Whitney U test when data were not normally distributed. Palmitate and endogenous glucose R_a AUC were calculated by using the trapezoidal method, and the significance of differences between groups was evaluated by using the Mann-Whitney U test. A P value of less than .05 was considered statistically significant. All values are expressed as means ± SE.

Table 2
Plasma insulin concentration (μU/mL) during each stage of the pancreatic clamp

	Lean subjects	Obese subjects with AN	P
Stage 1	1.1 ± 0.9	3.0 ± 0.7	.04
Stage 2	3.8 ± 0.6	17.3 ± 3.4	.01
Stage 3	15.1 ± 5.3	38.0 ± 11	.08
Stage 4	31.9 ± 7.6	65.2 ± 9.9	.05
Stage 5	598.2 ± 38	1842 ± 254.7	.01

Values are expressed as mean ± SE.

4. Results

4.1. Body composition

Body weight and BMI were 2-fold greater and total fat mass was 6-fold greater in obese subjects with AN than in lean subjects (Table 1).

4.2. Substrate and insulin concentrations

Mean basal plasma insulin concentration was more than 6-fold greater in obese subjects with AN than in lean subjects (Table 1). Plasma insulin concentration decreased during stage 1 of the pancreatic clamp, when somatostatin was infused without insulin replacement, and then increased progressively during each subsequent stage of the clamp procedure (Table 2). Plasma insulin concentration was greater in subjects with AN than in lean subjects during the clamp procedure because of the higher baseline concentration and higher rate of insulin infusion.

Plasma glucose and FFA concentrations during the pancreatic clamp procedure are shown in Table 3. Glucose infusion was not needed to maintain euglycemia in 4 of the 5 subjects with AN during stages 1 to 3 of the clamp procedure because plasma glucose concentrations were already greater than the target of 95 mg/dL (5.3 mmol/L). Although mean plasma FFA concentration was similar between groups during stage 1, plasma FFA concentrations were much greater in subjects with AN than in lean subjects during all other stages.

Table 3
Plasma glucose and FFA concentrations during each stage of the pancreatic clamp procedure

Clamp stage	Plasma glucose (mg/dL)		Plasma FFA (mmol/L)	
	Lean subjects	Obese subjects with AN	Lean subjects	Obese subjects with AN
Stage 1	77 ± 7	112 ± 8*	1.05 ± 0.11	1.16 ± 0.11
Stage 2	105 ± 4	129 ± 7*	0.43 ± 0.08	0.84 ± 0.12*
Stage 3	93 ± 4	113 ± 7	0.11 ± 0.01	0.50 ± 0.11*
Stage 4	92 ± 2	94 ± 2	0.05 ± 0.01	0.20 ± 0.04*
Stage 5	96 ± 2	100 ± 7	0.04 ± 0.01	0.07 ± 0.01*

Values are expressed as mean ± SE.

* P < .05, significantly different from corresponding value for lean subject.

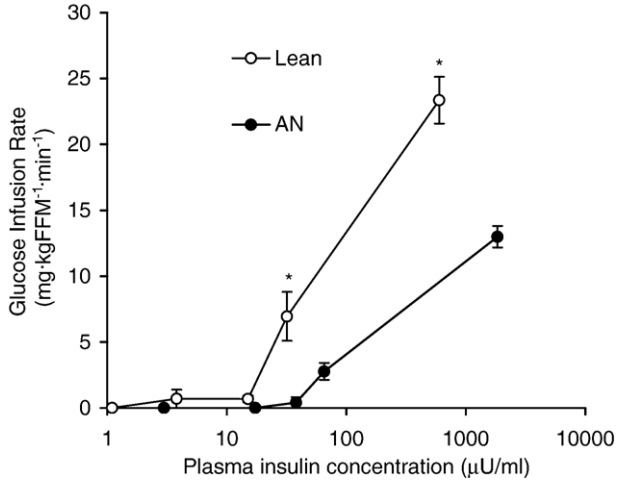

Fig. 2. Glucose infusion rate to maintain euglycemia during each stage of the isotope infusion/hyperinsulinemic-euglycemic pancreatic clamp study in patients with AN (closed circles) and healthy lean subjects (open circles). Values are expressed as means ± SE. *$P < .05$ vs patients with AN at same clamp stage.

