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Artificial induction of intravascular lipolysis by lipid-heparin infusion leads to insulin resistance in man

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Summary. Although extensive evidence indicates that free fatty acids can decrease glucose utilization in vitro, it is still controversial how an increase in lipolysis affects glucose metabolism in man. To test the hypothesis that an increase in lipolysis is related to insulin resistance, we examined the effect of lipid-heparin infusion on glucose metabolism in ten normal subjects by the euglycaemic glucose clamp technique and isotopic determination of glucose turnover. In the control euglycaemic clamp studies with insulin infusion at 0.2 and 1.0 $mU \cdot kg^{-1} \cdot min^{-1}$, endogenous glucose production was suppressed from the basal rate of 2.0 ± 0.3 $mg \cdot kg^{-1}min^{-1}$ to 1.1 ± 0.7 $mg \cdot kg^{-1} \cdot min^{-1}$ and -0.4 ± 0.7 $mg \cdot kg^{-1}min^{-1}$ respectively. Glucose utilization increased from the basal rate of 2.0 ± 0.3 $mg \cdot kg^{-1}min^{-1}$ to 2.3 ± 0.5 $mg \cdot kg^{-1}min^{-1}$ and 5.9 ± 1.8 $mg \cdot kg^{-1}min^{-1}$ respectively. When the euglycaemic clamp studies were coupled with lipid-heparin infusion at comparable low and high rates of insulin infusion, endogenous glucose production increased (1.8 ± 0.7 $mg \cdot kg^{-1} \cdot min^{-1}$, $p < 0.001$, and 0.3 ± 0.6 $mg \cdot kg^{-1} \cdot min^{-1}$, $p < 0.05$, respectively), and glucose utilization decreased (2.1 ± 0.3 $mg \cdot kg^{-1} \cdot min^{-1}$, not significant, and 3.2 ± 0.7 $mg \cdot kg^{-1} \cdot min^{-1}$, $p < 0.001$ respectively). These data suggest that the artificial induction of intravascular lipolysis by lipid-heparin infusion leads to a state of insulin resistance in man.

Key words: Insulin resistance, lipolysis, free fatty acids, glycerol, glucose clamp technique, glucose production, glucose utilization.

Insulin resistance is a common feature of many altered physiological and pathological states including diabetes mellitus [1]. Insulin sensitivity, as measured by the glucose disposal rate during euglycaemic glucose clamp studies [2], has been shown to improve after treatment with either insulin or oral hypoglycaemic agents in patients with diabetes mellitus [3]. Since the lowering of the plasma glucose levels in diabetic patients is usually associated with a simultaneous decrease in plasma levels of other metabolic substances such as free fatty acids (FFA), amino acids, and ketone bodies, it is tempting to relate the high plasma levels of these substances to the insulin resistance observed [4, 5].

Randle et al. initially reported that FFA inhibits glucose utilization in cardiac and diaphragm muscles [6] and showed that FFA inhibits muscle glucose utilization by decreasing glucose transport and inhibiting glycolysis and pyruvate oxidation [7]. Subsequently, FFA was also shown to stimulate gluconeogenesis from lactate, alanine or pyruvate [8]. However, the importance of these initial observations remained uncertain since elvated plasma FFA levels could be the result of insulin deficiency and/or insulin resistance rather than the cause of insulin resistance. Indeed, the data and the conclusion on the role of FFA in glucose metabolism in vivo have varied depending on the experimental conditions [9-11].

Recently, several investigators have reevaluated the in vivo effect of lipid infusion and high plasma levels of FFA on glucose metabolism [12-14]. Thiébaud et al. employed the euglycaemic clamp technique in combination with indirect calorimetry in man and showed that lipid infusion leads to a significant reduction in total body glucose uptake. This decrease in total body glucose uptake was the combined effect of an inhibition of both glucose storage and glucose oxidation [12]. Ferrannini et al. showed that the increased FFA levels lead to insulin resistance by two different mechanisms. In the hyperinsulinaemic state, the increased FFA levels competed with glucose for peripheral utilization, while in the insulin-deficient state, induced by somatostatin infusion, increased FFA levels led to hyperglycaemia by enhancing hepatic glucose production [13].

