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Effects of free fatty acids on plasma resistin and insulin resistance in awake rats☆

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Abstract

Resistin has been postulated to play a role in obesity-related insulin resistance. To explore this possibility, we have investigated effects of acute euglycemic (5.2 ± 0.1 mmol/L) hyperinsulinemia (96 ± 8 μU/mL) with and without concurrent infusion of lipid plus heparin (to raise or lower plasma free fatty acid [FFA] levels) on glucose turnover and plasma resistin levels in alert rats. Plasma FFA concentrations increased during lipid/heparin (L/H) infusion (from 0.82 to 2.86 mmol/L, $P < .001$) and decreased (from 0.83 to 0.21 mmol/L, $P < .001$) in controls who were infused with insulin but not with L/H. L/H infusion reduced insulin suppression of endogenous glucose production by ~90% (from 28.9 to 3.1 mg · kg^{-1} · min^{-1}, $P < .001$) and insulin-stimulated glucose uptake (glucose rate of disappearance) by 78% (from 30.8% to 6.9%, $P < .001$). Plasma resistin levels increased by 46% (from 39.9 to 58.4 μg/L, $P < .05$) during L/H infusion and did not change in controls (39.7 vs 39.3 μg/L). Plasma ghrelin levels decreased by 41% (from 892 to 584 ng/L, $P < .05$) in response to hyperinsulinemia, whereas concurrent L/H infusion had no additional effect on ghrelin levels (584 ± 67 vs 548 ± 82 ng/L). In summary, we found that FFA induced hepatic insulin resistance, and to a lesser extent, peripheral insulin resistance was associated with elevated plasma resistin levels. We conclude that FFA-induced release of resistin may contribute to the development of FFA-induced insulin resistance in rats.

1. Introduction

Resistin, also called FIZZ3 (found in inflammatory zone 3) or adipocyte-specific secretory factor, is a novel 12.5-kDa cysteine-rich polypeptide secreted by adipocytes [1,2]. It has been linked to energy homeostasis, diet-induced obesity, and insulin resistance [1-3]. Initial studies in mice suggested that resistin mediates insulin resistance by modulating 1 or more steps in the insulin signaling pathway [1-3]. However, conflicting results have since been reported in different animal models and in humans with respect to resistin gene expression and/or blood levels and relative to effects of obesity, hyperglycemia, hyperinsulinemia, and treatment with thiazolidinediones (drugs known to reduce insulin resistance) on plasma resistin levels [2-4]. Thus, the physiological role of resistin has remained uncertain.

Ghrelin was discovered as a protein that stimulated release of growth hormone from the anterior pituitary [5]. It was subsequently discovered that ghrelin had also significant effects on appetite and energy balance (for review, see Ref [2]). Ghrelin is a 28–amino-acid peptide (3.3 kDa) with an *n*-octanoylated serine (which is needed for biologic activity). It is secreted mainly from the stomach [5,6] but in smaller amounts also from pancreatic alpha cells [7]. Ghrelin is profoundly orexogenic and adipogenic, increasing food intake and body weight [8]. Ghrelin is currently the only food intake–stimulating signal originating from the stomach. Glucose administration or food intake has been shown to decrease plasma ghrelin concentrations [6,9,10].

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In humans, insulin infusions have been shown to decrease ghrelin concentrations [11]; however, in rats, insulin increased ghrelin mRNA [12]. In view of these discrepant findings and the postulated roles of resistin and ghrelin in insulin resistance and appetite regulation, we have assessed the effects of insulin and free fatty acid (FFA) on plasma resistin and ghrelin levels in rats during euglycemic-hyperinsulinemic clamping.

2. Materials and methods

2.1. Preparation of animals

Twenty male Sprague-Dawley rats weighing between 250 and 300 g were housed in individual cages and subjected to an environmentally controlled room with a 12-hour light/dark cycle, where they had free access to standard rat chow and water. Five to 7 days before the in vivo study, rats were anesthetized with an intraperitoneal injection of pentobarbital (50 mg/kg body weight). A silastic catheter (internal diameter 0.02 in) was inserted into the right internal jugular vein and extended to the level of the right atrium. Another catheter was advanced through the left carotid artery until its tip reached the aortic arch. The free ends of both catheters were attached to long segments of steel tubing and tunneled subcutaneously around the side of the neck to the back of the neck where they were exteriorized through a skin incision and then securely anchored to the skin by a standard wounded clip. At the end of the procedure, catheters were flushed with 300 μL isotonic saline containing heparin (20 U/mL) and ampicillin (5 mg/mL) and then filled with a viscous solution of heparin (300 U/mL) and 80% polyvinylpyrrolidone (PVP-10, Fisher Scientific, Pittsburgh, Pa) to prevent refluxing of blood into the catheter lumen.

