Effect of Interferon on Glucose Tolerance and Insulin Sensitivity

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Many viral infections induce interferon (IFN) production and cause insulin resistance. To examine the causal relationship between IFN and insulin resistance, we injected natural human leukocyte IFN-α (3×10^6 IU, i.m.) twice overnight in eight healthy subjects and determined oral (OGT) and intravenous (IVGT) glucose tolerance and sensitivity to insulin (287 nmol or 40 mU · m^{-2} · min^{-1} euglycemic insulin clamp) the following morning. IFN caused mild influenzalike symptoms and induced a rise in circulating glucose, insulin, hydrocortisone (cortisol), growth hormone, and glucagon concentrations ($P < .05$–$.001$). In the OGT test, the area under the glucose curve was 2.6-fold greater ($P < .02$), and the disappearance rate of intravenously administered glucose was reduced by 28% ($P < .05$) after IFN administration. The impairment in OGT and IVGT occurred despite augmented insulin response. Insulin-stimulated glucose disposal was reduced by 22% ($P < .005$), and insulin clearance increased by 18% ($P < .02$) after IFN administration. When the insulin-clamp study was repeated in patients with steady-state hyperinsulinemia that was 12% higher ($P < .005$) after IFN, the glucose disposal rate was still reduced by 15% ($P < .01$). These data indicate that IFN *1*) stimulates counterregulatory hormone secretion, *2*) impairs glucose tolerance and insulin sensitivity, and *3*) stimulates insulin clearance. Thus, IFN may be involved in the development of insulin resistance during viral infections. *Diabetes* 38:641–47, 1989

Interferons (IFNs) are a group of polypeptides with antiviral, cytostatic, and immunomodulatory properties (1). They cause changes in cell membranes, enzyme metabolism, and protein synthesis (1). IFNs are classified as α, β, and γ. IFN-α is predominantly produced when leukocytes are exposed to viruses (2). Consequently, during viral infections IFN-α has been detected in the circulatory system, urine, saliva, tears, nasal washings, and cerebrospinal or vesicle fluid (3–5). Viral infections may also be associated with insulin resistance (6,7). Under these conditions a rise in counterregulatory hormones (hydrocortisone [cortisol], growth hormone [GH], catecholamines) can contribute to insulin resistance (7–10). Whether IFN-α can stimulate counterregulatory hormone secretion and play a role in the development of insulin resistance during viral infections either directly or via insulin antagonists is not known. IFN-α is also often present in the circulatory system of patients with autoimmune diseases (11,12). These conditions can be associated with insulin resistance in the absence of a rise in counterregulatory hormones (13,14). Whether IFN-α contributes to insulin resistance in people with autoimmune diseases is not known.

The clinical use of IFN is increasing. IFN-α has been successfully used for several experimental and natural viral infections, e.g., herpes keratitis, the common cold, and hepatitis B and papilloma viruses (15). Various malignant diseases, particularly hairy cell leukemia, have also responded well to treatment with IFN-α (16).

Data on the metabolic effects of IFN are scant. In healthy subjects, the administration of human leukocyte IFN has profound effects on the metabolism of serum lipoproteins (17). We are not aware of any studies addressing the effect of IFN on insulin action or glucose tolerance in humans. A causal relationship between the production of IFN and the development of insulin resistance during viral infections is possible. Furthermore, the increasing clinical use of IFN calls for additional information about its metabolic effects. Consequently, we examined the effects of IFN administration on glucose tolerance by both oral (OGTT) and intravenous

C-peptide	1 nM = 0.331 ng/ml	Insulin	1 pM = 0.139 mU/ml
Glucose	1 mM = 18 mg/dl		

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(IVGTT) glucose tolerance tests and on body sensitivity to insulin as determined directly with the insulin-clamp technique.

MATERIALS AND METHODS

Subjects. We studied nine healthy subjects (6 men, 3 women), aged 22 ± 2 yr, with normal body weight (72 ± 3 kg) and height (179 ± 3 cm). The nature, purpose, and possible risks of the study were explained to all subjects before they gave their voluntary consent to participate. The study protocol was approved by the ethical committee of the Helsinki University Hospital.

