CMLS, Cell. Mol. Life Sci. 61 (2004) 727–732
1420-682X/04/060727-06
DOI 10.1007/s00018-003-3460-6

CMLS Cellular and Molecular Life Sciences

Research Article

C-peptide fragments stimulate glucose utilization in diabetic rats

Y. Sato[a, b], Y. Oshida[a, b], Y.-Q. Han[b], Y. Morishita[c], L. Li[d], K. Ekberg[e], H. Jörnvall[f] and J. Wahren[e, *]

[a] Research Centre of Health, Physical Fitness and Sports, Nagoya University, Nagoya (Japan)
[b] Department of Sports Medicine, Graduate School of Medicine, Nagoya University, Nagoya (Japan)
[c] Department of Internal Medicine, Anjyo Kosei Hospital, Anjyo (Japan)
[d] Department of Endocrinology, Second University Hospital, China Medical University, Shen Yang (China)
[e] Section of Clinical Physiology, Department of Surgical Sciences, Karolinska Hospital N1:05, Karolinska Institutet, 171 76 Stockholm (Sweden), e-mail: john.wahren@creativepeptides.se
[f] Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm (Sweden)

Received 18 December 2003; received after revision 19 January 2004; accepted 21 January 2004

Abstract. Studies of C-peptide cellular effects show that not only the full-length native peptide but also specific C-terminal fragments are biologically active in in vitro systems. In the present study, the effect of five C-peptide fragments and the native peptide on whole-body glucose turnover was studied in streptozotocin diabetic rats using the insulin clamp technique. Insulin was infused intravenously at 18 pmol kg^{-1} min^{-1} for 90 min and blood glucose concentration was clamped at 8 and 4 mM in diabetic and non-diabetic animals. A steady state was reached during the last 30 min of the study period. Rat C-peptide II and fragments comprising residues 27–31 and 28–31 were effective in augmenting glucose turnover in diabetic rats (+100% to 150%), while no significant effects were seen for segments 1–26, 11–19 and 11–15. The metabolic clearance rate for glucose during infusion of C-peptide or fragments 27–31 and 28–31 in diabetic rats was similar to that seen in non-diabetic animals. We conclude that C-terminal tetra- and pentapeptides, but not fragments from the middle segment of C-peptide, are as effective as the full-length peptide in stimulating whole-body glucose turnover in diabetic rats.

Key words. C-peptide analogue; glucose utilization; metabolic clearance rate for glucose; insulin clamp technique.

Studies during the last decade have demonstrated that proinsulin C-peptide, in contrast to previous views, exerts molecular and cellular effects of its own, showing that C-peptide is in fact a bioactive peptide [1, 2]. It binds specifically to cell membranes, activates a G-protein-coupled membrane receptor and Ca^{2+}-dependent intracellular signaling pathways, resulting in stimulation of both Na^+,K^+-ATPase [3, 4]. and endothelial nitric oxide synthase (eNOS) activities [5, 6]. In animal models and in patients with type 1 diabetes, administration of C-peptide in replacement doses has been found to exert beneficial effects on renal and nerve dysfunction [7–11], attesting to clinical effects and a possible therapeutic potential for C-peptide.

In addition to the above effects, administration of C-peptide to diabetic animals and to type 1 diabetes patients has been found to stimulate glucose utilization as studied by the glucose clamp technique [12–14]. Studies in isolated strips of human skeletal muscle indicate that the effect may be independent of the insulin receptor and tyrosine kinase activation [15]. Studies of other effects in in vitro systems have demonstrated that not only the native peptide but also specific fragments are biologically active. Thus, the C-terminal pentapeptide but not mid-molecule

* Corresponding author.

fragments are capable of displacing membrane-bound C-peptide [16], increasing intracellular Ca^{2+} concentrations [17] and stimulating Na^{+},K^{+}-ATPase activity [3]. The extent to which C-peptide fragments are biologically active under in vivo conditions has not been examined. Consequently, the aim of the present study was to evaluate under in vivo conditions in streptozotocin diabetic rats, the rate of glucose utilization during administration of the native rat C-peptide and five fragments comprising residues 27–31, 28–31, 11–15, 11–19, 1–26.

