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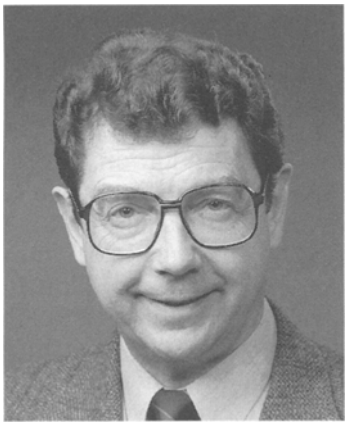
## John Wahren
## Minkowski Award, 1977, Geneva



John Wahren was born in 1937 in Uppsala, Sweden. He received his M.D. from the medical school at the Karolinska Institute, Stockholm in 1963. He trained as an intern and resident in clinical physiology and internal medicine at the Serfimer Hospital, Stockholm from 1964 to 1972. He presented his academic thesis in 1966 on the regulation of forearm muscle blood flow and oxygen uptake during physical exercise. He completed one year of postdoctoral training at the Joslin Clinic, Harvard Medical School, Boston, Mass., USA in 1968, participating in studies on the metabolic adaption to fasting. Upon returning to Sweden he was appointed Professor of Clinical Physiology at the Karolinska Institute and head of the corresponding Department at the Serafimer Hospital (1973) and subsequently at Huddinge Hospital (1976). Dr. Wahren enjoyed a sabbatical year at the Institute de Physiologie, Université de Lausanne, Switzerland from 1981 to 1982, learning methods for the evaluation of energy expenditure and participating in studies on the regulation of thermogenesis.

John Wahren's scientific effort mainly concerns problems related to clinical metabolism and energy expenditure in healthy humans in the basal state, during fasting and during physical exercise and in patients with Type 1 and 2 diabetes, obesity, hyperthyroidism, cirrhosis, uraemia, etc. Dr. Wahren is currently Professor of Clinical Physiology at the Karolinska Hospital, Stockholm.

# Does C-peptide have a physiological role?

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**Summary** Short-term administration of physiological amounts of C-peptide to patients with insulin-dependent diabetes was found to reduce the glomerular hyperfiltration in these patients as well as augment whole body glucose utilization. It could also be shown that C-peptide administration increases blood flow, oxygen uptake and capillary diffusion capacity of exercising forearm muscle in IDDM patients, probably by increasing capillary recruitment in the working muscle. Studies under in vitro conditions have shown that C-peptide stimulates glucose transport in skeletal muscle with its maximal effect within the physiological concentration range. The findings in a clinical study in which IDDM patients were given C-peptide and insulin or insulin alone for 4 weeks in a double-blind randomized study design, indicate that C-peptide improves renal function by reducing urinary albumin excretion and glomerular filtration, decreases blood retinal barrier leakage and improves metabolic control. Preliminary findings suggest that C-peptide administration on a short-term basis (3 h) may ameliorate autonomic neuropathy by restoring to near normal the heart rate variability in response to expiration and inspira-

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*Abbreviations:* IDDM, Insulin-dependent diabetes mellitus; GFR, glomerular filtration rate; NEFA, non-esterified fatty acids; CDC, capillary diffusion capacity.

tion. Insight into a possible mechanism of action of C-peptide is provided by the finding that C-peptide stimulates $Na^+K^+$-ATPase activity in renal tubular segments. In conclusion, the present results suggest that, contrary to the prevailing view, C-peptide possesses important physiological effects. [Diabetologia (1994) 37 [Suppl 2]: S 99–S 107]

**Key words** Blood-retinal barrier function, capillary diffusion capacity, glucose utilization, kidney function, insulin-dependent diabetes mellitus.

## Background

It was not until approximately 45 years after the discovery of insulin that its biosynthesis in the beta cell of the islets of Langerhans became known in some detail. Steiner et al. [1, 2] found that a single-chain polypeptide, proinsulin, is synthesized in the endoplasmatic reticulum and subsequently converted to proinsulin and stored in the Golgi apparatus [3, 4]. Proinsulin is then cleaved by membrane-bound proteases into equimolar amounts of insulin and connecting peptide (C-peptide). The peptides are stored in secretory granules which are transported to the cell membrane and their contents are eventually released to the circulation by exocytosis.