In contrast to these results, Wolfe et al. reported that FFA inhibits glucose production in dogs [14]. Combined alpha- and beta-adrenergic blockade under the condition of euglycaemia resulted in a fall in plasma FFA level and a rise in hepatic glucose production. This increase in glucose production by the adrenergic blockade was entirely prevented by the infusion of lipid and heparin.

Because of the aforementioned apparent discrepancies, and the potentially important role that lipids might play in the regulation of glucose metabolism, we reexamined the effect of artifically induced lipolysis on glucose metabolism in healthy subjects. In order to see the effects under the physiological insulin levels, two different rates of insulin infusion were chosen, i.e. low insulin infusion rate ($0.2\ mU \cdot kg^{-1} \cdot min^{-1}$) to achieve a normoinsulinaemia, and a high insulin infusion rate ($1.0\ mU \cdot kg^{-1} \cdot min^{-1}$) to achieve a sub-maximal physiological insulin level [15].

Subjects and methods

Ten young, healthy male medical doctors volunteered for the study (29 ± 1 years, range 27-31). Their body weight ranged from 55 to 83 kg (69 ± 7 kg; body mass index, 20.7-25.6 kg/m^2). None had a family history of diabetes mellitus. All subjects went on weight-maintaining diet and no subjects took any medication.

The studies were performed as paired-tests; e.g. a control experiment and a test experiment with the infusion of lipid and heparin, on different days. The order of the control and lipid-heparin infusion experiments was randomised. For the lipid-heparin infusion experiments, Intralipid (a 10% triglyceride emulsion) was given at 2 ml/min together with heparin (a bolus of 200 U plus $0.4\ U \cdot mg^{-1} \cdot kg^{-1}$) to induce intravascular lipolysis of the infused triglyceride by stimulating the activity of lipoprotein lipase in blood. All test procedures were identical in control and test experiments except for the infusion of lipid-heparin.

The nature, purpose, and possible risks of the study were carefully explained to all subjects and written consent was obtained. The experimental protocol was approved by the human experimental committee of the Department of Internal Medicine at the Seoul National University Hospital, South Korea.

Protocol of study

The subjects were studied at 08.00 hours after an overnight (12-14 h) fast. An 18 gauge catheter was inserted into an antecubital vein for the infusion of all test substances. Another catheter was inserted into the contralateral hand vein for blood sampling. The hand was kept in a heating pad at 60 °C to allow arterialization of the venous blood [16].

Primed (25 μCi) and continuous (0.25 μCi/min) infusion of [3-^{3}H] glucose (Amersham, Buckinghamshire, England, sp. act. = 5.0 Ci/mmol) was maintained throughout the studies, and blood samples were collected for isotopic determination of glucose turnover. After a 2-h period to attain an isotopic steady state, the subjects received an insulin infusion (Velosulin, Nordisk, Coppenhagen, Denmark) at 0.2 and $1.0\ mU \cdot kg^{-1} \cdot min^{-1}$ by an IMED pump in conjunction with the infusion of somatostatin (300 μg/h, Bachem, Torrance, California, USA) and glucagon ($0.45\ ng \cdot kg^{-1} \cdot min^{-1}$, Novo, Coppenhagen, Denmark) to suppress endogenous insulin secretion and to maintain physiological plasma glucagon levels. Insulin was infused at each rate for 2 h. Prior to initial insulin infusion, a priming intravenous bolus of insulin ($0.2\ mU \cdot kg^{-1}$) was given for 10 s, and another bolus of insulin ($7\ mU \cdot kg^{-1}$) was given prior to the subsequent increase in the insulin infusion rate. Concurrent with the insulin infusion, variable amounts of glucose were infused with another IMED pump to clamp the blood glucose levels at the basal level. The blood glucose level was monitored every 5 min, and the glucose infusion rate was adjusted accordingly, using the formula of DeFronzo et al. [2].

