Diabetologia (1984) 27: 358–363

Diabetologia

Originals

Infusion of insulin impairs human adipocyte glucose metabolism in vitro without decreasing adipocyte insulin receptor binding

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Summary. To determine whether hyperinsulinaemia can cause insulin resistance in man and, if so, whether this occurs at a receptor or post-receptor site, nine normal volunteers were infused with insulin for 6 h at a rate ($2\,mU \cdot kg^{-1} \cdot min^{-1}$) which resulted in steady-state plasma insulin concentrations of 140 ± 13 mU/l and four subjects were infused with saline (0.45%). Isolated adipocytes and monocytes were used as models for studying insulin binding, while adipocytes were also used to study insulin action in vitro. Adipocyte insulin binding did not decrease following infusion of insulin (4.6 ± 0.5 versus 4.4 ± 0.4% per 2×10^5 cells, before and after, respectively), whereas monocyte insulin binding did (7.2 ± 0.6 versus 6.2 ± 0.6% per 10^7 cells, $p < 0.05$). Initial rates of adipocyte 3-0-methyl glucose transport were decreased in the absence of insulin (basal) and at submaximally effective (33.3 pmol/l) but not at maximally effective insulin concentrations. At all insulin concentrations and in the absence of insulin, rates of glucose conversion to lipids were decreased more than 50% ($p < 0.05$), whereas rates of glucose oxidation were unaffected. This decrease in the rates of conversion of glucose to lipids could not be accounted for by the decrease in rates of glucose transport. These results suggest that hyperinsulinaemia can cause insulin resistance in man and that, at least initially, this occurs at a post-receptor site. Furthermore, the discordant effect of hyperinsulinaemia on monocyte and adipocyte insulin binding indicates that monocyte insulin binding may not always reflect insulin binding in insulin-sensitive tissues.

Key words: Hyperinsulinaemia, insulin resistance, adipocytes, receptors.

Insulin resistant states, such as obesity and Type 2 (non-insulin-dependent) diabetes mellitus, are often associated with hyperinsulinaemia, decreased insulin receptor binding, and post-receptor defects in insulin action [1]. It has been suggested that hyperinsulinaemia seen in human obesity may produce insulin resistance secondarily at a post-receptor site [1–4] as a consequence of prolonged compensatory hyperinsulinaemia due to an initial decrease in insulin receptor binding. Moreover, the post-receptor defect in insulin action, as well as the insulin receptor down-regulation found in Type 1 (insulin-dependent) diabetes, has been attributed to the hyperinsulinaemia resulting from insulin treatment [5].

Although studies in vitro indicate that insulin itself may cause insulin resistance at receptor as well as post-receptor sites [6–15], it is uncertain to what extent the hyperinsulinaemia observed in insulin resistant states in man may contribute to impaired insulin action or merely be a consequence of insulin resistance. Moreover, if hyperinsulinaemia does contribute to insulin resistance, it is unclear whether this occurs primarily at a receptor or post-receptor site.

Short-term infusions of insulin have been reported to decrease monocyte and erythrocyte insulin binding in normal human volunteers [16–19]. However, to date there is no direct evidence that insulin excess can cause insulin resistance. Studies in which rats were injected with insulin for 1–2 weeks [20–33] have yielded conflicting results, suggesting that either no insulin resistance resulted or insulin resistance occurred which was solely explicable by a decrease in insulin-receptor binding.

Because of the uncertainties regarding the role of hyperinsulinaemia in the pathogenesis of insulin resistance, the present study was undertaken to determine whether infusions of insulin can produce insulin resistance in man and, if so, whether this is due initially to a receptor or post-receptor defect.

Materials and methods

Subjects

Informed written consent was obtained from 13 normal volunteers after these studies were approved by the Mayo Clinic Human Studies Committee. All subjects were within 15% of their ideal body weight

(Metropolitan Life Insurance Company Tables) and had no family history of diabetes mellitus. For 72 h before the study, subjects ate standard meals at the Clinical Research Center consisting of 45% carbohydrate, 35% fat and 20% protein (30 kcal/kg).