Since the discovery of proinsulin and C-peptide, it has generally been held that the main role of C-peptide is to facilitate the folding of the proinsulin molecule in a manner that allows the formation of the disulphide bonds between the cysteine amino acid residues of the A and B chains of the insulin molecule [4]. Subsequent to its discovery, C-peptide was screened with regard to possible metabolic effects. Specifically, insulin-like effects of C-peptide were looked for in rat adipose tissue and none were found [5–8]. Only a few studies were carried out in humans and with negative results [9, 10]. The C-peptide molecular structure varies greatly between different species with regard both to its chain length and its amino acid sequence [4], a fact that together with the negative experimental results have tended to support the view that C-peptide does not exert biological effects.

Since the 1970s C-peptide measurements have been used extensively in the clinical evaluation of residual insulin secretion in patients with IDDM [11, 12]. Specifically, C-peptide has been a most important research tool in the study of the natural course of beta-cell destruction in IDDM and of therapeutic interventions to arrest or delay this process.

Our interest in possible physiological effects of C-peptide stems from the following considerations. Some patients with IDDM maintain a level of pancreatic beta-cell activity for many years after onset of the disease [13, 14]. Residual insulin secretory activity in these patients has been suggested to be associated with improved blood glucose control in comparison with patients without such activity [15, 16]. Likewise, early signs of microvascular lesions are observed more frequently in patients without signs of residual beta-cell activity [14]. In addition, a negative relationship has been demonstrated between endogenous beta-cell activity and early signs of diabetic retinopathy, as evaluated by vitreous fluorophotometry; this correlation was more marked than that between glycaemic control and early retinopathy [17]. The above findings have generally been interpreted to indicate that the remaining endogenous insulin levels exert a beneficial effect on glycaemic control and a protective influence with regard to development of diabetic complications. While this may be true it is not immediately apparent why endogenous insulin should be more beneficial than exogenous, and one may consider another aspect of the above findings. Residual beta-cell activity implies not only remaining insulin secretion but also preserved production of C-peptide. We may therefore consider the theoretical possibility that C-peptide rather than the endogenous insulin might exert a beneficial influence on both blood glucose control and the development of microvascular complications in IDDM.

In view of the above considerations, we have undertaken a series of studies involving short-term (1–2 h) and long-term (4 weeks) administration of C-peptide as well as in vitro measurements in order to examine the possibility that C-peptide may not be biologically inert.

## C-peptide and kidney function

It is well established that patients with IDDM show glomerular hyperfiltration during the first years after onset of the disease [18–20], yet the underlying mechanism is not known. The hyperfiltration is not readily corrected by insulin administration [21] and it is present in patients with insulin-dependent but not in non-insulin-dependent diabetes. Since the latter patients but not the former have circulating levels of C-peptide and in view of the fact that a major proportion of C-peptide uptake and catabolism occurs in the kidney [22–24], the question could be raised as to whether C-peptide may modify renal function and the GFR.

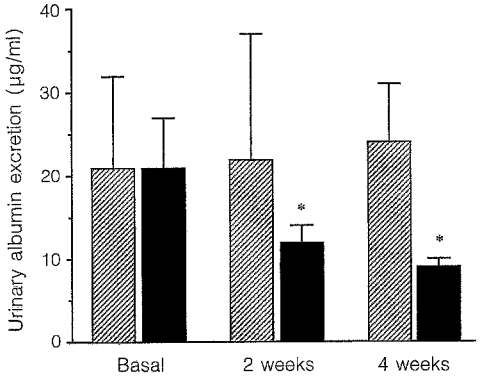


**Fig. 1.** Urinary albumin excretion in the C-peptide group (■) and the insulin-treated group (▨) before and after 2 and 4 weeks of treatment. * The probability that the difference from the basal value is caused by random factors, $p < 0.05$. Mean values ± SEM are presented