Interferon. The natural human leukocyte IFN-α was produced and purified as described previously (18,19). It had a mixture of ~10 subtypes of human INF-α. The main impurity was human serum albumin. No detectable interleukin 1 (IL-1), IL-2, or tumor necrosis factor was present. The preparation used had a specific activity of 1×10^6 IU/mg protein. The IFN vials contained 3×10^6 IU of IFN in 0.5 ml of physiological phosphate-buffered saline (PBS; pH 7.3). The placebo vials contained an equivalent amount of human serum albumin in 0.5 ml of PBS. For control experiments, IFN was further purified by natural killer 2 (NK2) monoclonal antibodies as described previously (20). The virtually pure product (NK2-IFN) had a specific activity of 2×10^8 IU/mg protein. It contained 3×10^6 IU in 0.5 ml physiological saline. Human serum albumin was added to a final concentration of 0.5% to stabilize the product. The two IFN preparations have strikingly similar effects on pulse rate, body temperature, and total and differential white blood cell counts (21). The influenzalike symptoms caused by the two IFN preparations are also indistinguishable (21).

Design. The effects of IFN on oral (OGT) and intravenous glucose tolerance (IVGT), body sensitivity to insulin (glucose clamp), and glucose kinetics were studied. The studies were done during six different hospitalizations, each at least 1 wk apart. The subjects were asked to eat a weight-maintaining diet for at least 2 days before each study. On the day before the study, the subjects were admitted to the hospital at 1700. They ate a standard meal of ~600 cal at 1800 and an evening snack (200 cal) at 2100. At 2000 and 0400, the subjects were given either 3×10^6 IU IFN or placebo intramuscularly. In each of the three studies (OGTT, IVGTT, and glucose clamp), nocturnal blood samples were taken for the measurement of glucose and glucoregulatory hormones as indicated in Table 1. The mean of the three determinations at each time point represents the subject's nocturnal changes in glucose and glucoregulatory hormones after IFN or placebo injection. At 0800, the studies began for the determination of OGT or IVGT or glucose kinetics and sensitivity to insulin. Glucose kinetics and insulin sensitivity were determined on the same occasion. Each pair of studies (IFN or placebo) was performed in a randomized, double-blind fashion. The same eight subjects were studied for glucose kinetics and insulin sensitivity, whereas seven of them participated in the OGTT and IVGTT. To confirm the results on insulin sensitivity obtained with the partially purified IFN, two subjects (1 from the earlier group and 1 other) were treated with the pure NK2-IFN, and insulin sensitivity was measured as described. Axillar temperature (at 2000, midnight, and 0700) and possible influenzalike symptoms after IFN or placebo administration were recorded by a nurse at the ward and were not made known to the investigators who performed the studies.

Oral glucose tolerance. The subjects ingested 75 g of glucose diluted in 200 ml water within 5 min. Venous samples were taken for the measurement of glucose, insulin, C-peptide, and glucagon at intervals shown in Fig. 1.

Intravenous glucose tolerance. The subjects were given injections of 25 g of glucose (50% solution), and samples were taken for the measurement of serum glucose, insulin, C-peptide, and glucagon at intervals shown in Fig. 2.

Insulin sensitivity. Sensitivity to insulin was determined with the euglycemic insulin-clamp technique (22,23). An indwelling catheter was inserted into an antecubital vein for glucose and insulin infusions. A second catheter was inserted into a hand vein for blood sampling. The hand was kept in a heated chamber at 70°C to arterialize the venous blood. A primed continuous infusion of human short-acting insulin (Actrapid HM, Novo, Bagsvaerd, Denmark) was given. The priming dose was infused in a logarithmically falling manner for 10 min, followed by a continuous infusion at a rate of 287 nmol or 40 $mU \cdot m^{-2} \cdot min^{-1}$. The same infusion rate was used in all subjects. Because the steady-state plasma insulin levels after IFN turned out to be lower than after placebo study, six of the subjects underwent repeat studies. They were injected with IFN as before, but the insulin infusion rate during the clamp study was 22% higher than in the first study. Plasma glucose was determined at 5-min intervals, and the concentration was maintained at a fasting level by a variable infusion of 20% glucose.