Experimental design

At 0700 h on the day of study the subjects, having fasted for 12–14 h, were admitted to the Clinical Research Center. Monocyte and adipocyte insulin binding, as well as adipocyte glucose transport, oxidation and conversion to lipids were determined before and after a 6-h infusion of insulin (eight women, one man, mean ± SEM age 25 ± 1 years) or a 6-h infusion of 7.7 mmol/l saline (three women, one man, mean age 35 ± 5 years). During this insulin infusion ($2\,mU \cdot kg^{-1} \cdot min^{-1}$), plasma glucose was maintained at approximately 5.3 mmol/l using the glucose clamp technique described previously [16].

Monocyte and adipocyte isolation

Mononuclear cells were isolated from heparinized blood by a modification of the Ficoll-Hypaque technique [24]. Adipocytes were isolated by a modification [25] of the collagenase digestion method of Rodbell [26]. Approximately 6 g of subcutaneous adipose tissue were obtained from the left or right lower abdominal quadrant from the centre of an area anaesthetized locally in a square-field fashion [3]. The tissue was washed in warm (37 °C) saline (0.15 mmol/l) and 1 g portions, which had been cut into 5 × 5 mm pieces, were placed into polyethylene vessels. Each vessel contained 6 ml Krebs-Ringer-bicarbonate buffer (pH 7.6, at 37 °C after gassing with 95% O_2:5% CO_2) containing 3.5% bovine plasma albumin (Armour Pharmaceuticals, Kankakee, Illinois, USA), glucose (5 mmol/l), and collagenase (3 mg) (Worthington Biochemicals, Millipore Corporation, Freehold, New Jersey, USA). After shaking for 1 h at 37 °C, the digested tissue was passed first through a 1000 micron and then through a 250 micron nylon mesh. Care was taken to maintain the adipocytes at 37 °C at all times. The cells were then centrifuged at 100 *g* in 16 × 100 mm polyethylene tubes (A/S Nunc, Roskilde, Denmark), the infranatant was removed, and an equal volume of Krebs-Ringer bicarbonate buffer at 37 °C was added. After resuspension by gentle inversion, the cells were recentrifuged and washed twice in the same manner into a Hepes buffer [(N-2-hydroxyethyl-piperazine-N'-2-ethane sulphonic acid) (10 mmol/l); NaCl, (135 mmol/l); KCl (4.8 mmol/l); $CaCl_2 \cdot H_2O$ (2.5 mmol/l); $MgSO_4$ (1.7 mmol/l); NaH_2PO_4 (1.0 mmol/l); pH 7.6, at 37 °C after gassing)] containing 5% bovine plasma albumin with bacitracin (0.5 mg/ml) (Sigma, St. Louis, Missouri) and either glucose (5 mmol/l) (for determination of insulin binding) or no glucose (see below, for determination of [^{14}C]-3-0-methyl-D-glucose transport or incorporation of U-[^{14}C]-D-glucose into [^{14}C]-CO_2 or [^{14}C]-lipids). Following final resuspension of the cells, an aliquot was fixed in 1% osmium tetroxide in collidine-HCl buffer for cell counting and size analysis by a modification of the method of Hirsch and Gallian [27].

Insulin binding

[^{125}I]-insulin was prepared to a Sp. act. of 150–200 μCi/μg from purified crystalline pork insulin (courtesy of Dr M. Root, Eli Lilly, Indianapolis, Indiana) as outlined by De Meyts [28], using stepwise addition of chloramine-T. Monocyte insulin binding was determined as previously described, using 1% bovine serum albumin (Sigma) [18]. Adipocyte insulin binding was determined by a modification of the method of Pedersen and Hjollund [5]. Isolated adipocytes (approximately 5×10^5/ml) were incubated in triplicate in a final volume of 0.5 ml Hepes buffer, containing 0.825 U/l [^{125}I]-insulin and 0, 5 or 625 mU/l unlabelled insulin. Non-specific binding was defined as [^{125}I]-insulin bound in the presence of 250 000 mU/l unlabelled insulin and was subtracted from all other values. Incubations were carried out with slow shaking at 37 °C for 1 h. Following incubation, duplicate 0.2 ml aliquots were removed from each tube and centrifuged at 10 000 *g* for 90 s through 0.2 ml silicone oil (Silwet 722, Union Carbide, Danbury, Connecticut) in a Beckman microcentrifuge (Palo Alto, California, USA). The portion of the centrifuge tube containing the cells, which float on the oil, was excised and its radioactivity was counted using a gamma counter (Searle, Des Plaines, Illinois, USA). Results were expressed as insulin bound specifically/2×10^5 adipocytes. Incubations carried out in this manner result in steady-state binding at 30–45 min which is maintained for at least 150 min.