With this background the influence of C-peptide infusion on renal function was studied in a group of IDDM patients [25]. They were young individuals [18–26 years] who had had diabetes for less than 15 years; they were C-peptide negative and had no signs of diabetic complications but showed glomerular hyperfiltration. The patients were given an i.v. insulin infusion during the night before the study and were euglycaemic at the time of the examination. The GFR and effective renal plasma flow were measured by clearance techniques using constant-rate infusions of inulin and sodium para-aminohippurate. After baseline measurements C-peptide was infused in 11 patients during two 60-min periods at rates of 5 and 30 pmol · $kg^{-1}$ · $min^{-1}$. Biosynthetic human C-peptide (Eli Lilly Co, Indianapolis, Ind., USA) was used. The purity of the substance as determined by HPLC analysis was greater than 99 %. In a control study 0.9 % NaCl was infused during two 60-min periods in 10 IDDM patients. The GFR was found to decrease by 7 % ($p < 0.001$), and effective renal plasma flow increased by 3 % ($p < 0.05$) during infusion of C-peptide at the lower rate, which resulted in plasma C-peptide levels of approximately 0.8 nmol/l. When the infusion rate was increased six-fold the plasma concentration rose to about 2.1 nmol/l but no further changes in GFR or effective plasma flow were observed. The patient group infused with NaCl showed an unaltered GFR and effective renal plasma flow.

It is noteworthy that the influence of C-peptide on renal function had already occurred with the low-dose infusion, which had been estimated to restore basal physiological concentrations of C-peptide. Only a small further decrease in GFR took place when the C-peptide infusion rate was increased and the plasma concentration rose almost three-fold. Thus, a dose-response relationship could not be established between GFR and plasma C-peptide concentrations in diabetic patients. The possibility should be considered that the experimental procedure itself, by virtue of the prolonged study period (5 h) during which the subjects rested in the supine position without ingestion of food, and/or the slow rate of insulin infusion (0.5 mU · $kg^{-1}$ · $min^{-1}$) may have influenced renal function. However, in the patients who received NaCl rather than C-peptide under otherwise identical conditions, no change in GFR could be observed. In addition, four patients who participated in both study groups all had a greater fall in GFR during low-dose C-peptide infusion compared with 60 min of NaCl infusion ($p < 0.05$). These observations thus support the notion that C-peptide does in fact exert a direct effect on renal function in IDDM patients, at least during short-term infusion.

The observations regarding renal function during short-term infusion of C-peptide have been extended to include more prolonged administration of C-peptide. In a double-blind randomized prospective study patients with IDDM received either insulin plus equimolar amounts of C-peptide or insulin alone by subcutaneous pump infusion for 4 weeks [26]. The patients were monitored with regard to several variables, notably GFR and urinary albumin excretion. All patients showed glomerular hyperfiltration (142–147 ml · $min^{-1}$ · 1.73 $m^2$) and the average microalbuminuria was 21±6 µg/ml at the start of the study. In the C-peptide treated group the GFR decreased by on average 6 % after 2 weeks and remained at this level during the remainder of the study period. In contrast, the group receiving insulin only exhibited no change in GFR during the course of the study. Moreover, the group given C-peptide showed a reduction in the level of albumin excretion to approximately half of the basal value, while no significant change was seen in the control group (Fig. 1). These observations thus confirm and extended the previous results with regard to short-term effects of C-peptide [25].

The above findings probably reflect a direct influence of C-peptide on renal function but the possible mechanism behind this effect is not apparent. It should also be considered that in the long-term study there was a simultaneous improvement in metabolic control in the C-peptide group, as evidenced by reduced $HbA_{1c}$ and fructosamine concentrations (see further), which may have contributed to the improvement in renal function. However, a similar decrease in GFR was also seen during short-term C-peptide infusion, despite the fact that blood glucose concentrations were clamped at constant levels [25]. In addition, the observed improvements in renal function were not significantly correlated to changes in any of the variables describing glycaemic control. Other factors such as a diminished blood pressure may have played a role, but blood pressure measurements did not indicate a drop in the C-peptide treated group [26].