Materials and methods

Animals

Male Wistar rats weighing 185–235 g were used for the study. The animals were housed in individual cages in a room maintained at 23 °C with an alternating 12-h light/dark cycle and had free access to standard diet and water. The rats were anesthetized with an intraperitoneal (i.p.) injection of 50 mg/kg sodium pentobarbital. The right jugular vein and the left carotid artery were then exposed and isolated through a middle neck incision. Thin silastic catheters were introduced and secured with a silk ligature. The free ends of the catheters were attached to short segments of steel tubing and tunneled subcutaneously around the side of the neck to the top of the skull where they were threaded through a skin incision to the exterior. After patency had been checked, the catheters were flushed with 300 µl 0.9% NaCl containing heparin (40 U/ml), and 500 µl sodium penicillin G (10,000 U/ml) was injected. The catheters were then filled with a viscous solution, 50% polyvinypyrrollidon (PVP K-30; Nacalai, Tesque, Kyoto, Japan) and capped. Two days after surgery, streptozotocin (50 mg/kg body weight; Sigma, St. Louis, Mo.) was injected i.p., and citrate buffer was given to the control rats. Animal care conformed with the standards set by the 'Principles of Laboratory Care' (NIH publication No. 85-23, revised 1985) and the 'Guidelines for Animal Experimentation' (Nagoya University 1988).

Insulin clamp procedure

The animals were allowed to recover from surgery for at least 5 days. The clamp studies were then carried out in the morning after a 16-h overnight fast. Throughout the study, rats were awake and allowed to move freely within a large cage. The PVP solution was aspirated from the catheters, and the venous catheter was then used for infusion of glucose (20%), insulin (Actrapid MC; Novo Nordisk, Bagsvaerd, Denmark) by infusion pumps (STC-521; Terumo, Tokyo, Japan). The blood samples for glucose (25 µl per sample) and insulin (0.5 ml) determinations were drawn from the arterial cannula. In the clamp studies, insulin was infused at a rate of 18 pmol kg^{-1} min^{-1} (3.0 mU kg^{-1} min^{-1}) for 90 min. Blood glucose concentrations were measured every 10 min and the glucose level of the diabetic and normal rats was maintained at approximately 8 and 4 mM, respectively. Simultaneously, rat C-peptide fragments including positions 1–26 (EVEDPQVPQLELGGGPEAGDLQTLAL); positions 27–31 (EVARQ); positions 28–31 (VARQ); positions 11–19 (ELGGGPEAG); positions 11–15 (EL GGGP) or rat C-peptide II (all from Sigma Genosys, Cambridge, UK; purity >98%) were infused at rates of 50 pmol kg^{-1} min^{-1} and saline was administered in the control study. Each fragment or C-peptide was infused into five animals. Total blood loss during the experimental procedure did not exceed 1.5 ml. All of the infused glucose was considered to be taken up by body tissues and under these steady-state conditions, glucose input was considered equal to glucose utilization. The glucose disposal rate (GDR, mg kg^{-1} min^{-1}) was calculated every 10 min during the clamp study. The metabolic clearance rate for glucose (MCR, ml kg^{-1} min^{-1}) was then obtained from GDR divided by the corresponding blood glucose concentration.

Analytical methods

Blood glucose concentrations were determined with a YSI 23 A glucose analyzer (Yellow Spring Instruments, Yellow Springs, Ohio). Blood samples for insulin assay were immediately separated into plasma and cell portions in a centrifuge at 4 °C and stored at –70 °C until analysis. Plasma insulin concentrations were determined by a radioimmunological technique (Phadeseph Insulin RIA; Pharmacia AB, Uppsala, Sweden).

Statistical analyses

All data in the text, tables and figures are expressed as means ± SE. The concentrations of blood glucose and plasma insulin were compared between normal and diabetic rats with an unpaired Student's t test. A one-way analysis of variance (ANOVA) was used to evaluate the differences in GDR and MCR in the presence or absence of C-peptide or its fragments during the clamp procedure. The differences between individual groups were tested using Fischer's PLSD test.