TABLE 1
Nocturnal glucose, glucoregulatory hormone, and serum free–fatty acid (FFA) concentrations and axillar temperature after injection of human leukocyte interferon (IFN) or placebo (P) at 2000 and 0400

	2000		Midnight		0400		0700	
	IFN	P	IFN	P	IFN	P	IFN	P
Blood glucose (mM)	5.0 ± 0.1	5.0 ± 0.2	4.6 ± 0.2	4.8 ± 0.2	4.8 ± 0.4	4.5 ± 0.1	4.7 ± 0.1*	4.3 ± 0.1
Serum insulin (pM)	187 ± 64	151 ± 57	108 ± 43	106 ± 41	79 ± 28	50 ± 21	79 ± 29†	50 ± 29
Serum C-peptide (pM)	1166 ± 100	1065 ± 120	810 ± 90	966 ± 99	633 ± 67	630 ± 65	599 ± 67	532 ± 33
Serum hydrocortisone (nM)	173 ± 30	164 ± 22	299 ± 40‡	67 ± 9	415 ± 14‡	153 ± 32	525 ± 45	556 ± 36
Serum growth hormone (μg/L)	3.2 ± 1.5	2.3 ± 0.8	9.2 ± 4.1	4.1 ± 1.6	7.7 ± 1.5†	2.6 ± 0.5	2.6 ± 0.8	3.4 ± 2.5
Plasma glucagon (ng/L)							377 ± 30†	302 ± 39
Serum FFA (mM)							0.53 ± 0.19	0.35 ± 0.07
Axillar temperature (°C)	36.8 ± 0.1	36.9 ± 0.1	37.2 ± 0.1§	36.4 ± 0.1			37.5 ± 0.1‡	36.0 ± 0.1

The mean of 3 measurements per subject is used for the calculations. Dose of IFN was 3×10^6 IU i.m.
*$P < .02$, †$P < .05$, ‡$P < .001$, §$P < .005$, vs. placebo.