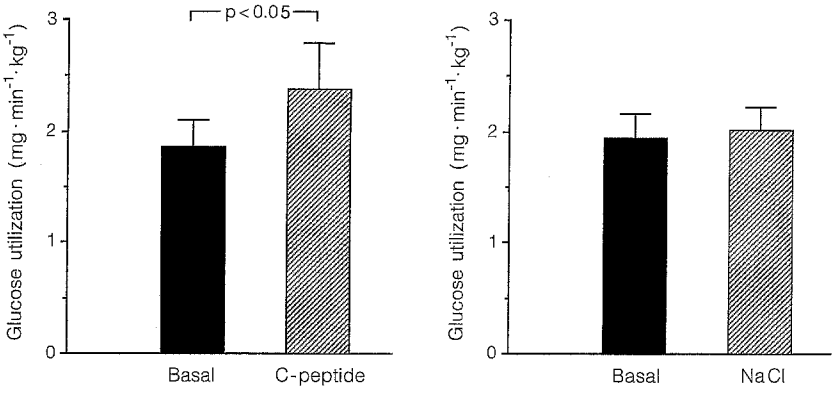


**Fig. 2.** Glucose utilization in IDDM patients before and during 60 min of C-peptide infusion (left panel) and before and during 60 min of NaCl infusion (right panel). Mean values ± SEM are presented

## C-peptide and blood-retinal barrier function

The findings presented here regarding C-peptide and microalbuminuria suggest that C-peptide may influence the function of the glomerular membrane and, hence, possibly also other membranes or barrier functions related to the diabetic disorder. To examine this possibility we included measurements of the leakage of fluid across the blood-retinal barrier in the study involving 4 weeks' administration of C-peptide described above [26]. The diabetic patients underwent vitreous fluorophotometry [27] before the start of the study and after 4 weeks of treatment. The results show that fluorescein leakage across the blood-retinal barrier decreased from 88±16–63±16 ng/ml ($p< 0.05$) in the C-peptide treated group, whereas it remained unchanged in the group that received insulin only (41±7 and 47±11 ng/ml, NS) [26].

The vitreous fluorophotometry technique provides a sensitive measurement of the blood-retinal barrier function and an early indication of developing retinopathy [28, 29]. The present results indicating that C-peptide may improve blood-retinal barrier function could thus be of interest in the pathogenesis of diabetic eyeground changes. This formulation receives support from the observation that there is a significant negative relationship between blood-retinal barrier function and fasting C-peptide levels in young IDDM patients [17]. However, it is recognized that by chance the baseline values for the groups in the study by Johansson et al. [26] were somewhat different with regard to blood-retinal barrier leakage prior to the study, the C-peptide treated group showing a greater leakage at the onset of the study compared to the control groups. The possibility cannot be excluded that the difference in baseline values may have influenced the outcome. In this context it should also be noted that the blood-retinal barrier function is reported to deteriorate during the initial phase of insulin pump treatment [30]. In the current investigation 12 of the patients had not been on insulin pumps prior to the study (6 in each group). Nevertheless, a significant improvement in blood-retinal barrier function could be demonstrated in the C-peptide group. Finally, as in the case of the improvements in renal function, it cannot be ruled out that the amelioration in glycaemic control in the C-peptide group may have contributed to the decrease in the blood-retinal barrier leakage of fluid [31].

## C-peptide and glucose metabolism

*Glucose utilization in vivo.* In the experiments described above, where C-peptide was infused in IDDM patients at two different infusion rates (5 and 30 pmol · $kg^{-1}$·$min^{-1}$) in order to evaluate its effect on kidney function [25], the peripheral glucose utilization rate could also be evaluated. Insulin was infused at a low rate and the blood glucose level was kept constant by means of a variable glucose infusion and blood glucose measurements every 5 min. The rate of glucose infusion had to be increased by approximately 25 % during the low-dose C-peptide infusion in order to maintain an unchanged blood glucose level, but could be kept unaltered during the corresponding time period with NaCl infusion (Fig. 2). The high-dose C-peptide infusion resulted in a further 15 % rise in glucose utilization. The arterial insulin levels remained unchanged during this period, and it is unlikely that the observed increase in glucose utilization was caused by insulin stimulation. These findings suggest that C-peptide exerts a stimulatory effect on whole body glucose utilization in IDDM patients, although the possibility of an inhibition of hepatic glucose production by C-peptide could not be excluded.