Results

Body weights, blood glucose and insulin concentrations before and during the insulin clamp procedure are presented in table 1. During the clamp, the blood glucose was maintained at approximately 8 mM in the diabetic groups and at about 4 mM in the non-diabetic control group throughout the study procedure by periodic adjustments of the glucose infusion rate. As expected, basal in-

Table 1. Body weight, blood glucose and plasma insulin concentrations in normal and diabetic rats before and during the insulin clamp procedure.

	Body weight (g)	Glucose (mM)		Insulin (pM)	
		basal	clamp	basal	clamp
Normal rats	221 ± 4	4.4 ± 0.1	4.4 ± 0.2	42 ± 6	216 ± 66
Diabetic rats					
Saline	205 ± 8	13.8 ± 0.8	8.1 ± 0.2	30 ± 6	198 ± 60
Fragment 1–26	205 ± 6	12.2 ± 0.7	8.1 ± 0.1	24 ± 6	126 ± 18
Fragment 27–31	198 ± 6	12.2 ± 0.3	8.1 ± 0.1	30 ± 6	120 ± 24
Fragment 28–31	211 ± 8	12.9 ± 0.6	7.7 ± 0.1	36 ± 6	240 ± 36
Fragment 11–19	219 ± 14	12.6 ± 0.4	7.9 ± 0.1	34 ± 10	174 ± 16
Fragment 11–15	225 ± 5	12.1 ± 0.7	7.6 ± 0.2	30 ± 5	204 ± 20
C-peptide II	209 ± 6	12.1 ± 0.3	7.7 ± 0.2	24 ± 5	294 ± 24

Mean values ± SE are shown.

sulin concentrations were lower (30 ± 4 pM) in the diabetic rats than in the controls (42 ± 6 pM, $p < 0.01$) but during the clamp procedure, insulin levels were similar in the two groups.

The GDR rose gradually in all study groups during the course of the insulin clamp procedure and reached a plateau during the last 20–30 min (fig. 1). The level of GDR at the end of the infusion period in the diabetic rats receiving saline was approximately 40% less than that of the non-diabetic controls ($p < 0.05$ (fig. 2). Infusion of C-peptide to diabetic animals significantly increased GDR, as previously shown [14]. Fragments 27–31 and 28–31 both caused GDR to rise on average 105% ($p < 0.005$) and 150% ($p < 0.001$), respectively, above the level observed in diabetic animals infused with saline. In the case of fragment 28–31, the rise in GDR even exceeded the level observed in the non-diabetic group ($p < 0.01$). Fragments 1–26, 11–19 and 11–15 all failed to significantly stimulate GDR above the level observed in the diabetic rats receiving saline.

The average MCR values at the end of the clamp period are presented in figure 3. As expected, streptozotocin-induced diabetes caused a marked reduction in MCR compared to the non-diabetic state (–65%, $p < 0.001$). Infusion of the full-length C-peptide gave rise to a 110% ($p < 0.005$) increase in MCR compared to that of saline-infused diabetic rats. Infusion of fragments 27–31 and 28–31 resulted in significant increases in MCR (+108%, $p < 0.005$ and +160%, $p < 0.001$, respectively) above the level observed in saline-infused diabetic rats. Administration of fragment 28–31 resulted in MCR levels similar to that observed in the non-diabetic con-trol rats, while infusion of fragment 27–31 increased MCR to a level slightly lower than that of the non-diabetic animals ($p < 0.05$). No significant change in MCR was seen during infusion of fragments 1–26, 11–19 or 11–15.