Analytical procedure

During the euglycaemic glucose clamp study, blood samples were collected at regular intervals for the determination of plasma glucose, ^{3}H-glucose specific activity, insulin, glucagon, FFA and glycerol. Blood samples for plasma glucose and ^{3}H-glucose specific activity determination were collected at 15-min intervals in the tubes containing EDTA, and immediately centrifuged. Plasma and blood glucose levels were measured by the glucose oxidase methods using the YSI glucose analyzer (Yellow Springs Instrument Co., Yellow Springs, Ohio, USA).

A 2.5 ml portion of the plasma was deproteinized by adding 0.5 ml of chilled perchloric acid, and triplicate samples of the 0.5 ml of supernatant were evaporated to dryness at 60 °C in scintillation vials under compressed air to eliminate ^3H-H_2O. A 5 ml of Instagel (Packard, Downers Grove, Illinois, USA) was added and the radioactivity was measured in a liquid scintillation spectrophotometer (Beckman, Palo Alto, California, USA). The external standards ratio method was used to correct for quenching during counting of [3-^{3}H] glucose radioactivity. The percent recovery of tritiated glucose from the deproteinization procedure was determined by adding aliquots of tritiated glucose to 12 aliquots of plasma and to 12 aliquots of distilled water. The plasma samples were deproteinized, radioactivity was measured, and compared with that of water samples, which yielded a recovery of $98 \pm 1\%$. The plasma glucose specific activity was calculated by dividing the corrected radioactivity by the plasma glucose concentration.

Blood samples for insulin, C-peptide and glucagon assays were collected every 30 min in a prechilled tube containing EDTA and aprotinin (a trypsin inhibitor, 500 U/ml), immediately centrifuged and stored at −60 °C until analysed. Plasma insulin concentrations ware measured by radioimmunoassay using the commercial kits from Dainabot, Tokyo, Japan. Plasma C-peptide and glucagon levels were measured by radioimmunoassay kits from Daiichi, Tokyo, Japan.

Blood samples for FFA and glycerol determination were collected every 20 min in pre-chilled tubes containing EDTA and Paroxon (diethyl p-nitrophenyl phosphate, Sigma, St. Louis, Montana, USA a lipoprotein lipase inhibitor, 0.275 mg/ml of blood). Plasma FFA and glycerol levels were measured by enzymatic assays [17, 18].

Isotopic determination of glucose turnover

The rates of total glucose appearance (Ra; endogenously produced and exogenously infused) and disappearance (Rd) were determined isotopically using the equation of Steele in their derivative form [19, 20]. Values obtained during basal state and over the 2nd and 4th h of each clamp procedure were used for statistical analysis.

In the basal state, the rate of glucose turnover (Rt) was calculated by the isotopic dilution equation, $Rt = F/SA$ (Eq. 1), where F is the rate of infusion of labelled glucose (μCi/min) and SA is the specific activity of glucose. Under this condition, Rt equals Ra and Rd.

In nonsteady states, Ra and Rd were calculated according to the equations (2) and (3).

$$Ra = \left(F - pv\,\frac{C_1 + C_2}{2} \times \frac{SA_2 - SA_1}{t_2 - t_1} \times \frac{1}{1/2\,(SA_2 + SA_2)}\right) \quad (2)$$

$$Rd = Ra - pv\,\frac{C_2 - C_1}{t_2 - t_1} \quad (3)$$

where C_1 and C_2 are the plasma glucose concentrations (mg/ml), SA_1 and SA_2 are the glucose specific activities (μCi/mg) at the time

t_1 and t_2, respectively, v is the glucose distribution volume (25% of body weight, ml) and p is the pool fraction (0.65, according to Cowan and Hetenyi [21]).

Endogenous glucose production was obtained by subtracing the amount of infused glucose from the isotopically determined total glucose appearance rate. The evaluation of glucose turnover using euglycaemic glucose clamp and isotopic dilution methods has been validated [20] except for the frequent underestimation of endogenous glucose production [22, 23].

Statistical analysis

Data in text and tables are given as mean ± SD, and data in figures are given as mean ± SEM. Paired Student's t-test was used in statistical analysis.