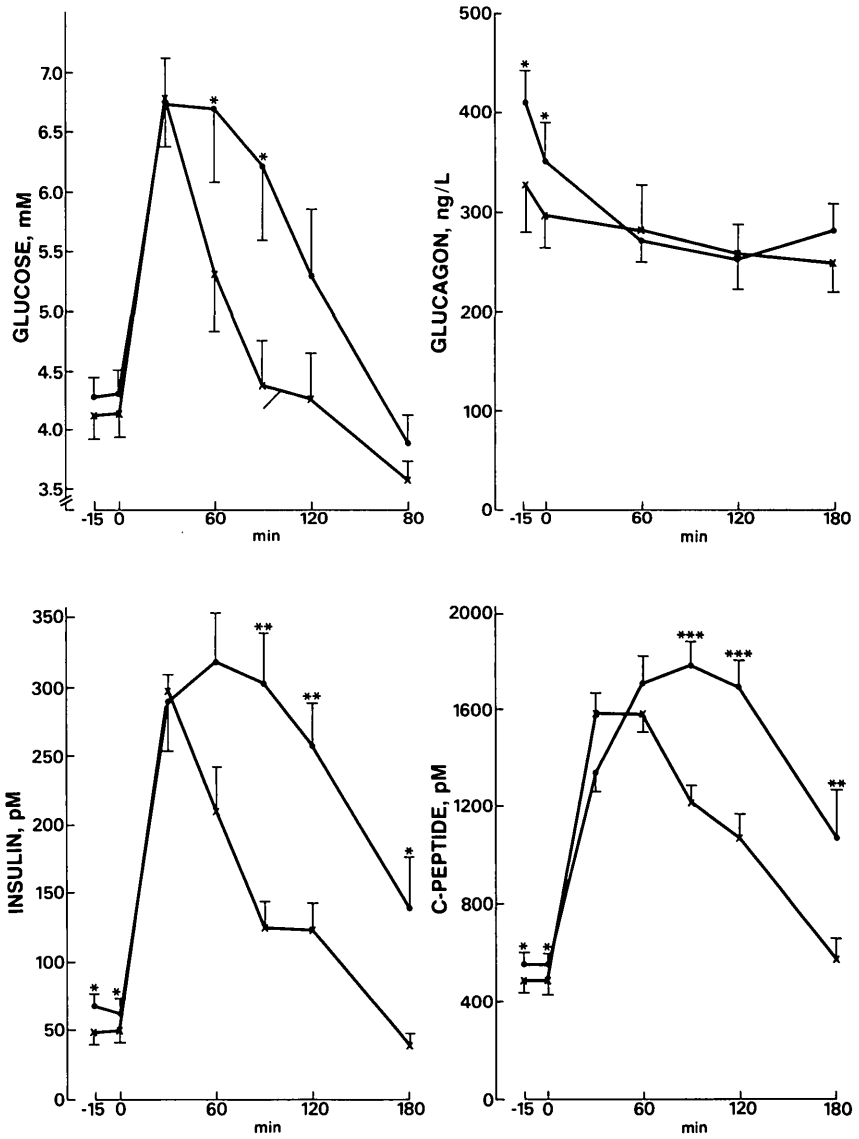

FIG. 1. Blood glucose (*$P < .02$), plasma glucagon (*$P < .05$), serum insulin (*$P < .05$, **$P < .005$), and C-peptide (*$P < .05$, **$P < .01$, ***$P < .005$) responses to oral glucose load in 7 healthy subjects after intramuscular overnight administration of human leukocyte interferon-α (3×10^6 IU twice, ●) or placebo (x). P values refer to differences between studies.

Glucose kinetics. For 120 min before the clamp study was begun, the glucose pool was labeled with a primed continuous infusion of D-[3-^{3}H]glucose (sp act 5 Ci/mmol, Amersham, Buckinghamshire, UK). The priming dose was given as a 20-μCi (in 20 ml of saline) bolus injection followed by a constant infusion of 0.2 μCi/min throughout the rest of the study. Plasma samples for the determination of steady-state glucose specific activity were taken at 5-min intervals from 90 to 120 min and at 10-min intervals thereafter. A steady-state plateau of glucose specific activity was obtained in all subjects before the beginning of the insulin-clamp study. The rate of glucose appearance (R_a) in the basal state was calculated by dividing the rate of [3-^{3}H]glucose infusion (cpm/min) by the steady-state level of glucose specific activity (cpm/mg). During glucose and insulin infusion, when glucose specific activity was not in the steady-state condition, the R_a was calculated with Steele's equation with a pool fraction of 0.65 (24). The rate of hepatic glucose production was calculated by subtracting the glucose infusion rate from the isotopically determined R_a. During hyperinsulinemia with a high rate of glucose utilization, the isotopically determined rate of glucose disappearance (R_d) may underestimate true rates of glucose utilization (25). Therefore, the exogenously infused glucose was used as a measure of glucose disposal rate after the endogenous R_a was suppressed to zero.

Analytical procedures. Nocturnal glycemia and blood glucose response to oral or intravenous glucose were measured in whole blood (Auto-Analyzer, Technicon, Tarrytown, NY), whereas during the clamp studies, glucose concentration was measured in the plasma via the glucose oxidase method (Glucose Analyzer II, Beckman, Fullerton, CA). Serum insulin (26), hydrocortisone (27), GH (28), and C-peptide (29) and plasma glucagon (30) concentrations were all determined with a radioimmunoassay. For glucagon determinations we used a kit from Cambridge Medical Diagnostics (Billerica, MA), with cross-reactivity with gut glucagon <3%. Serum free fatty acids (FFAs) were measured by the microfluorometric method (31). D-[^{3}H]glucose activity was determined after deproteinization with $Ba(OH)_2$-$ZnSO_4$ and evaporation of ^{3}H as described (32). The glucose R_d (K value) in the IVGTT was calculated according to Lundbaek (33) and is expressed as percent per minute. Statistical analysis was