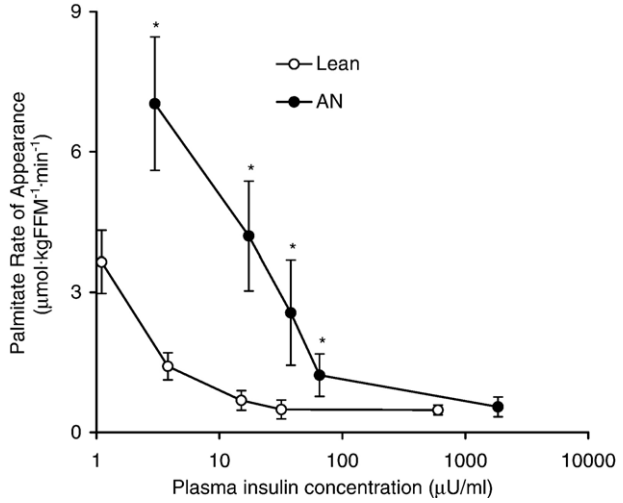

Fig. 4. Palmitate R_a during each stage of the isotope infusion/hyperinsulinemic-euglycemic pancreatic clamp study in obese subjects with AN (closed circles) and lean subjects (open circles). Values are expressed as means ± SE. *$P < .05$ vs lean subjects at same clamp stage.

4.3. Glucose kinetics

Glucose R_d (glucose disposal) increased during stages 3 to 5 of the pancreatic clamp in both groups. Maximal glucose R_d was lower in subjects with AN than in lean subjects (13.0 ± 0.8 vs 23.4 ± 1.8 mg · kg FFM^{-1} · min^{-1}, $P = .01$) despite much greater plasma insulin concentrations in the AN group (Fig. 2). One lean subject was unable to complete stages 4 and 5 of the experimental protocol and was not included in calculations for glucose disposal.

Endogenous glucose R_a (endogenous glucose production rate) was greater in subjects with AN than in lean subjects during stage 1 (3.7 ± 0.3 vs 2.6 ± 0.3 μmol · kg FFM^{-1} · min^{-1}, $P = .04$) and declined with increasing insulin concentrations in both groups (Fig. 3). Endogenous glucose R_a AUC above baseline during the entire clamp procedure was also greater in subjects with AN (1920 ± 374 μmol · kg FFM^{-1} · 600 min^{-1}) than lean subjects (1032 ± 88 μmol · kg FFM^{-1} · 600 min^{-1}, $P = .02$) despite higher insulin concentrations in the AN group.

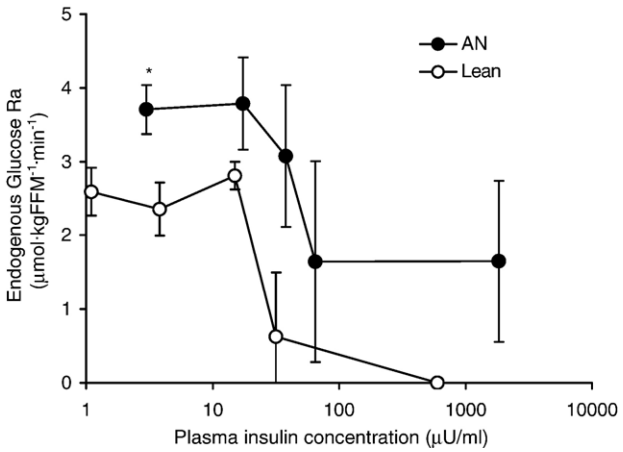

Fig. 3. Endogenous glucose R_a during each stage of the isotope infusion/hyperinsulinemic-euglycemic pancreatic clamp study in obese subjects with AN (closed circles) and lean subjects (open circles). Values are expressed as means ± SE. *$P < .05$ vs lean subjects at same clamp stage.