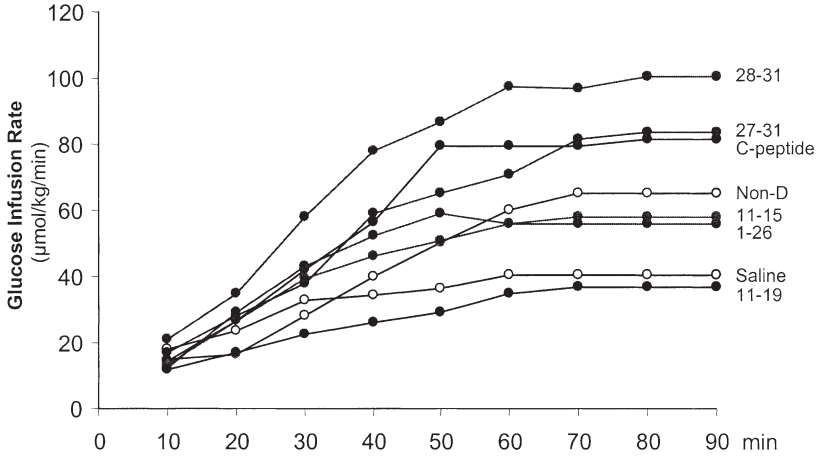

Figure 1. Glucose infusion rates during the 90-min clamp procedure involving infusion of insulin (18 pmol kg^{-1} min^{-1}) and either fragments 27–31, 28–31, 11–15, 11–19, 1–26 or C-peptide (50 pmol kg^{-1} min^{-1}), or saline in streptozotocin diabetic rats and saline in non-diabetic controls.

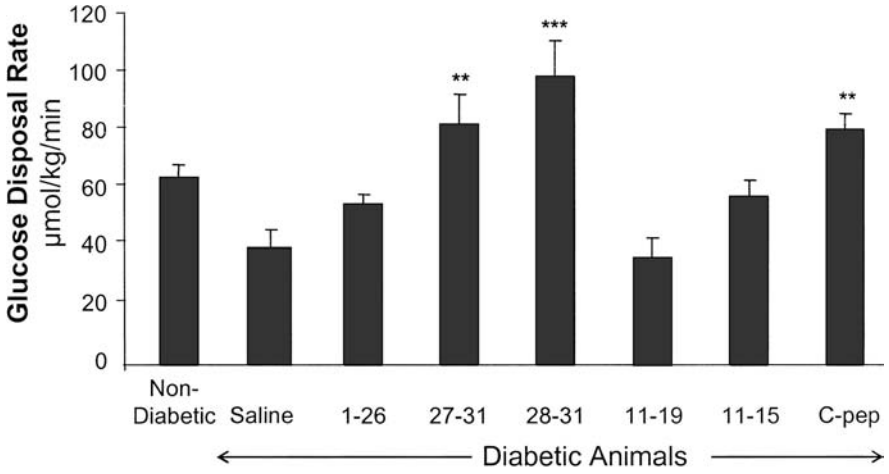

Figure 2. GDRs (average values for the last 20 min of the 90-min insulin clamp) for non-diabetic control rats and streptozotocin diabetic rats infused with insulin (18 pmol kg^{-1} min^{-1}) and either C-peptide, five different fragments (50 pmol kg^{-1} min^{-1}) or saline. Mean values ± SE are indicated. **$p < 0.01$ and *** $p < 0.001$ versus the corresponding value for saline-infused diabetic animals.