2.2. Euglycemic-hyperinsulinemic clamp study

Euglycemic-hyperinsulinemic clamps were performed with awake and unrestrained rats as described previously [13]. Briefly, glucose concentrations were clamped at euglycemic levels by a variable rate infusion of 25% glucose. Blood glucose levels were monitored with a portable glucometer, and glucose infusion rates (GIRs) were adjusted every 5 to 10 minutes as needed. 3-[^{3}H] glucose (Amersham Inc, Los Angeles, Calif) was infused through the jugular vein catheter starting at 60 minutes with a bolus (6 μCi) followed by a continuous infusion (0.2 μCi/min $\times$ 3 hours). Starting at 0 minute, a 15% lipid emulsion containing heparin (lipid/heparin [L/H]) (20 U/mL, Fresenius-Kabi Inc, Berlin, Germany) was infused at a rate of 1.5 mL/h. Insulin (4.8 mU/kg per minute) was infused through the jugular vein catheter from 120 to 240 minutes. Controls received saline infusions instead of L/H. Blood samples (~400 μL) were obtained from the carotid artery at 0, 120, 200, 220, 230, and 240 minutes for determination of insulin, resistin, ghrelin, FFAs, and specific activity of tritiated glucose. Each blood sample was replaced by the same volume of fresh whole blood from a donor rat. Glucose rates of appearance (G_{Ra}) were determined with 3-[^{3}H] glucose as described [13]. G_{Ra} and the glucose rate of disappearance (G_{Rd}) were calculated using the non–steady-state equation of Steele et al [14]. The distribution volume for glucose was assumed to be 150 mg/kg. Hepatic glucose production (HGP) was calculated as the difference between the isotopically determined G_{Ra} and the rate of glucose infused to maintain euglycemia (GIR). Endogenous glucose production (EGP) = HGP = GRa − GIR.

2.3. Analytical procedures

Plasma insulin was measured in deproteinized serum by radioimmunoassay using rat insulin as standard (Linco, St Charles, Mo). FFA was measured in plasma with a kit (Wako Pure Chemicals, Richmond, Va). Resistin and ghrelin were measured in plasma containing EDTA (1 mg/mL of blood) and protease inhibitor (aprotinin 100 U/mL of blood) by enzyme-linked immunosorbent assay (Phoenix Pharmaceuticals, Belmont, Calif) in plasma specimens that had been stored at −20°C. The limit of detection was 0.2 μg/L and 100 ng/L, intra-assay coefficient of variation was 5.4% and 4.6%, and interassay coefficient of variation was 6.6% and 7.0%, respectively, for resistin and ghrelin.

2.4. Statistical analysis

Data are presented as mean ± SE. Comparisons between groups were made by the 2-tailed Student t test. Within-group comparisons were made using the paired Student t test. The associations between plasma resistin and ghrelin levels and metabolic parameters were calculated using Pearson correlation coefficients. Differences were statistically significant at $P < .05$. All analyses were performed using SPSS 8.0 software (SPSS Inc, Chicago, Ill). The study protocol was reviewed and approved by the institutional animal care and use committee of Chongqing Medical University.

3. Results

3.1. Euglycemic-hyperinsulinemic clamp studies

The 2 groups of rats were similar with respect to body weight, basal glucose, insulin, FFA, resistin, and ghrelin levels (Table 1).

Table 1
General characteristics of control and lipid-infusion rats

Groups	Controls	L/H group
n	10	10
Body weight (g)	279 ± 19	286 ± 17
Fasting blood glucose (mmol/L)	5.2 ± 0.1	5.1 ± 0.2
Fasting plasma insulin (mU/L)	30.3 ± 2.4	27.9 ± 2.2
Fasting FFAs (μmol/L)	672.5 ± 92.2	741.9 ± 50.6
Fasting plasma resistin (μg/L)	39.3 ± 3.6	39.9 ± 3.1
Fasting plasma ghrelin (μg/L)	8.9 ± 0.9	9.4 ± 0.8

All $P > .05$.