In another study involving rhythmic forearm exercise in patients with IDDM, i.v. C-peptide administration was found to induce a marked increase in glucose uptake by exercising muscle [32]. Patients given NaCl instead of C-peptide, as well as healthy control subjects receiving C-peptide or NaCl, showed largely unaltered glucose exchange during a corresponding exercise period (Fig. 3). Even though the baseline

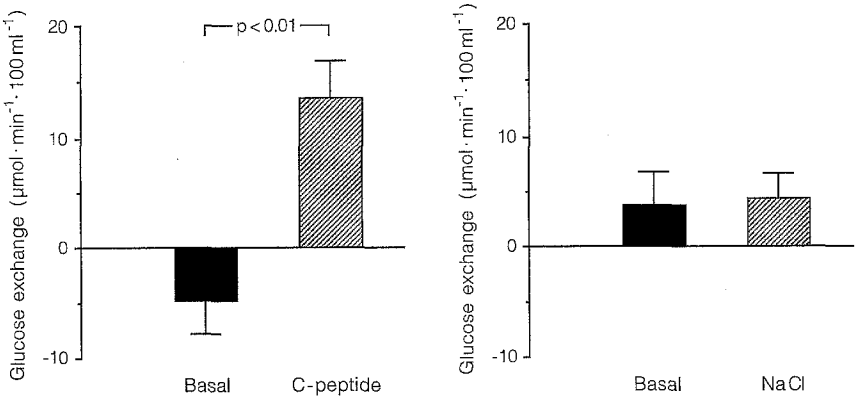


**Fig. 3.** Glucose exchange across the exercising forearm before and during C-peptide infusion (left panel) or NaCl infusion (right panel) in patients with IDDM. Mean values ± SEM are presented

values in these studies were variable, the above results thus confirm a stimulatory effect of C-peptide on glucose utilization in skeletal muscle and demonstrate that this effect becomes quite marked during exercise, perhaps as a consequence of the increased blood flow and augmented number of open capillaries.

The effect of C-peptide on carbohydrate metabolism in IDDM patients has previously been evaluated following a small, single i.v. C-peptide injection (24–36 nmol) in diabetic and control subjects. No significant changes in the concentration of glucose, lactate, alanine, $\beta$-hydroxybutyrate, glycerol or NEFA were observed in this report [9]. However, infusion of rat C-peptide in pharmacological doses has been found to increase and prolong the hypoglycaemic effect of exogenous insulin in alloxan diabetic rats [33]. The latter finding taken together with the observed effects of physiological concentrations of C-peptide on the peripheral glucose utilization rate in IDDM patients, raised the possibility that C-peptide may affect glucose homeostasis in diabetic individuals.

*Blood glucose control.* In order to examine the possible effects of C-peptide on blood glucose control during prolonged administration, a clinical trial over a 1-month period was undertaken in IDDM patients, as presented here [26]. Blood samples for determination of glycaemic control were collected in the fasting state before the beginning of the study and after 2 and 4 weeks during the study. Blood glucose at the onset of the study was 9.6±1.6 mmol/l in the group receiving C-peptide (Group 1) and 9.4±2.0 mmol/l in the group treated with insulin alone (Group 2); during the study the glucose concentration tended to fall by approximately 20 % in Group 1 ($p < 0.1$) and increased by 26 % in Group 2 ($p < 0.05$), which resulted in a significant inter-group difference in blood glucose concentration at the end of the study. $HbA_{1c}$ and fructosamine values both decreased by 9–16 % in Group 1 ($p < 0.05$) but not in Group 2. Thus, glycaemic control was improved in the IDDM patients treated with equimolar amounts of C-peptide and insulin, while the patients receiving insulin alone showed no improvement.