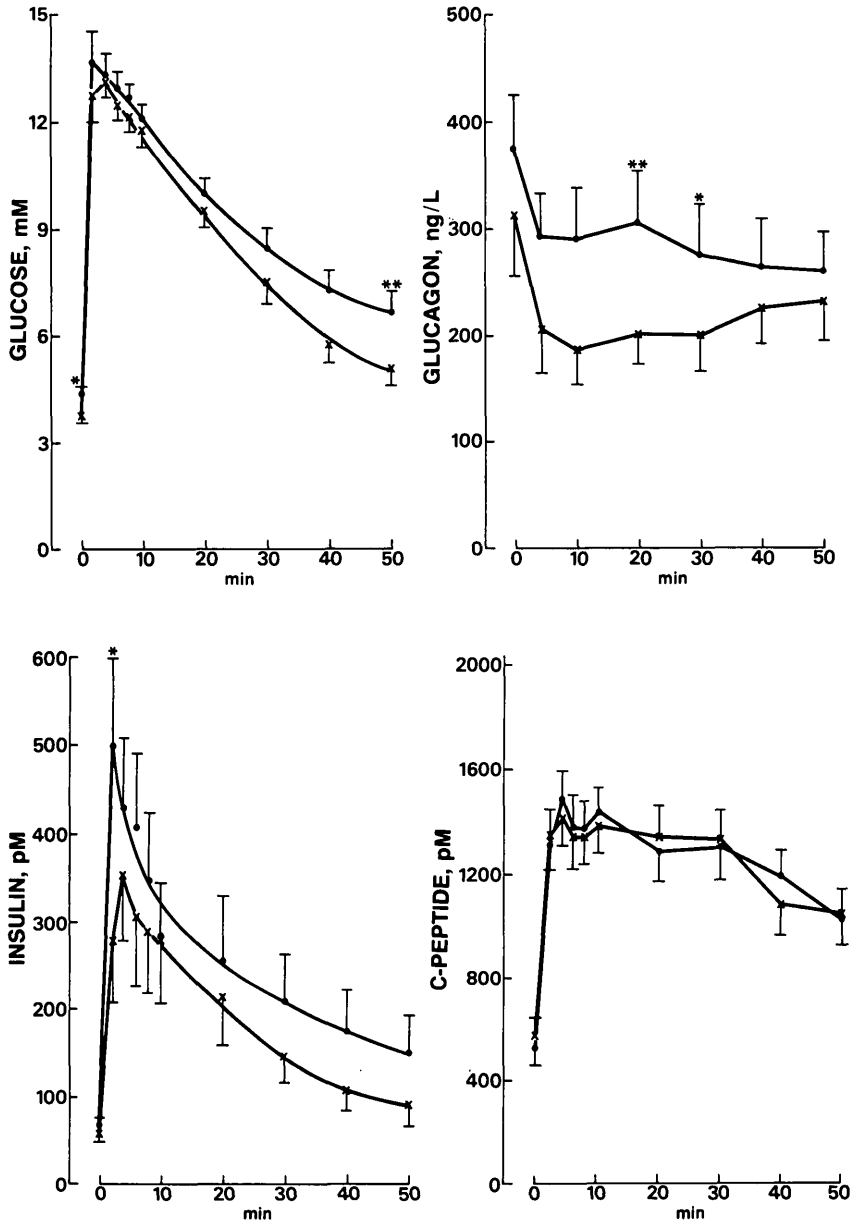

FIG. 2. Blood glucose, plasma glucagon, insulin, and C-peptide responses to intravenous glucose injection in 7 healthy subjects after overnight administration of human leukocyte interferon-α (3×10^6 IU twice, ●) or placebo (x). *$P < .05$, **$P < .01$, differences between studies.

done with analysis of variance for repeated measures with BMDP computer program 2V (34) and with paired *t* tests. The results are given as means ± SE.

RESULTS

Nocturnal glucose, FFA, and hormone levels. Changes in blood glucose and glucoregulatory hormones after IFN or after placebo injection are shown in Table 1. In the morning, blood glucose, serum insulin, and plasma glucagon levels were significantly higher after IFN than after placebo administration. In addition, after IFN administration, nocturnal serum hydrocortisone concentration was 4.5-fold and GH 3-fold higher when compared with placebo. Serum FFA concentrations were not significantly different in the IFN and placebo studies.