[^{14}C]-3-0-methyl-D-glucose transport

Adipocyte glucose transport was assessed using [^{14}C]-3-0-methyl-D-glucose, a non-metabolizable glucose derivative [29, 30]. Transport of this analogue was determined following exposure in duplicate of isolated adipocytes (approximately 10^6/ml) to 0, 5 or 625 mU/l insulin in 16 × 100 mm polyethylene tubes in a final volume of 0.4 ml Hepes buffer (without glucose) at 37 °C with slow shaking for 1 h. Following exposure to insulin, triplicate 50 μl aliquots of cells were pipetted directly into 20 μl of 0.15 mol/l NaCl containing 0.4 μCi [^{14}C]-3-0-methyl-D-glucose (New England Nuclear Corporation, Cambridge, Massachusetts) and 0.4 μCi [^{3}H]-L-glucose (New England Nuclear) as a marker for non-specific cell-associated radioactivity. The final concentration of 3-0-methyl-D-glucose was 17.4 μmol/l. Reactions were terminated after 15 s by the rapid addition with a syringe pipettor of 8 ml cold 0.3 mmol/l phloretin (Sigma) in 0.15 mol/l NaCl to block further glucose transport. Approximately 2 ml silicone oil was layered onto the phloretin cell mixture, and the tubes were centrifuged at 1500 *g* for 30 s. The adipocytes, which float on top of the oil, were collected and placed in scintillation fluid (Safety Solve Research Products International, Mount Prospect, Illinois) for dual counting of tritium and carbon-14. Results corrected for non-specific cell associated radioactivity were expressed as pmol 3-0-methyl-D-glucose transported per 2×10^5 adipocytes in 15 s. Using this technique, transport rates were found to be linear for approximately 20 s following exposure to 3-0-methyl-D-glucose.

Glucose oxidation

Production of [^{14}C]-CO_2 from U-[^{14}C]-D-glucose (New England Nuclear) was determined by incubating isolated adipocytes (approximately 5×10^5 cells/ml) in duplicate in 16 × 100 mm polyethylene tubes in a volume of 0.5 ml Hepes buffer (without unlabelled glucose) containing 0.033 or 4.25 nmol/l insulin and labelled glucose (1 μCi, final concentration 5.6 μmol/l) at 37 °C with slow shaking for 90 min. After the addition of adipocytes, tubes were sealed with rubber stoppers through which plastic centre wells were suspended. After incubation, 0.2 ml H_2SO_4 (0.5 mol/l) was injected through the stopper into the cell suspension to liberate [^{14}C]-CO_2, and 0.2 ml 1 N hyamine hydroxide was injected through the stopper into the centre well for collection of [^{14}C]-CO_2. After radioactivity was collected overnight, the centre wells were removed and placed in scintillation fluid for counting. Results were expressed as pmol glucose converted to $CO_2/2 \times 10^5$ cells in 90 min. [^{14}C]-CO_2 produced by tissue blanks was less than 1% of values in the presence of adipocytes.

Incorporation of glucose into lipids

Incorporation of U-[^{14}C]-D-glucose into total adipocyte lipids was determined by incubating isolated adipocytes (approximately 5×10^5 cells/ml) in duplicate in 16 × 100 mm polyethylene tubes in a volume of 0.5 ml Hepes buffer (without unlabelled glucose) containing 0, 0.033 or 4.25 nmol/l insulin and labelled glucose (1 μCi) at 37 °C with slow shaking for 90 min. After the incubation, total lipids in 0.4 ml aliquots of cell suspension from each tube were extracted in chloroform-methanol [31]. The resulting organic phase was dried with air and redissolved in scintillation fluid for counting. [^{3}H]-triolein (0.1 μCi, New England Nuclear) was added to each extraction tube to determine extraction recoveries. These recoveries ranged from 75 to 95%, and all data were corrected accordingly.