*Glucose transport in vitro.* In view of the above findings regarding a stimulation by C-peptide on in vivo peripheral glucose utilization, it was of interest to further define the interaction between C-peptide and glucose transport in skeletal muscle. The effect of increasing concentrations of C-peptide or insulin on the rate of 3–0-methylglucose transport in incubated human muscle strips was studied under in vitro conditions [34]. Muscle specimens were obtained with an open muscle biopsy technique from the vastus lateralis portion of the quadriceps femoris muscle in healthy male subjects and incubated in vitro [35]. C-peptide stimulated the rate of 3–0-methylglucose transport in a dose-dependent manner (Fig. 4). The lowest concentration of C-peptide (0.5 nmol/l), did not significantly alter the rate 3–0-methylglucose transport, but when the medium concentration of C-peptide was increased to 1.0 or 2.5 nmol/l, the rate of basal glucose transport was increased by more than 60 %. In the presence of insulin, glucose transport increased 1.8-fold over the basal rate with a maximum effect obtained at 0.3 nmol/l of insulin. In keeping with the above findings the muscle strips exposed to C-peptide demonstrated glycogen levels which were 22 % higher than the basal values after a 2-h in vitro incubation [34].

Muscle strips from a group of well-controlled patients with IDDM has been demonstrated to respond to insulin with an increase in the rate of 3–0-methylglucose transport which is comparable to that observed for samples from young age-matched, control subjects [36]. Thus, at the level of glucose transport, muscle strips obtained from well-controlled IDDM patients display no sign of insulin resistance. Despite the chronic deprivation of endogenous C-peptide which is at hand in IDDM, an in vitro exposure of skeletal muscle strips from these patients to a low physiological (0.6 nmol/l) concentration of C-peptide increased the basal rate of glucose transport by approximately 60 % [36].

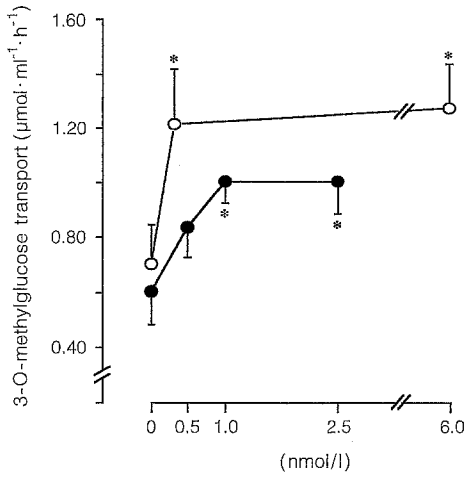


**Fig. 4.** Dose-response curves for insulin (○) or C-peptide (●) stimulated human skeletal muscle strips. * $p < 0.05$ vs non-stimulated skeletal muscle strips. Values are expressed per ml of intracellular water. Data are means ± SEM for 5–7 muscle strips

The above findings demonstrate that physiological concentrations of C-peptide stimulate glucose transport in healthy human skeletal muscle in a dose-dependent manner with a $V_{max}$ at approximately 1.0 nmol/l. In addition, insulin-sensitive muscle from IDDM patients responded to C-peptide in a similar fashion to healthy muscles. The increase in skeletal muscle glycogen content following a maximal C-peptide stimulation was similar in magnitude when compared to the effect of 0.6 nmol/l of insulin. Thus, the observed increase in glucose transport and glycogen content of the in vitro incubated human skeletal muscle strips provides evidence for a regulatory role of C-peptide in the muscular glucose transport process.

The mechanism by which C-peptide induces an increase in muscular glucose transport appears at least in part to operate via the insulin-mediated glucose transport pathway. A combination of maximal concentrations of insulin and C-peptide does not elicit an additive effect on the glucose transport rate in incubated muscle strips obtained from healthy young individuals [36].