Oral glucose tolerance. Fasting serum insulin, C-peptide, and plasma glucagon concentrations were slightly higher after IFN than after placebo administration (Fig. 1). IFN substantially impaired OGT. The area under the blood glucose curve was 2.6-fold greater after IFN than after placebo administration ($P < .02$; Fig. 1). This occurred despite augmented insulin and C-peptide responses to glucose stimulus after IFN injections. Plasma glucagon levels were suppressed by oral glucose. In the IFN study, all the glucagon values from 60 to 180 min were below baseline ($P < .02$–$.001$; Fig. 1). In the placebo study, plasma glucagon concentration fell below baseline 120 min after glucose administration ($P < .05$).

Intravenous glucose tolerance. Fasting blood glucose and plasma glucagon concentrations were higher after IFN than after placebo administration (Fig. 2). In response to a bolus of glucose, blood glucose was slightly higher in the IFN study, although the difference was not significant until 50 min after the glucose injection. Glucose R_d (K value) was 28% lower after IFN administration (1.82 ± 0.17%/min) than after placebo injections (2.58 ± 0.31%/min, $P < .05$). The area under the serum insulin curve after intravenous glucose was 36% greater in the IFN study than in the placebo study

($P < .05$). The rise in C-peptide concentration was similar in the IFN and control studies, whereas glucagon levels remained higher 20–30 min after IFN injection when compared with placebo (Fig. 2).

Insulin-clamp study. Fasting plasma glucose concentration was higher after IFN (5.3 ± 0.1 mM) than after placebo injections (4.9 ± 0.1 mM, $P < .02$). During the insulin-clamp study, the steady-state plasma glucose levels were identical in the two studies (4.8 ± 0.1 mM), with a coefficient of variation (C.V.) of 7 ± 1% in both. Serum insulin levels in the beginning of the insulin-clamp study were slightly higher after IFN than after placebo administration (89.4 ± 3.6 vs. 55.3 ± 2.9 pM, respectively, $P < .02$). During the insulin clamp, serum steady-state insulin (SSSI) concentrations were 18% lower in the IFN than in the placebo study (462.4 ± 28.7 vs. 560.0 ± 21.5 pM, respectively, $P < .02$; Fig. 3, IFN I), with a C.V. of 7 ± 1 and 10 ± 1%, respectively. The decline in SSSI after IFN occurred in 7 of 8 subjects, and in both of those who had received the pure NK2-IFN (Fig. 3). The rate of insulin-stimulated glucose disposal (M) in each individual is shown in Fig. 3. The M value fell in 9 of 10 subjects in the IFN study; the only exception was the subject with a low value in the placebo study. The mean M value after IFN administration (0.35 ± 0.01 mmol · kg^{-1} · min^{-1}) was 22% lower than in the placebo study (0.45 ± 0.03 mmol · kg^{-1} · min^{-1}) ($P < .02$). In the two subjects treated with the pure NK2-IFN, the decline in M was 30 and 47%, respectively (Fig. 3).

Due to the differences in steady-state hyperinsulinemia, we repeated the insulin-clamp study in six subjects after IFN administration. The insulin infusion rate was 22% higher than in the first study, resulting in SSSI concentrations of 617.5 ± 14.4 pM, which was slightly higher than during the placebo study with the same subjects (552.9 ± 21.5 pM, $P < .005$; Fig. 3, IFN II). Despite the higher SSSI levels, the M value in the second IFN study was 15% lower ($P < .01$) than in the placebo study in the same subjects (0.40 ± 0.02 vs. 0.47 ± 0.02 mmol · kg^{-1} · min^{-1}, respectively).

Serum C-peptide levels in the beginning of the insulin-clamp study were similar in the IFN (632.7 ± 67.0 pM) and placebo studies (566.1 ± 67.0 pM). During steady-state hyperinsulinemia, C-peptide concentration fell to 266.0 ± 33.0 pM after IFN and to 366.0 ± 33.0 pM after placebo administration ($P < .005$ between the groups). Thus, the decline in the C-peptide concentration induced by hyperinsulinemia was 2-fold greater after IFN than after placebo treatment ($P < .05$). In the two subjects injected with the pure NK2-IFN, the decline in C-peptide was 1.4- and 1.9-fold greater after IFN than after placebo.