Table 2
Plasma parameters and glucose turnover data in the control and lipid-infusion rats during clamping steady-state

Group	FFA (μmol/L)		Blood glucose (mmol/L)		Insulin (mU/L)	
	Basal	Steady state	Basal	Steady state	Basal	Steady state
Controls	673 ± 92	213 ± 11*	5.2 ± 0.1	5.2 ± 0.1	30.3 ± 2.4	86.9 ± 2.5*
L/H group	741 ± 51	2868 ± 138*·**	5.1 ± 0.2	5.3 ± 0.1	27.9 ± 2.2	96.3 ± 8.3*

Values are presented as mean ± SE. GIR indicates glucose infusion rate; G_{Rd}, glucose disappearance rate.
* $P < .01$ versus basal.
** $P < .01$ versus control.

During the clamps, serum insulin concentrations increased from 22.3 ± 5.8 to 96.3 ± 8.3 μU/mL in the L/H group and from 20.8 ± 6.4 to 86.9 ± 2.5 μU/mL in the control group. The difference between the 2 groups was not statistically significant.

Plasma glucose concentrations in the L/H and saline-infused rats were maintained at 5.3 ± 0.1 and 5.2 ± 0.1 mmol/L, respectively.

Plasma FFA concentrations increased from 0.82 ± 0.06 to 2.86 ± 0.13 mmol/L in the L/H group ($P < .001$) and decreased from 0.83 ± 0.19 to 0.21 ± 0.01 mmol/L in the control group ($P < .001$, Table 2).

3.2. Glucose turnover

In response to hyperinsulinemia, the GIR needed to maintain euglycemia increased from 0 to 12.6 ± 0.3 mg · kg^{-1} · min^{-1} ($P < .001$) in the L/H group. By comparison, GIR increased 63% less (from 0 to 34.1 ± 0.7 mg · kg^{-1} · min^{-1}) in the control group. The difference in GIR between the 2 groups was highly significant ($P < .001$) (Fig. 1).

G_{Rd} increased in the control group from 19.0 ± 4.5 to 37.8 ± 3.3 mg · kg^{-1} · min^{-1} ($P < .01$) and in the L/H group from 18.7 ± 3.0 to 35.5 ± 2.1 mg · kg^{-1} · min^{-1} ($P < .01$). The difference between the 2 groups, however, was not statistically significant. In response to hyperinsulinemia, EGP (HGP) decreased by ~85% (from 19.0 ± 4.5 to 3.1 ± 1.8 mg · kg^{-1} · min^{-1}, $P < .001$) in the control group. In contrast, EGP did not decrease (from 18.7 ± 3.0 to 23.2 ± 3.1 mg · kg^{-1} · min^{-1}) in the L/H group. The difference between the 2 groups was highly significant ($P < .001$) (Fig. 2).

3.3. Resistin and ghrelin

Plasma resistin levels increased by 46% (from 39.9 ± 3.1 to 58.4 ± 4.7 μg/L, $P < .05$) in the L/H group. By comparison, resistin levels did not change in the control group (39.7 ± 2.9 vs 39.3 ± 3.6 μg/L, not significant) (Fig. 3).

Plasma ghrelin levels decreased in the L/H group by 35% (from 8.9 ± 0.9 to 5.8 ± 0.6 μg/L, $P < .05$) and by 41% (from 9.4 ± 0.8 to 5.5 ± 0.8 μg/L, $P < .05$) in the control group. The difference between the 2 groups was not statistically significant (Fig. 3).

Plasma resistin levels correlated positively with plasma FFA levels ($r = 0.68$, $P < .01$, Fig. 4) and with glucose concentrations ($r = 0.66$, $P < .01$) (Fig. 5).