Results

Plasma insulin, C-peptide, glucagon concentrations

Fasting plasma insulin, C-peptide and glucagon levels of the subjects studied were similar in control and lipid-heparin infusion experiments, and were maintained at similar levels, as shown in Figure 1. Fasting plasma insulin levels were 12 ± 7 μU/ml (control study) and 14 ± 5 μU/ml (lipid-heparin study), and were raised to 19 ± 7 μU/ml and 21 ± 7 μU/ml, respectively, during the 2nd h (low dose insulin infusion), and to 88 ± 18 μU/ml and 84 ± 12 μU/ml, respecitvely, during the 4th h (high dose insulin infusion) of studies (Fig. 1 A). Fasting plasma C-peptide levels were 1.4 ± 0.3 ng/ml (control) and 1.4 ± 0.4 ng/ml (lipid-heparin), and declined to 0.4 ± 0.1 ng/ml and 0.4 ± 0.1 ng/ml, respectively, during the 2nd h, and to 0.3 ± 0.1 ng/ml and 0.3 ± 0.1 ng/ml, respectively, during the 4th h of studies (Fig. 1 B). Plasma glucagon concentrations were 102 ± 15 pg/ml (control) and 109 ± 24 pg/ml (lipid-heparin) in the fasting state, and were maintained during the 2nd h at 94 ± 30 pg/ml and 93 ± 24 pg/ml, respectively, and at 90 ± 29 pg/ml and 87 ± 19 pg/ml, respectively, during the 4th h of studies (Fig. 1 C).

Plasma free fatty acids and glycerol concentrations

In the fasting state (Fig. 1 D), plasma FFA concentrations were similar in the two studies, averaging 0.42 ± 0.16 μmol/ml (control) and 0.44 ± 0.16 μmol/ml (lipid-heparin). In control experiments, FFA levels decreased to 0.17 ± 0.07 μmol/ml during the 2nd h, and to 0.07 ± 0.03 μmol/ml during the 4th h of studies. In contrast, lipid-heparin infusion caused a marked increase in plasma FFA to an average of 1.43 ± 0.16 μmol/ml and 1.54 ± 0.32 μmol/ml during the 2nd and 4th h of studies respectively (Fig. 1 D). Similarly, plasma glycerol levels of the control experiments fell from the basal value of 0.06 ± 0.02 μmol/ml to a plateau of 0.03 ± 0.01 μmol/ml during the 2nd and 4th h of studies. In the lipid-heparin infusion experiments, plasma glycerol concentrations increased from the basal value of 0.06 ± 0.03 μmol/ml to an average of 0.37 ± 0.11 μmol/ml and 0.35 ± 0.12 μmol/ml during the 2nd and 4th h of studies respectively (Fig. 1 E).