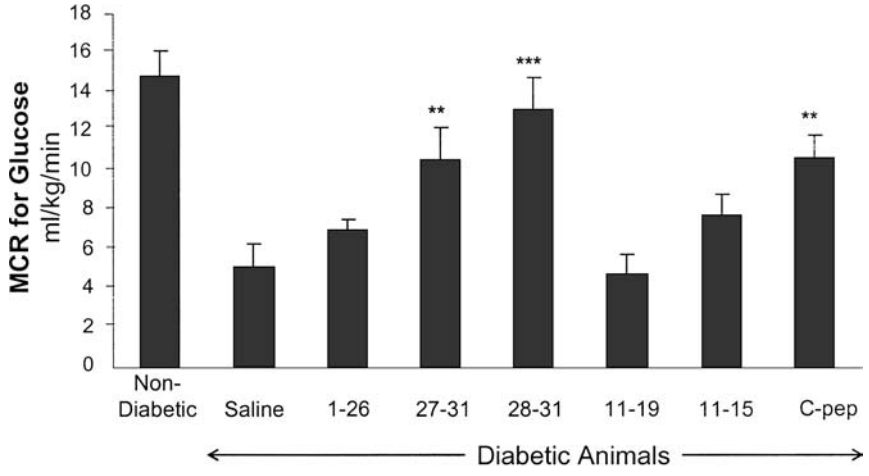

Figure 3. MCR for glucose (average values for the last 20 min of the 90-min insulin clamp) for non-diabetic control rats and streptozotocin-diabetic rats during infusion of insulin (18 pmol kg^{-1} min^{-1}) and either C-peptide, five different fragments (50 pmol kg^{-1} min^{-1}) or saline. Mean values ± SE are indicated. ** $p < 0.01$ and *** $p < 0.001$ versus the corresponding value for saline-infused diabetic animals.

Discussion

Previous studies have established that C-peptide administered intravenously to streptozotocin-diabetic rats elicits a substantial increase in whole-body glucose turnover [12, 14]. The present findings confirm the previous results and demonstrate that fragments of C-peptide composed of residues 27–31 and 28–31, but not residues 11–15, 11–19 or 1–26, exert a stimulatory influence on glucose turnover. The effect of fragments 27–31 and 28–31 was similar to that of the native full-length peptide.

The present in vivo findings for the C-terminal pentapeptide comprising residues EVARQ confirm and extend previous in vitro observations. Thus, this peptide has been shown to competitively displace bound C-peptide in cell membrane binding sites [16], to elicit a rise in intracellular Ca^{2+} concentrations [17], to cause phosphorylation of ERK1/2 [18] and to stimulate Na^+,K^+-ATPase activity [3]. It is also capable of eliciting an improvement in the deformability of red blood cells from type 1 diabetes patients [19]. All of these effects suggest that the native C-peptide and its C-terminal pentapeptide are equipotent with regard to the observed in vitro effects, and the present data indicate that this applies also to the in vivo effect on glucose turnover in diabetic rats.

However, the present findings for the C-terminal tetrapeptide (VARQ) are partly inconsistent with previous observations. The tetrapeptide stimulates glucose utilization in vivo (fig. 2) and Na^+,K^+-ATPase activity in vitro as effectively as the native full-length C-peptide [3], but it does not displace bound C-peptide [20]. Further-

more, the N-terminal Glu residue (E) of the pentapeptide has been found to be essential for the displacement effect [20] and even free Glu can elicit part of the cellular effects [3, 17]. One can speculate that the differential effects of the C-terminal penta- and tetrapeptides are compatible with the involvement of two types of receptors, one requiring the N-terminal Glu residue, the other not. Alternatively, the divergent results for the tetrapeptide may be related to species differences. Failure of the tetrapeptide to displace bound C-peptide was observed for the human tetrapeptide (GSLQ) and its effect on homologous C-peptide bound to human cells [20]. In the case of fragment 1–26, however, the present results agree well with previous findings. This fragment fails to displace bound C-peptide [20], it does not increase intracellular Ca^{+2} concentrations [13], nor does it stimulate Na^+,K^+-ATPase activity [3]. Finally, fragments 11–15 and 11–19 showed no significant effect on GDR or MCR in the present study, in keeping with previous findings that these fragments fail to displace membrane bound C-peptide [20] or to improve red blood cell deformability [19]. However, both fragments 11–15 and 11–19 have been reported to elicit limited stimulation of Na^+,K^+-ATPase of renal tubular segments, although these effects have been ascribed to complex and non-specific interactions [3].