4.4. Palmitate kinetics

Palmitate R_a was greater in subjects with AN than in lean subjects during stages 1 through 4 of the clamp procedure (Fig. 4). However, differences between groups decreased with progressive increases in insulin concentration, and values for palmitate R_a were not significantly different between groups during stage 5 of the pancreatic clamp procedure. At intermediate plasma insulin concentrations (stage 3 in subjects with AN, plasma insulin = 38.0 ± 11.0μU/mL, and stage 4 in lean subjects, plasma insulin = 31.9 ±7.6 μU/mL; P = not significant), plasma palmitate R_a was more than 5-fold greater in subjects with AN than lean subjects (2.6 ± 1.1 vs 0.5 ± 0.2 μmol · kg FFM^{-1} · min^{-1}, $P = .05$). The AUC for palmitate R_a above baseline during the entire clamp procedure was greater in subjects with AN than in lean subjects (1867 ± 501 vs 663 ± 75, $P = .03$).

5. Discussion

Acanthosis nigricans is a marker of hyperinsulinemia and insulin resistance. In this study, we carefully evaluated insulin action in vivo in extremely obese, young adult subjects with AN. A 5-stage euglycemic-hyperinsulinemic pancreatic clamp, in conjunction with stable isotopically labeled tracer infusions, was used to evaluate insulin sensitivity and responsiveness in adipose tissue (lipolysis), liver (glucose production), and skeletal muscle (glucose disposal) across a range of insulin concentrations from below basal to supraphysiologic concentrations. Lipolytic rate during intermediate plasma insulin concentration and the

AUC above baseline for substrate kinetics during the entire clamp procedure were used to assess insulin sensitivity. Although all subjects with AN had normal oral glucose tolerance, and basal plasma glucose and FFA concentrations, they demonstrated marked insulin resistance in all tissues. These data demonstrate that insulin hypersecretion in extremely obese young adults with AN adequately compensates for impaired insulin action and results in normal glucose and fatty acid metabolism during basal conditions.

The mechanism(s) responsible for insulin resistance in patients with AN is not clear. Although increased circulating FFA has been proposed as an important mechanism for insulin resistance in obese persons [19], we found that basal plasma FFA concentrations were normal, and plasma FFA concentration was low during stages 4 and 5 of the clamp procedure when insulin sensitivity was impaired. In addition, data from other studies have found that neither the degree of obesity nor hyperandrogenism seen in patients with AN fully account for insulin-resistant glucose metabolism [13,20].

The combination of a decrease in insulin sensitivity and maximal responsiveness of glucose disposal suggests there are defects in both insulin-binding and postreceptor action in patients with AN. In fact, defects in insulin binding [21] and post–insulin receptor function [22], and genetic defects within the insulin receptor gene have been documented in patients with AN [23,24]. These abnormalities are consistent with data from several studies showing both impaired insulin-receptor autophosphorylation [25-27] and reduced numbers of insulin receptors in subjects with AN [13,28]. Although obesity has been found in some, but not all, studies to be associated with down-regulation of insulin receptors [29] and diminished intracellular signaling [30], obesity is unlikely to be solely responsible for the insulin resistance seen in subjects with AN [28]. In fact, 4 of our 5 subjects with AN reported that AN appeared before the presence of extreme obesity, suggesting insulin resistance and hyperinsulinemia preceded the development of extreme obesity. All insulin receptor gene exons were sequenced in these subjects and, although silent polymorphisms were identified, no missense or truncation codons were found (unpublished data).

It is likely that marked hyperinsulinemia in our subjects with AN prevented basal hyperglycemia. However, these subjects became hyperglycemic when insulin secretion was suppressed by somatostatin infusion, although plasma insulin concentrations were 2- to 4-fold higher than basal values observed in our lean subjects. The increase in glucose concentration was likely caused by the increase in endogenous glucose production rates. In addition, it is possible that defects in non–insulin-dependent glucose uptake also contributed to the hyperglycemia observed during stages 1 to 3 of the pancreatic clamp. We have previously found that subjects with AN have abnormal expression of the insulin-independent glucose transporters, GLUT1 and GLUT3 [31], in skeletal muscle.