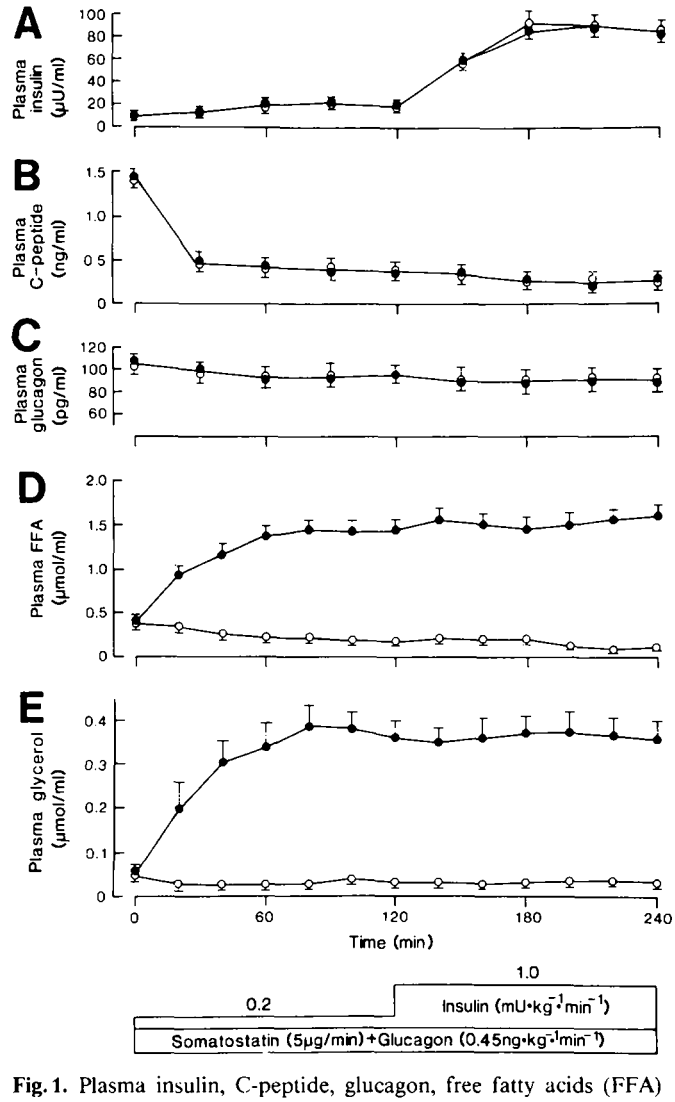

Fig. 1. Plasma insulin, C-peptide, glucagon, free fatty acids (FFA) and glycerol concentrations during the 4-h euglycaemic glucose clamp studies with (●) and without (○) lipid-heparin infusion. Vertical bars indicate 1 SEM

Plasma glucose levels and plasma glucose specific activities

Fasting plasma glucose levels averaged 4.89 ± 0.33 mmol/l in the control experiments, and 4.89 ± 0.22 mmol/l in the lipid-heparin infusion experiments (Fig. 2 A). The mean plasma glucose concentration during the 4 h glucose clamp was 5.17 ± 0.33 mmol/l (mean coefficient of variance = 4.9 ± 1.7%) in the control experiments, and was 5.22 ± 0.33 mmol/l (mean coefficient of variance = 6.6 ± 2.3%) in the lipid-heparin infusion experiments.

In the control experiments, plasma glucose specific activity declined from the basal rate of 1.86 ± 0.20 μCi/g to 1.57 ± 0.27 μCi/g during the 2nd h, and

to 0.70 ± 0.23 μCi/g during the 4th h of studies (Fig. 2 B). The mean plasma glucose specific activities during the 2nd and 4th h were 1.70 ± 0.23 μCi/g and 1.25 ± 0.33 μCi/g, respectively, in the lipid-heparin infusion experiments.

Glucose infusion, glucose production and utilization

Exogenous glucose infusion rate (Fig. 2 C). In the control experiments, glucose infusion rates to clamp the plasma glucose at euglycaemia during the 2nd and 4th h of studies were 1.3 ± 0.7 and 6.0 ± 2.6 mg·k^{-1}·min^{-1} respectively. Glucose infusion rates in the lipid-heparin infusion experiments were significantly lower than in the control experiments; namely 0.4 ± 0.4 ($p < 0.001$, 31% of control experiments) and 2.8 ± 1.2 mg·kg^{-1}·min^{-1} ($p < 0.001$, 47% of control experiments) at the 2nd and 4th h of studies respectively.

Endogenous glucose production rate (Fig. 2 D). In the control experiments, glucose production was suppressed from a basal rate of 2.0 ± 0.3 mg·kg^{-1}·min^{-1} to 1.1 ± 0.7 mg·kg^{-1}·min^{-1} during the 2nd h and was nearly completely suppressed (-0.4 ± 0.7 mg·kg^{-1}·min^{-1}) during the 4th h of studies. Glucose production in the lipid-heparin infusion experiments was 1.8 ± 0.5 mg·kg^{-1}·min^{-1} and 0.3 ± 0.6 mg·kg^{-1}·min^{-1} during the 2nd and 4th h of studies respectively ($p < 0.001$ and $p < 0.05$, respectively, compared with control experiments).

Glucose utilization rate (Fig. 2 E). In the control experiments, glucose utilization increased from a basal rate of 2.0 ± 0.3 mg·kg^{-1}·min^{-1} to 2.3 ± 0.5 mg·kg^{-1}·min^{-1}, and to 5.9 ± 1.8 mg·kg^{-1}min^{-1} during the 2nd and 4th h of studies respectively. In the lipid-heparin infusion experiments, glucose utilization rate was 2.1 ± 0.3 mg·kg^{-1}·min^{-1} during the 2nd h (statistically not significant), and was 3.2 ± 0.7 mg·kg^{-1}·min^{-1} during the 4th h of studies ($p < 0.001$, compared with control experiments).