The mechanism by which C-peptide or its C-terminal fragments elicit an increase in glucose utilization is not clear. C-peptide bound to human cell membranes cannot be displaced by insulin [16] and there is no evidence that C-peptide binds to the insulin receptor. However, insulin-like effects of C-peptide have been reported for rat skeletal muscle (L6 myoblasts) [21]. These effects involve activation of insulin receptor tyrosine kinase, IRS-1 phosphorylation, PI-3 kinase and MAPK activation [21], and inhibition of protein tyrosine phosphatase activity with an associated increase in the phosphorylation of the insulin receptor and IRS-1 [22]. The above findings suggest that C-peptide may interact with the insulin signaling pathway at a proximal level, resulting in enhanced glucose uptake. Alternative mechanisms may be considered. C-peptide has been shown to stimulate NO release from endothelial cells both under in vitro [5, 6] and in vivo [23] conditions. Several studies report that muscle glucose transport can be activated by NO and that NOS in its endothelial and neural isoforms is expressed in rat skeletal muscle [24–27]. In keeping with this hypothesis, administration of an NOS blocker was found to abolish the C-peptide-mediated increase in glucose utilization in diabetic rats [14].

The effect of C-peptide and its C-terminal fragments on glucose utilization in diabetic rats is quite marked (figs. 1–3). In contrast, in some but not all studies of type 1 diabetes patients a short-term effect of C-peptide on glucose utilization has been observed [13, 28], whereas long-term (3 months) administration in type 1 diabetes patients does not result in lowered blood glucose concentrations or reduced HbA1c levels [8, 11]. Studies in isolated human skeletal muscle strips indicate that C-peptide does not interact with the insulin receptor, nor does it cause tyrosine kinase activation [15], indicating a species difference between human and rat with regard to C-peptide-mediated effects on glucose utilization.

Although C-peptide concentrations were not measured in the present investigation, a previous study using the same technique, protocol and rate of C-peptide infusion would suggest that the present C-peptide concentrations were in the range 2–4 nM [14]. The rate of infusion was the same (50 pmol kg^{-1} min^{-1}) for C-peptide and for the fragments. The concentration for the fragments could not be measured in this study. However, the pentapeptide is known to have a shorter half-life than C-peptide in serum, and C-peptide undergoes endopeptidase degradation while the pentapeptide, and probably the other small fragments also are degraded by aminopeptidases in serum [29]. Taken together, these considerations suggest that the concentrations of the fragments are likely to have been lower than that of the full-length peptide. Since the effect of fragments 27–31 and 28–31 on glucose utilization was similar to that of C-peptide, the findings attest to the same or greater bioactivity of the C-terminal fragments compared to C-peptide.

Acknowledgements. The authors would like to express deep gratitude to Drs N. Fuku and K. Kitakoshi for their excellent technical assistance.

1 Wahren J., Ekberg K., Johansson J., Henriksson M., Pramanik A., Johansson B.-L. et al. (2000) Role of C-peptide in human physiology. Am. J. Physiol. Endocrinol. Metab. **278:** E759–E768
2 Johansson J., Ekberg K., Shafqat J., Henriksson M., Chibalin A., Wahren J. et al. (2002) Molecular effects of proinsulin C-peptide. Biochem. Biophys. Res. Commun. **295:** 1035–1040
3 Ohtomo Y., Bergman T., Johansson B.-L., Jörnvall H. and Wahren J. (1998) Differential effects of proinsulin C-peptide fragments on Na^+,K^+-ATPase activity of renal tubule segments. Diabetologia **41:** 287–291
4 Tsimaratos M., Roger F., Chabardès D., Mordasini D., Hasler U., Doucet A. et al. (2003) C-peptide stimulates Na,K-ATPase activity via PKC alpha in rat medullary thick ascending limb. Diabetologia **46:** 124–131
5 Wallerath T., Kunt T., Forst T., Closs E., Lehmann R., Flohr T. et al. (2003) Stimulation of endothelial nitric oxide synthase by proinsulin C-peptide. Nitric Oxide. **9:** 95–102
6 Kitamura T., Kimura K., Makondo K., Furuya T., Suzuki M., Yoshida T. et al. (2003) Proinsulin C-peptide increases nitric oxide production through mitogen-activated protein kinase-dependent transcriptional enhancement of endothelial nitric oxide synthase in rat aortic endothelial cells. Diabetologia **46:** 1698–1705
7 Samnegård B., Jacobson S., Jaremko G., Johansson B.-L. and Sjöquist M. (2001) Effects of C-peptide on glomerular and renal size and renal function in diabetic rats. Kidney Int. **60:** 1258–1265
8 Johansson B.-L., Borg K., Fernqvist-Forbes E., Kernell A., Odergren T. and Wahren J. (2000) Beneficial effects of C-peptide on