Glucose kinetics. During the steady-state period for the calculation of baseline glucose kinetics (90–120 min of [3-^{3}H]glucose infusion), plasma glucose averaged 5.1 ± 0.1 mM in the IFN and 5.0 ± 0.1 mM in the placebo study. The rate of glucose production was also similar after IFN and placebo administration (0.13 ± 0.01 vs. 0.12 ± 0.01 mmol · kg^{-1} · min^{-1}, respectively). During hyperinsulinemia, glucose production was totally suppressed after 20–40 min of insulin infusion and was similar in the IFN and placebo studies.

Symptoms after IFN. In each subject, IFN injections caused mild influenzalike symptoms (headache, muscle pain), and the axillar temperature in the morning was higher than after placebo (Table 1). In the two subjects injected with the pure NK2-IFN, axillar temperature rose to peak levels of 37.4 and 37.7°C, which was similar to peak levels in the others.

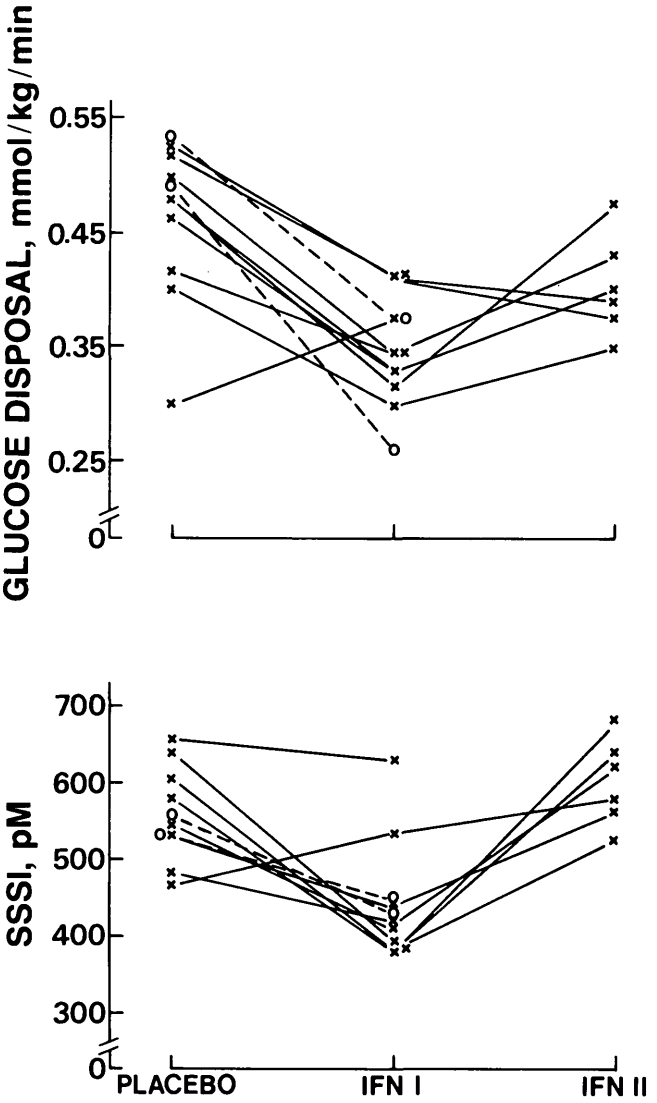

FIG. 3. Individual values for rate of insulin-mediated glucose disposal (M value) and steady-state serum insulin (SSSI) levels during euglycemic insulin-clamp procedure performed morning after overnight intramuscular administration of human leukocyte interferon-α (IFN-α) (3 × 10^6 IU twice) or placebo. ○, Two subjects who received pure natural killer cell 2 IFN. In 1st IFN study, glucose disposal rate ($P < .02$) and SSSI ($P < .02$) were lower than in placebo study. In 2nd IFN study, SSSI was higher ($P < .005$) but glucose disposal rate remained lower ($P < .01$) than in placebo study in same subjects.

DISCUSSION

Viral infections can induce leukocyte IFN production (2) and insulin resistance (6,7). This raises the possibility that IFN could be involved in the development of insulin resistance. To examine this possibility, we injected IFN into healthy subjects at a dose that results in blood IFN concentrations comparable to those seen during viral infections (3,4).