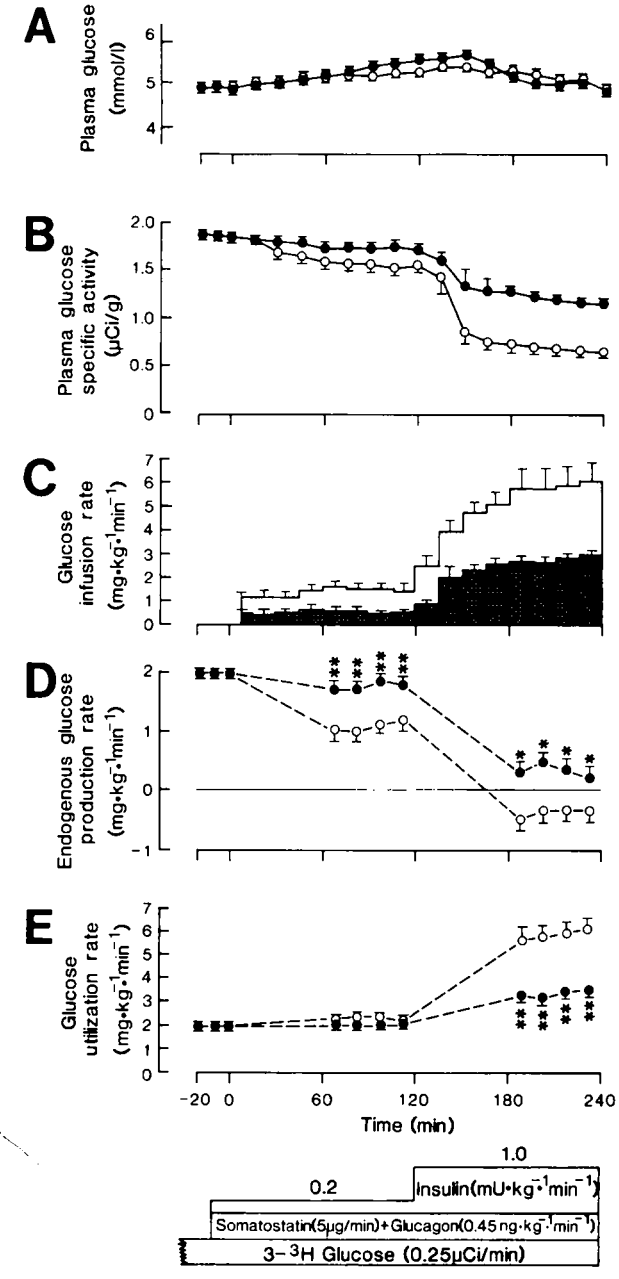

Fig. 2. Plasma glucose concentrations, glucose specific activities, glucose infusion rates, endogenous glucose production rates and glucose utilization rates during the glucose clamp studies with (●) and without (○) lipid-heparin infusion. Vertical bars indicate 1 SEM. * $p < 0.05$, ** $p < 0.001$ for difference between control and lipid-heparin infusion experiments

Discussion

The present study has demonstrated that the initiation of intravascular lipolysis by a lipid-heparin infusion results in insulin resistance in man. The data are in good agreement with the earlier observation [13] that elevated plasma FFA levels lead to suppression of peripheral glucose utilization in the hyperinsulinaemic state and to the enhancement of hepatic glucose production in the insulin-deficient state. In the present study, lipid-heparin infusion appeared to influence both glucose production and utilization simultaneously at physiological insulin levels. Lipid-heparin infusion led to an increase of glucose production and a decrease of glucose utilization at the submaximal physiological insulin level (~85 μU/ml), and led to an increased glucose production at normoinsulinaemia (~20 μU/ml). Although the difference was not statistically significant, lipid-heparin also inhibited almost 60% of the increase in the mean glucose utilization rate above the basal rate at normoinsulinaemia.