4. Discussion

4.1. FFA and insulin resistance

In the present study, we have explored effects of sustained 2-hour elevations of serum insulin and 4-hour elevations of plasma FFA levels on plasma levels of resistin and ghrelin. In keeping with previous reports [13,15], we found that increasing plasma FFA levels during euglycemic-hyperinsulinemic clamping caused profound hepatic insulin resistance. The inhibitory effect of FFA on peripheral insulin-stimulated glucose uptake (G_{Rd}) was less pronounced, probably because of the relatively short time

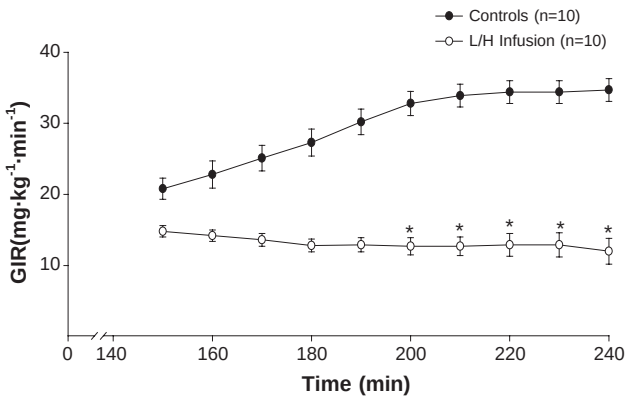

Fig. 1. Time course of GIR during the hyperinsulinemic euglycemic clamp. Shown are means ± SE. *$P < .01$ compared with controls.

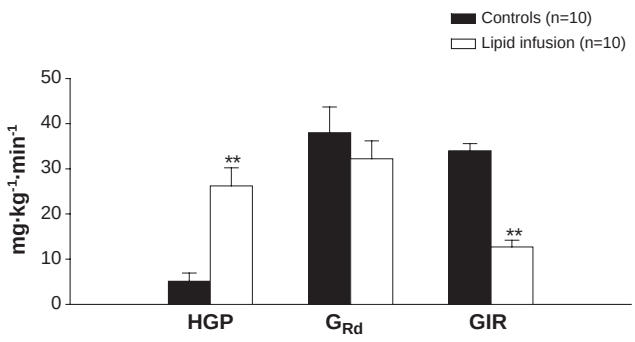

Fig. 2. Steady-state HGP, glucose disappearance rate, GIR during the hyperinsulinemic-euglycemic clamp in control and lipid-infusion rats. **$P < .01$ compared with controls.

Table 2 Cont'd

GIR (mg · kg^{-1} · min^{-1})		HGP (mg · kg^{-1} · min^{-1})		G_{Rd} (mg · kg^{-1} · min^{-1})	
Basal	Steady state	Basal	Steady state	Basal	Steady state
0	34.1 ± 0.7	19.0 ± 4.5	3.1 ± 1.8*	19.0 ± 4.5	37.8 ± 3.3*
0	12.6 ± 0.3**	18.7 ± 3.0	23.2 ± 3.1**	18.7 ± 3.0	35.5 ± 2.1*

(120-240 minutes) during which FFA and insulin were infused together. Four hours has been shown to be insufficient for the FFA-induced peripheral insulin resistance to be fully expressed [13,15]. The delay in the onset of FFA-induced insulin resistance in human and rat muscle and liver has been shown to be related to the accumulation of fat and of intermediate metabolites of fatty acid esterification such as long-chain acyl-CoA and diacylglycerol [16,17].

4.2. FFA and resistin

Of interest was the finding that lipid infusion during euglycemic hyperinsulinemia increased plasma resistin levels, whereas hyperinsulinemia alone did not. We are not aware of previous reports on effects of FFA on resistin. Studies of resistin responses to acute changes in serum insulin or glucose have yielded inconsistent results. For instance, hyperinsulinemia has been reported to decrease resistin mRNA in mouse adipocytes and in 3T3-L1 cells [18-21] and to increase resistin secretion from 3T3-L1 adipocytes [22].

The mechanism by which FFA increased resistin levels is not known. Because resistin is produced by adipocytes and monocytes/macrophages and is present in areas of inflammation, resistin has been proposed to be a member