## C-peptide and skeletal muscle circulation

The influence of C-peptide administration on skeletal muscle blood flow, oxygen uptake and CDC has been examined in IDDM patients. A double indicator technique based on the administration of $^{51}$Cr-EDTA and indocyanine green [37] was employed to determine forearm blood flow and CDC of the exercising forearm before and during either C-peptide administration (6 pmol · kg$^{-1}$ · min$^{-1}$) or NaCl infusion [32]. Teflon catheters were inserted percutaneously into a brachial artery and a deep forearm vein in the retrograde direction. The patients performed strenous rhythmic exercise on a hand ergometer during 5-min periods. The two indicators were injected as a bolus into the brachial artery after 4–5 min exercise and the washout was followed in blood samples obtained each second from the deep forearm vein for 30 s. Blood flow was calculated from the dilution curve of the plasma-bound indicator (indocyanine green) and the extraction of the permeable tracer, estimated from the difference between the two washout curves, was used in the calculation of CDC [37].

The diabetic subjects showed initially approximately 30 % lower blood flow and CDC of the exercising forearm as compared to age- and sex-matched healthy control subjects. During i.v. C-peptide administration in the patients resulting in a rise in C-peptide concentration to physiological levels (approximately 1 nmol/l) both forearm blood flow and CDC rose to values similar to those for the healthy control subjects (Fig. 5). This was a consistent finding, all patients showing a similar response. In contrast, in the healthy control subjects, who showed higher blood flow and CDC before infusion compared to the diabetic patients, C-peptide exerted no significant influence on blood flow. Likewise, NaCl infusion in the diabetic patients did not influence either forearm blood flow or CDC.

These findings are consistent with the view that in C-peptide negative diabetic patients replacement of this peptide elicits microcirculatory effects, while in healthy subjects increments of C-peptide levels above the endogenous basal concentration do not exert similar effects. In parallel with the arteriolar dilatation and the increase in blood flow in exercising forearm muscle an augmentation of the CDC was seen in the patients infused with C-peptide. CDC expresses the total unidirectional flux of tracer over the exchange vessels and it is thus a reflection not only of the number of perfused capillaries (capillary surface area), which is regulated by the precapillary sphincters, but also of the permeability per unit surface area of the capillary membrane. By measurements of CDC for one tracer molecule only, it is not possible to determine whether the enhanced CDC in the C-peptide-treated diabetic patients reflects an increase in the number of perfused capillaries, an increase in the vascular permeability per se, or a combination of the two. The finding that the forearm muscle extraction of $^{51}$Cr-EDTA increased during C-peptide administration in the face of a marked rise in blood flow suggests the possibility that capillary permeability may have increased. On the other hand, a rise in forearm uptake of oxygen and glucose as well as relatively greater production of lactate by the forearm tissues was also observed in the diabetic patients receiving C-peptide. Since neither oxygen nor glucose or lactate are likely to be restricted in their passage over the capillary membrane, it seems unlikely

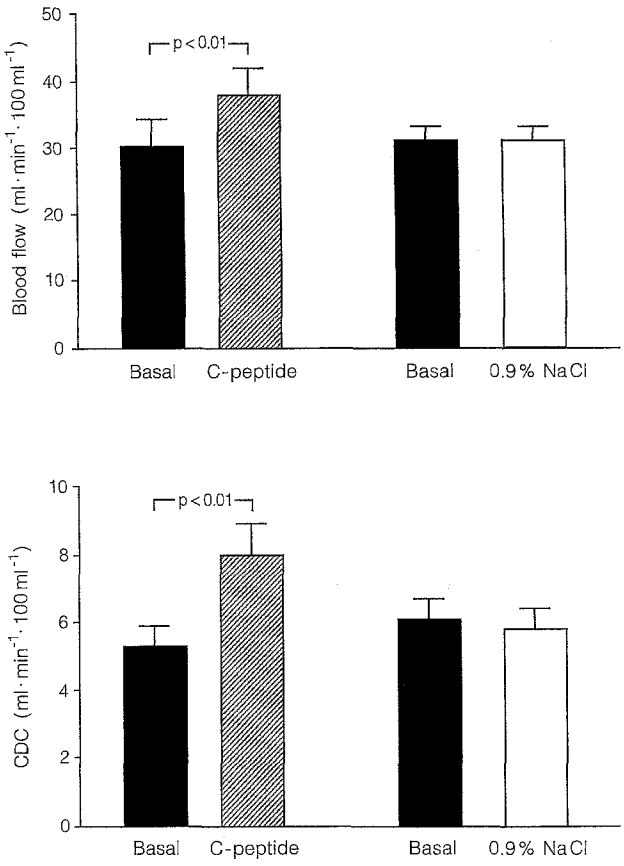


**Fig. 5.** Forearm blood flow (upper panel) and capillary diffusion capacity (CDC) (lower panel) during exercise before (■) and during infusion of C-peptide (▨) or NaCl (□) in patients with IDDM. Mean values ± SEM are presented