The results obtained during the low dose insulin infusion studies are in apparent contrast with a previous study. Wolfe et al. [14] reported that FFA inhibits glucose production in dogs under the condition of adre-

nergic blockade. The exact cause of the discrepancy is presently unclear. Except for the adrenergic blockade, similar experimental protocols were employed to attain similar plasma insulin, glucagon and FFA levels. In the present study, plasma C-peptide levels in control and lipid-heparin experiments showed similar suppression throughout the study period. Since plasma C-peptide levels reflect the endogenous insulin secretion, these data suggest similar portal plasma insulin levels in both parts of the experiments.

Adrenergic blockade can influence hepatic glucose metabolism directly. However, since adrenergic blockade was employed in both the control and lipid-heparin infusion experiments, it is less likely that the inhibition of glucose production by lipid-heparin infusion was due to the direct effect of adrenergic blockade on the liver. Another, and more probable, mechanism is that adrenergic blockade led to an inhibition of fatty acid release from fat stores. Since FFA is the major fuel used in the postabsorptive state [24], this may have resulted in the relative deficiency of the energy fuels that can be used in the body. This in turn may have led to the breakdown of muscle protein and the release of amino acids into the blood. High levels of plasma amino acids including gluconeogenic amino acid, alanine, might have resulted in the increase in glucose production [25].

During the high dose insulin infusion studies, the mean endogenous glucose production rate of control experiments was negative. Frequent underestimations of endogenous glucose production during hyperinsulinaemic glucose clamp and unlabelled glucose infusion have been reported. This has been attributed to: the discrepant metabolism of the tracer in comparison with native glucose (isotopic effect) [22]; the "mixing problem" of unlabelled glucose infusate and tracer [23]; or, error arising from the incorrect model structure [26].

Glycerol contributes little to the oxidative reactions in the body [27]. Therefore, it seems that elevated FFA are the main cause of the inhibition of glucose utilization by lipid-heparin infusion. In contrast, the observed enhancement of glucose production under lipid-heparin may be due to an increased release of both FFA and glycerol. FFA can stimulate gluconeogenesis either by supplying the energy necessary for gluconeogenesis or by activating key enzymes for gluconeogenesis [28]. On the other hand, glycerol can serve as a direct substrate for gluconeogenesis [27]. It would be of interest to estimate the relative contribution of glycerol to the observed difference in endogenous glucose production between the control and the lipid-heparin infusion experiments [29]. If the splanchnic glycerol clearance is taken to be ~1.0 l/min [30], a difference in glycerol levels of ~0.3 μmol/ml makes for a difference in splanchnic glycerol uptake of ~0.3 mmol/min. This would yield a maximum of ~0.4 $mg \cdot kg^{-1} \cdot min^{-1}$ of glucose, compared with an observed mean difference of ~0.7 $mg \cdot kg^{-1} \cdot min^{-1}$ (1.8 vs 1.1 $mg \cdot kg^{-1} \cdot min^{-1}$ during the 2nd h, and −0.4 vs 0.3 $mg \cdot kg^{-1} \cdot min^{-1}$ during the 4th h). Although this calculation may not be absolutely correct, the estimated data are consistent with previous data [13].

The clinical implication of our study is that the elevated plasma levels of FFA and glycerol may play a role in the insulin resistance observed in several clinical states including diabetes mellitus. Among the primary causes of insulin resistance, the defects of insulin action at either receptor or postreceptor levels have been identified and extensively studied [31, 32]. In addition to the primary insulin resistance, increased lipolysis observed in both insulin-dependent and non-insulin-dependent diabetes mellitus may lead to secondary insulin resistance. Although the present data from healthy subjects may not fully apply to diabetic patients, recent investigations on obese subjects have supported our contention [29, 33].

In conclusion, a simultaneous infusion of lipid and heparin induced a state of relative insulin resistance in healthy subjects. This insulin resistance appears to be caused by the combined effects of the intravascular lipolytic products, FFA and glycerol, which inhibit insulin-stimulated glucose utilization by peripheral tissues and counteract the insulin-mediated suppression of hepatic glucose production.

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