Marked hyperinsulinemia also maintained normal basal rates of lipolysis and plasma FFA concentrations in our subjects with AN. However, lipolytic rates and plasma FFA concentrations were more than 2- to 4-fold greater in the AN group than in the lean group when insulin secretion was suppressed by somatostatin infusion, although plasma insulin concentrations were much higher than basal values observed in our lean subjects.

Several potential limitations of our study should be considered. First, our subjects with AN were younger and contained more women than the lean control group. The duration and complexity of using the multistage pancreatic clamp technique to assess insulin sensitivity in vivo made it difficult for us to recruit lean adolescent subjects for this study, and we were only able to recruit an older healthy control group. However, the age difference between groups strengthens our conclusions of blunted insulin sensitivity in subjects with AN because insulin sensitivity tends to decline with age [32,33]. It is also unlikely that the absence of men in the group with AN affected our results because large studies suggest that sex does not affect insulin sensitivity [34] and, there is some evidence that peripheral insulin sensitivity may actually be greater in women than men [32]. Second, we did not demonstrate a plateau in glucose disposal rate. However, the plasma insulin concentration achieved during the last stage of the pancreatic clamp was sufficient to maximally stimulate glucose disposal [35].

In summary, insulin action in skeletal muscle, liver, and adipose tissue is defective in extremely obese subjects with AN. However, marked hyperinsulinemia was able to compensate for impaired insulin function and prevent basal abnormalities in glucose and lipid metabolism.

Acknowledgments

This study was supported by National Institutes of Health grants DK 37948 and DK 56341 (Clinical Nutrition Research Unit).

The authors thank the nurses of the General Clinical Research Center for their help in performing the studies and the study subjects for their participation.

References

[1] NHLBI Obesity Education Initiative, Expert Panel on the Identification, Evaluation and Treatment of Overweight and Obesity in Adults. Clinical guidelines on the identification, evaluation, and treatment of overweight and obesity in adults—the evidence report. National Institutes of Health. Obes Res 1998;6(Suppl 2):51S-209S.

[2] Field AE, Coakley EH, Must A, et al. Impact of overweight on the risk of developing common chronic diseases during a 10-year period. Arch Intern Med 2001;161:1581-6.

[3] Mokdad AH, Ford ES, Bowman BA, et al. Prevalence of obesity, diabetes, and obesity-related health risk factors, 2001. JAMA 2003;289:76-9.

[4] Stuart CA, Gilkison CR, Smith MM, et al. Acanthosis nigricans as a risk factor for non-insulin dependent diabetes mellitus. Clin Pediatr (Phila) 1998;37:73-9.