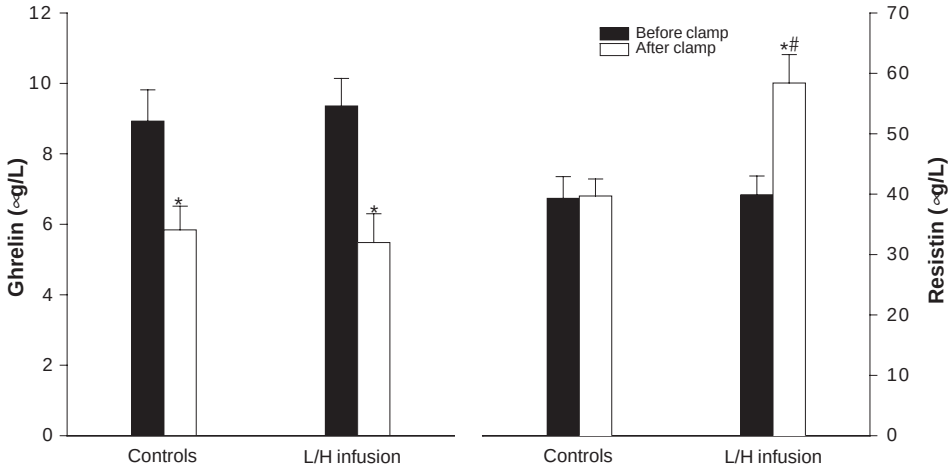

Fig. 3. The changes of ghrelin and resistin levels before and after clamp. $^{\#}P < .01$ compared with controls, $^{*}P < .05$ compared with 0 minute.

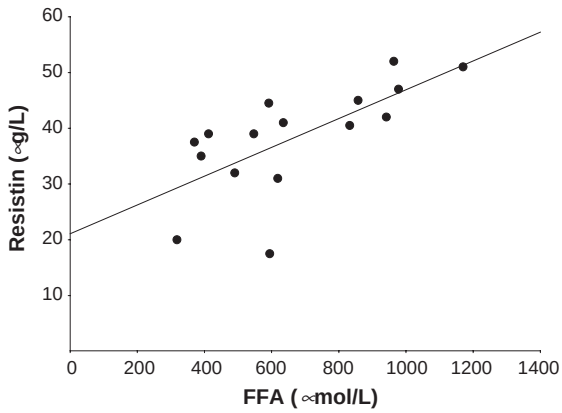

Fig. 4. The relationship between the fasting plasma resistin and FFA levels ($r = 0.68$, $P < .01$).

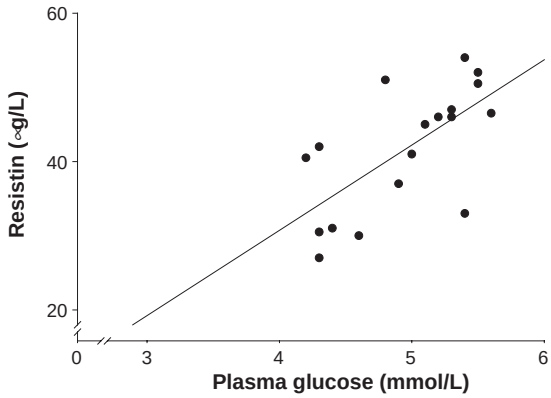

Fig. 5. The relationship between the fasting plasma glucose and resistin levels ($r = 0.66$, $P < .01$).

of a new class of proinflammatory cytokines and a link between obesity and inflammation [2,3,23]. This may be relevant because of the recent recognition that increased plasma FFA levels not only cause insulin resistance but also activate the proinflammatory NFκB pathway [17]. Hence, it is conceivable that the FFA-associated increase in plasma resistin may have been a consequence of FFA-induced and NFκB-mediated inflammation. On the other hand, resistin has also been demonstrated to cause hepatic insulin resistance in rats [24]. The resistin levels in that study, however, were higher than in our study (increased by 200%-1500% vs 50%). Thus, the FFA-mediated increase in resistin may have contributed to the FFA-induced hepatic insulin resistance.

4.3. FFA and ghrelin

Another finding was that hyperinsulinemia was associated with a decrease in plasma ghrelin but that ghrelin was not influenced by the combination infusion of L/H. These results confirmed a report by Mohlig et al [25] who showed in 4 human subjects that euglycemic hyperinsulinemia with or without lipid infusion decreased ghrelin levels. They are also in agreement with previous published reports showing that oral ingestion of glucose, fat, or mixed meals resulted in a decrease in plasma ghrelin [2,8]. Thus, our findings are compatible with the generally held concept that food intake, which in most cases results in an increase in insulin, lowers plasma ghrelin concentrations [8]. The mechanism by which increased insulin lowered plasma ghrelin remains to be explored. However, as ghrelin release has been demonstrated to be regulated by parasympathetic activation [26] and because hyperinsulinemia is known to decrease the parasympathetic tone, we believe that at least part of the observed decrease in plasma ghrelin may have been due to insulin-induced decreased parasympathetic activation.

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