Table 1. Glucose infusion rates necessary to maintain euglycaemia during 6-h insulin infusions

	Time (h)					
	1	2	3	4	5	6
Glucose infusion rates ($mg \cdot kg^{-1} \cdot min^{-1}$)	6.07 ±0.99	6.72 ±0.86	7.6 ±0.67	8.66 ±0.66	9.28 ±0.64	9.47 ±0.68

Results expressed as mean ± SEM for nine experiments

Table 2. Effect of hyperinsulinaemia on monocyte and adipocyte insulin binding

	Insulin binding	
	(5 mU/l)[a]	(625 mU/l)[a]
Monocytes (% per 10^7 cells)		
Saline infusion ($n=4$)		
Before	8.9 ± 0.6	1.4 ± 0.1
After	9.0 ± 1.1	1.6 ± 0.3
Insulin infusion ($n=9$)		
Before	7.2 ± 0.6	1.3 ± 0.1
After	6.2 ± 0.6[b]	1.1 ± 0.1[b]
Adipocytes (% per 2×10^5 cells)		
Saline infusion ($n=4$)		
Before	4.0 ± 0.8	0.7 ± 0.1
After	3.8 ± 0.8	0.7 ± 0.2
Insulin infusion ($n=9$)		
Before	4.6 ± 0.5	0.8 ± 0.1
After	4.4 ± 0.4	0.8 ± 0.1

Results expressed as mean ± SEM; [a] concentration of insulin present in binding assay; [b] $p < 0.05$, significant change from before insulin infusion

Plasma glucose and insulin determinations

Plasma glucose and insulin determinations were made on arterialized-venous specimens using a glucose analyzer (Yellow Springs Instruments, Yellow Springs, Ohio) and a dextran-coated charcoal technique [32], respectively.

Statistical analyses

Data are given as mean ± SEM. Statistical evaluation was performed using two-way analysis of variance for repeated measures and posteriori t-tests [33]. A p value < 0.05 was considered significant.

Results

Plasma glucose and insulin concentrations

Baseline plasma glucose and insulin concentrations prior to infusions of insulin and saline were comparable (5.3 ± 0.1 versus 5.2 ± 0.1 mmol/l and 10 ± 1 versus 11 ± 1 mU/l). During infusion of insulin, plasma insulin increased to a stable concentration of 140 ± 13 mU/l, and plasma glucose was maintained at 5.3 ± 0.1 mmol/l (coefficient of variation 5%). During infusion of saline, plasma glucose and plasma insulin averaged 5.3 ± 0.1 mmol/l and 8 ± 1 mU/l, respectively. Glucose infusion rates necessary to maintain euglycaemia are given at hourly intervals in Table 1. Glucose infusion rates increased progessively until reaching near-steadystate between 4 and 5 h. Values at 4 h were not significantly different from those at the end of the 6-h infusion.

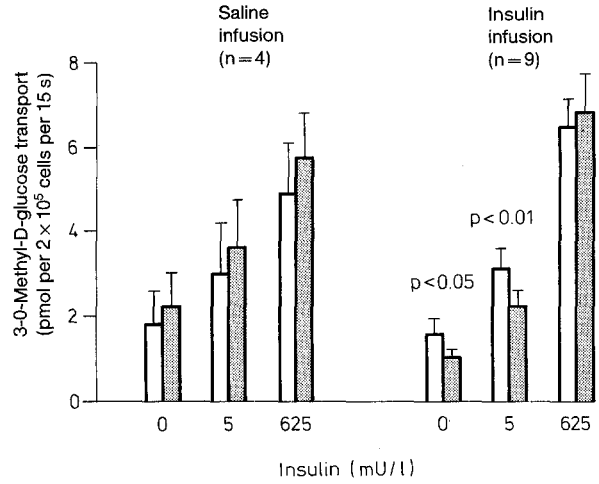

Fig. 1. Effect of infusion of insulin or saline in normal volunteers on adipocyte glucose transport in vitro. □ : before infusion; ▨ : after infusion (mean ± SEM)