After the overnight IFN administration, the subjects had moderately elevated fasting glucose and insulin concentrations, and both OGT and IVGT were impaired. In addition,

insulin responses to glucose challenges were augmented after IFN. Increased blood glucose concentrations in the basal state and after glucose stimulus despite hyperinsulinemia suggests the presence of insulin resistance after IFN administration.

Impaired insulin action after IFN administration was confirmed by the insulin-clamp technique, revealing a 22% lower insulin-stimulated glucose disposal rate than in the placebo study. This effect was confirmed with the IFN purified by monoclonal antibodies. The comparison between IFN and placebo studies was complicated by lower insulin levels after IFN administration. However, after IFN treatment, the glucose disposal rate remained significantly reduced when the clamp study was repeated with higher insulin infusion rates, and SSSI concentrations were higher than those in the placebo study. The site of IFN-induced insulin resistance was probably peripheral, because the rate of hepatic glucose production was similar in the basal state and equally suppressed by insulin in the IFN and placebo studies. The mechanisms of insulin resistance induced by IFN can be either direct effects or mediated by other immunomodulators or factors that are stimulated by IFN.

When lymphoblastoid Daudi cells were incubated with IFN-α, insulin binding was reduced by more than 50% (35,36). However, using adipocytes from healthy humans, we have not been able to demonstrate a decline in insulin binding to adipocytes by IFN-α (Kolaczynski JW, Taskinen M-R, Cantell K, Koivisto VA, unpublished observations). Although adipocytes are not a major target tissue for insulin-stimulated glucose disposal, our in vitro data do not support the direct inhibitory effect of IFN on insulin-stimulated glucose uptake.

IFNs induce other lymphokines and immunomodulators, such as IL-1 (37). When IL-1 was induced by administration of etiocholanolone in normal humans (38), or when IL-1 was injected into a rabbit (39), no effect on glucose clearance was observed. These data fail to support the contribution of IL-1 in the IFN-induced insulin resistance.

A rise in serum FFA concentration impaired glucose utilization during steady-state hyperinsulinemia (40), whereas both IVGT and OGT remained unchanged, when serum FFA concentration was raised five- to sevenfold above normal (41). In this study there was no significant difference in the fasting FFA levels after IFN and placebo administration. Thus, the insulin resistance after IFN cannot be explained by a rise in serum FFA levels.

Previous studies have demonstrated that a rise in serum hydrocortisone levels occurs after IFN administration (42). This was also observed in our study. In addition, we found that IFN induced a significant rise in serum GH and plasma glucagon concentrations. These hormones can stimulate hepatic glucose production, and hydrocortisone and GH can also reduce insulin-mediated glucose disposal (8,9). Thus, the increment in counterregulatory hormones may have played a role in the development of morning hyperglycemia and peripheral insulin resistance after IFN administration. A possible stimulatory effect of these hormones on glucose production had, however, vanished by the time the steady-state equilibrium for plasma [3-^{3}H]glucose was obtained, because plasma glucose concentration and the rate of glucose production were similar in the IFN and placebo studies.

After IFN administration, the clamp study showed that SSSI levels were lower than in the control study. In addition, the suppression of C-peptide concentration was greater after IFN than after placebo administration. The small difference in the remaining endogenous insulin secretion cannot, however, explain the lower SSSI concentrations after IFN administration. Consequently, the lower insulin concentrations are probably due to augmented insulin clearance induced by IFN.

A major site for insulin clearance is the liver. The relative amount of insulin retained by the splanchnic bed is 40–75% (43,44). In the liver, both Kupffer cells and hepatocytes bind insulin (45). A blockade of Kupffer cell phagocytosis can lead to a 2- to 2.5-fold increase in peripheral insulin levels (45). In addition, there are insulin receptors in monocytes (46) and macrophagelike cells (47). All of these cells can be activated by IFN-α. Thus, the enhanced insulin clearance could be explained by a stimulation of both hepatic and peripheral cells involved in the extraction of insulin.

In summary, administration of human leukocyte IFN-α stimulated counterregulatory hormone secretion and insulin clearance and impaired glucose tolerance and insulin sensitivity in healthy subjects. The impaired glucose metabolism can be at least partly explained by a rise in counterregulatory hormones. These findings raise the possibility that IFN is involved in the development of insulin resistance observed during viral infections.

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