[5] Hud Jr JA, Cohen JB, Wagner JM, et al. Prevalence and significance of acanthosis nigricans in an adult obese population. Arch Dermatol 1992;128:941-4.
[6] Rook A, Wilkinson D, Ebling FJG. Acanthosis nigricans. Textbook of dermatology. Oxford, UK: Blackwell Scientific; 1979.
[7] Stuart CA, Pate CJ, Peters EJ. Prevalence of acanthosis nigricans in an unselected population. Am J Med 1989;87:269-72.
[8] Stuart CA, Smith MM, Gilkison CR, et al. Acanthosis nigricans among Native Americans: an indicator of high diabetes risk. Am J Public Health 1994;84:1839-42.
[9] Bener A. Acanthosis nigricans, hyperinsulinaemia and risk factors for cardiovascular disease; 2000.
[10] Lestringant GG, Bener A, Frossard P, et al. Association of acanthosis nigricans with risk of diabetes mellitus and hormonal disturbances in females. Int J Gynaecol Obstet 2000;71:267-9.
[11] Kohrt WM. Body composition by DXA: tried and true? Med Sci Sports Exerc 1995;27:1349-53.
[12] Jensen MD, Heiling VJ. Heated hand vein blood is satisfactory for measurements during free fatty acid kinetic studies. Metabolism 1991; 40:406-9.
[13] Stuart CA, Peters EJ, Prince MJ, et al. Insulin resistance with acanthosis nigricans: the roles of obesity and androgen excess. Metabolism 1986;35:197-205.
[14] Morgan DR, Lazarow A. Immunoassay of insulin: two antibody system. Diabetes 1963;12:115-26.
[15] Patterson BW, Zhao G, Klein S. Improved accuracy and precision of gas chromatography/mass spectrometry measurements for metabolic tracers. Metabolism 1998;47:706-12.
[16] Horowitz JF, Coppack SW, Paramore D, et al. Effect of short-term fasting on lipid kinetics in lean and obese women. Am J Physiol 1999;276:E278-84.
[17] Steele R. Influences of glucose loading and of injected insulin on hepatic glucose output. Ann N Y Acad Sci 1959;82:420-30.
[18] Wolfe RR. Radioactive and stable isotope tracers in biomedicine: principles and practice of kinetic analysis. New York: Wiley-Liss; 1992. p. 324-5.
[19] Wolfe BM, Klein S, Peters EJ, et al. Effect of elevated free fatty acids on glucose oxidation in normal humans. Metabolism 1988;37: 323-9.
[20] Schwenk WF, Rizza RA, Mandarino LJ, et al. Familial insulin resistance and acanthosis nigricans. Presence of a postbinding defect. Diabetes 1986;35:33-7.
[21] Flier JS, Eastman RC, Minaker KL, et al. Acanthosis nigricans in obese women with hyperandrogenism. Characterization of an insulin-resistant state distinct from the type A and B syndromes. Diabetes 1985;34:101-7.
[22] Leme CE, Wajchenberg BL, Lerario AC, et al. Acanthosis nigricans, hirsutism, insulin resistance and insulin receptor defect. Clin Endocrinol (Oxf) 1982;17:43-9.
[23] Rique S, Nogues C, Ibanez L, et al. Identification of three novel mutations in the insulin receptor gene in type A insulin resistant patients. Clin Genet 2000;57:67-9.
[24] Moller DE, Cohen O, Yamaguchi Y, et al. Prevalence of mutations in the insulin receptor gene in subjects with features of the type A syndrome of insulin resistance. Diabetes 1994;43: 247-55.
[25] Grunberger G, Zick Y, Gorden P. Defect in phosphorylation of insulin receptors in cells from an insulin-resistant patient with normal insulin binding. Science 1984;223:932-4.
[26] Grigorescu F, Flier JS, Kahn CR. Defect in insulin receptor phosphorylation in erythrocytes and fibroblasts associated with severe insulin resistance. J Biol Chem 1984;259:15003-6.
[27] Stuart CA, Pietrzyk RA, Peters EJ, et al. Autophosphorylation of cultured skin fibroblast insulin receptors from patients with severe insulin resistance and acanthosis nigricans. Diabetes 1989;38:328-32.
[28] Fukushima N, Matsuura N, Nohara Y, et al. A case of insulin resistance associated with acanthosis nigricans. Tohoku J Exp Med 1984;144:129-38.
[29] Arner P, Einarsson K, Backman L, et al. Studies of liver insulin receptors in non-obese and obese human subjects. J Clin Invest 1983; 72:1729-36.
[30] Nawrocki AR, Scherer PE. The delicate balance between fat and muscle: adipokines in metabolic disease and musculoskeletal inflammation. Curr Opin Pharmacol 2004;4:281-9.
[31] Stuart CA, Wen G, Williamson ME, et al. Altered GLUT1 and GLUT3 gene expression and subcellular redistribution of GLUT4: protein in muscle from patients with acanthosis nigricans and severe insulin resistance. Metabolism 2001;50:771-7.
[32] Borissova AM, Tankova T, Kirilov G, et al. Gender-dependent effect of ageing on peripheral insulin action. Int J Clin Pract 2005; 59:422-6.
[33] Petersen KF, Befroy D, Dufour S, et al. Mitochondrial dysfunction in the elderly: possible role in insulin resistance. Science 2003;300: 1140-2.
[34] Wagenknecht LE, Langefeld CD, Scherzinger AL, et al. Insulin sensitivity, insulin secretion, and abdominal fat: the Insulin Resistance Atherosclerosis Study (IRAS) Family Study. Diabetes 2003; 52:2490-6.
[35] Kolterman OG, Insel J, Saekow M, et al. Mechanisms of insulin resistance in human obesity: evidence for receptor and postreceptor defects. J Clin Invest 1980;65:1272-84.