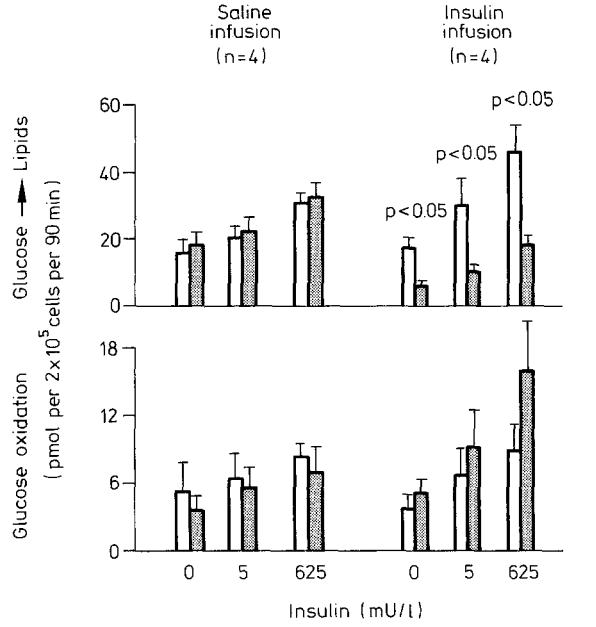

Fig. 2. Effect of infusion of insulin or saline in normal volunteers on adipocyte lipogenesis and glucose oxidation in vitro. □ : before infusion; ▨ : after infusion (mean ± SEM)

Monocyte and adipocyte insulin binding

Monocyte insulin binding decreased 15% after infusion of insulin and did not change after infusion of saline ($p < 0.05$). In contrast, neither infusion of insulin nor infusion of saline altered adipocyte insulin binding or di-

ameter (mean ± SEM: 91 ± 2 versus 90 ± 3 μm before and after insulin; 87 ± 11 versus 90 ± 11 μm before and after saline; Table 1).

Adipocyte glucose transport

After infusion of insulin, glucose transport in the absence of insulin and at a submaximally effective insulin concentration (5 mU/l) were both decreased approximately 30% ($p < 0.05$ and 0.01, respectively), whereas glucose transport at a maximally effective insulin concentration (625 mU/l) was unaffected (Fig. 1). Since basal (0 mU/l insulin) glucose transport rates were decreased following infusion of insulin, glucose transport data were expressed in terms of increment above basal per pmol insulin bound. When these data were analyzed, the increment in glucose transport at the submaximally effective insulin concentration (5 mU/l) was still significantly decreased following hyperinsulinaemia (720 ± 120 versus 1140 ± 180 pmol/pmol insulin bound, $p < 0.05$). After infusion of saline, glucose transport in the presence or absence of insulin was not decreased.

Adipocyte glucose conversion to lipids and glucose oxidation

Conversion of glucose to lipids was decreased more than 50% after infusion of insulin in the absence of insulin and at both submaximally and maximally effective insulin concentrations ($p < 0.05$; Fig. 2); no change was observed after infusion of saline. In contrast, both basal and insulin-stimulated adipocyte glucose oxidation were unaltered after infusion of insulin or saline.

Discussion

In the present study, hyperinsulinaemia decreased monocyte insulin binding but did not alter adipocyte insulin binding. Furthermore, the hyperinsulinaemia decreased adipocyte glucose transport and conversion of glucose to lipids but did not affect glucose oxidation. These results indicate that hyperinsulinaemia may cause insulin resistance and that, at least initially, this insulin resistance is due to cellular abnormalities not involving the insulin receptor, i.e., a so-called post-receptor defect. It is possible that more prolonged hyperinsulinaemia may have resulted in decreased adipocyte insulin binding, since other workers found that prolonged experimentally-induced hyperinsulinaemia decreased adipocyte insulin binding in rats [22, 23].

The present study confirms that short-term hyperinsulinaemia can decrease monocyte insulin binding [16–18]. Similar insulin-induced decreases in erythrocyte insulin binding have been reported in some [18, 19] but not all studies [16]. However, in the present study, adipocyte insulin binding was not affected by short-term hyperinsulinaemia. Pedersen and Hjollund [5] also found a dissociation between monocyte and adipocyte insulin binding. Taken together, these observations suggest that monocyte insulin binding, which is commonly thought to reflect insulin binding in insulin-sensitive tissues, may not always do so.

Theoretically it is possible that a small decrease in adipocyte insulin binding may not have been detected in the present study. However, it is unlikely for several reasons, that such a decrease, if present, could account for the observed impairment in glucose metabolism. A decrease in insulin binding alone would have been expected to result in a decrease in intracellular glucose metabolism which was proportional to an accompanying decrease in glucose transport. However, in the present study, insulin stimulated glucose oxidation was not decreased whereas glucose transport was. Moreover, in the absence of added insulin both glucose transport and conversion to lipids were decreased, further suggesting that short-term hyperinsulinaemia had produced a cellular defect. It should be pointed out though, that some of the decrease in glucose conversion into lipids may have been due to anti-lipolysis induced by the insulin infusion. This could have decreased the availability of intracellular glucose-derived glycerol into lipids.