that an increase in the capillary permeability alone would enhance their uptake or release. The more likely explanation is that C-peptide administration resulted in an increase in recruitment of capillaries in the muscle. Thus, the present circulatory and metabolic results taken together are compatible with the view that C-peptide increases the perfusion to compartments within the exercising skeletal muscle, which may have been poorly or not at all perfused in the diabetic patients before C-peptide administration.

The precise mechanism whereby C-peptide stimulates blood flow and increases CDC remains to be determined. In fact, the physiological variables that regulate the functional hyperaemia in skeletal muscle during contraction have not been fully elucidated, as described in a recent review [38]. It is possible that the effects of C-peptide may be exerted via a specific receptor, since there is a significant uptake of this peptide to the forearm muscle in the resting state and a release during exercise [32]. This finding may possibly indicate that C-peptide is bound to receptors in the resting state and subsequently released from the muscle during exercise. Similar findings have been described previously for insulin [39]. A C-peptide receptor in skeletal muscle has not been demonstrated but a specific receptor for homologous C-peptide has been found in rat beta cells [40].

## Mechanism of C-peptide action

From these studies it can be inferred that C-peptide, when given to IDDM patients, has the capacity to improve membrane function in the glomeruli [25, 26], to diminish the leakage of fluorescein across the blood-retinal barrier [26] and to stimulate oxygen uptake and blood flow to working skeletal muscle [32]. A physiological mechanism common to these phenomena is not apparent but indirectly, the above findings may be related to the activity of the $Na^+K^+$-ATPase, bound to different cell membranes [41].

In an attempt to examine the possibility that C-peptide may interact with $Na^+K^+$-ATPase activity, single renal tubule segments from rats were microdissected and incubated with rat C-peptide or NaCl. Preparation of the renal tubular segments and determination of $Na^+K^+$-ATPase activity were performed as described by Doucet et al. [42]. The preliminary findings indicate that C-peptide stimulates $Na^+K^+$-ATPase in a dose-dependent manner. Thus, $10^{-7}$ M C-peptide increased $Na^+K^+$-ATPase activity by on average 23 % and the corresponding value for $10^{-6}$ M C-peptide was 85 % [43].

If the above findings are confirmed and extrapolated to other tissues they may possibly help explain previous observations following C-peptide administration to patients with IDDM [25, 26, 32]. The above results may also be of interest in view of the fact that in diabetes disturbances in $Na^+K^+$-ATPase activity have been demonstrated in several tissues, particularly in nerve tissue [44]. In a preliminary study we have examined the possible influence of C-peptide administration on autonomic nervous function in patients with known diabetic autonomic neuropathy. Seven patients were studied with regard to heart rate variations following deep expiration and inspiration, providing an index primarily of vagus function. The measurements were done before and after 3 h of C-peptide infusion at a rate (6 pmol · $kg^{-1}$ · $min^{-1}$) calculated to restore the C-peptide level to physiological concentrations or NaCl infusion. Before the study all patients showed values confirming the presence of autonomic dysfunction (expiration/inspiration RR-interval ratio 1.14±0.01, normal value > 1.21). Following C-peptide infusion for 3 h all patients improved their heart rate variability and the above ratio increased significantly to a near-normal range (1.23±0.02, $p < 0.001$) while no statistically significant change occurred in the patients given NaCl [45]. These preliminary observations during short-term infusion thus suggest the possibility that C-pep-

tide administration may improve autonomic nerve function in diabetic patients. However, further studies will be required to confirm and define such an influence by C-peptide.

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