The finding of a metabolic defect induced by insulin in vivo, which could not be accounted for by a decrease in insulin binding, is in accord with studies in vitro demonstrating that exposure of rat adipocytes and myoblasts to increased insulin concentrations can produce post-receptor defects in glucose metabolism [12, 15]. Conventionally, an abnormality in insulin action is referred to as a receptor defect if there is decreased insulin receptor binding and is considered a post-receptor defect if subsequent events are involved. Such events may include physico-chemical alteration of the receptor, generation of an intracellular mediator and responses of cellular enzymes or organelles to the mediator. The fact that both basal and insulin-stimulated glucose transport were decreased following infusion of insulin suggest that certain cellular processes distal to insulin receptor binding (e.g. availability of glucose transporters) may have been adversely affected by the antecedent hyperinsulinaemia in such a way as to render them less responsive to insulin, i.e. a post-receptor defect.

Our findings have implications concerning the pathological sequence of events leading to the association of hyperinsulinaemia and decreased insulin binding in obesity. It is currently thought that in obesity [1–4], hyperinsulinaemia due to over-eating may initially cause insulin resistance by down-regulating insulin receptors and that, as insulin resistance progresses, a post-receptor defect may emerge. Our results suggest that hyperinsulinaemia may first cause a post-receptor defect. A non-rate-limiting post-receptor defect in insulin action would result in only a shift to the right of an insulin dose-reponse curve [37–42]. Thus, if subsequent down-regulation of insulin receptors were to occur, a modest post-receptor defect might not be detected even

when the biological responses to insulin were related to insulin receptor occupancies. This might explain the apparent presence of only a receptor defect in many hyperinsulinaemic patients with obesity and Type 2 diabetes [1–4]. Similarly the decreased insulin receptor binding found in some hyperinsulinaemic patients with Type 1 [5] or Type 2 diabetes [1, 37, 43], in association with a post-receptor defect, could be a later event which has been preceded by a post-receptor defect. Such a postulate is supported by the observation that post-receptor defects may occur in diabetic patients in the apparent absence of decreased adipocyte insulin binding [37]. Moreover, down-regulation of insulin binding by insulin is considered to be dependent upon post-receptor actions of insulin [6, 44–46].

It should be pointed out that in the present studies glucose was infused along with insulin to maintain euglycaemia. However, according to current knowledge [47] only trivial amounts of the glucose infused in the present studies would have been taken up by adipose tissue. In any study of induced hyperinsulinaemia in man, such an infusion of glucose is a necessary but potentially confounding variable, since increased glucose uptake itself may influence the outcome of such experiments. However, the increased glucose uptake is a direct effect of hyperinsulinaemia so it is not clear whether the mechanisms for changes induced by these intimately linked events can, or should, be differentiated.

Finally, our finding that hyperinsulinaemia may cause insulin resistance provides an explanation why insulin therapy may not always improve [48, 49] or completely normalize [50] insulin resistance in Type 2 diabetes. Although insulin therapy may correct defects in insulin action due to insulin deficiency [50], if it results in hyperinsulinaemia, it may conceivably itself produce insulin resistance.

In summary, the present studies indicate that hyperinsulinaemia can cause insulin resistance in man and that, at least initially, this is due to a post-receptor defect. Thus decreased insulin receptor binding, when accompanied by hyperinsulinaemia and a post-receptor defect, may be a secondary event.

Acknowledgements. We thank J. Kahl, J. King, T. Lund, R. Westland, W. Blanchard, K. Kluge, and the staff of the Clinical Study Unit for their excellent technical assistance, and P. Voelker for her editorial help. Funded in part by grants from the United States Public Health Service (AM-20411, AM-07352, RR00585, AM-29953), the American Diabetes Association and the Mayo Foundation. Dr. Mandarino is the recipient of the Rosalyn Yalow Research and Development Award from the American Diabetes Association.

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Received: 14 July 1983
and in revised form: 12 June 1984

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