incipient nephropathy and neuropathy in patients with type I diabetes – a three-month study. Diabet. Med. **17:** 181–189
9 Sima A., Zhang W., Sugimoto K., Henry D., Li Z., Wahren J. et al. (2001) C-peptide prevents and improves chronic type 1 diabetic neuropathy in the BB/Wor rat. Diabetologia **44:** 889–897
10 Johansson B.-L., Borg K., Fernqvist-Forbes E., Odergren T., Remahl S. and Wahren J. (1996) C-peptide improves autonomic nerve function in IDDM patients. Diabetologia **39:** 687–695
11 Ekberg K., Brismar T., Johansson B.-L., Jonsson B., Lindström P. and Wahren J. (2003) Amelioration of sensory nerve dysfunction by C-peptide in patients with type 1 diabetes. Diabetes **52:** 536–541
12 Wu W., Oshida Y., Yang W.-P., Ohsawa I., Sato J., Iwao S. et al. (1996) Effect of C-peptide administration on whole body glucose utlilization in STZ-induced diabetic rats. Acta. Physiol. Scand. **157:** 253–258
13 Johansson B.-L., Sjöberg S. and Wahren J. (1992) The influence of human C-peptide on renal function and glucose utilization in type I (insulin-dependent) diabetic patients. Diabetologia **35:** 121–128
14 Li L., Oshida Y., Kusunoki M., Yamanouchi K., Johansson B.-L., Wahren J. et al. (1999) Rat C-peptide I and II stimulate glucose utilization in STZ-induced diabetic rats. Diabetologia **42:** 958–964
15 Zierath J., Handberg A., Tally M. and Wallberg-Henriksson H. (1996) C-peptide stimulates glucose transport in isolated human skeletal muscle independent of insulin receptor and tyrosine kinase activation. Diabetologia **39:** 306–313
16 Rigler R., Pramanik A., Jonasson P., Kratz G., Jansson O., Nygren P.-Å. et al. (1999) Specific binding of proinsulin C-peptide to human cell membranes. Proc. Natl. Acad. Sci. USA **96:** 13318–13323
17 Shafqat J., Juntti-Berggren L., Zhong Z., Ekberg K., Köhler M., Berggren P.-O. et al. (2002) Proinsulin C-peptide and its analogues induce intracellular Ca^{2+} increases in human renal tubular cells. Cell. Mol. Life Sci. **59:** 1185–1189
18 Chibalin A., Zhong Z., Dumitrescu A., Ehrén I., Ekberg K. and Wahren J. (2003) C-peptide stimulates ERK1/2 phosphorylation via specific activation of PKC isoforms delta and epsilon in human renal tubular cells. Diabet. Metab. **29:** 45127
19 Hach T., Johansson B.-L., Ekberg K., Forst T., Kunt T. and Wahren J. (2003) C-peptide and fragments thereof restore impaired red blood cell deformability in type 1 diabetics. Diabetes **52:** A120
20 Pramanik A., Ekberg K., Zhong Z., Shafqat J., Henriksson M., Tally M. et al. (2001) C-peptide binding to human cell membranes: importance of Glu27. Biochem. Biophys. Res. Commun. **284:** 94–98
21 Grunberger G., Qiang X., Li Z., Mathews S., Sbrissa D., Shisheva A. et al. (2001) Molecular basis for the insulinomimetic effects of C-peptide. Diabetologia **44:** 1247–1257
22 Li Z.-G., Qiang X., Mathews S., Sima A. and Grunberg G. (2001) C-peptide inhibits protein tyrosine phosphatase and promotes glycogen synthesis. Int. J. Exp. Diabet. Res. **2:** 161
23 Johansson B.-L., Sundell J., Ekberg K., Jonsson C., Seppänen M., Raitakari O. et al. (2004) C-peptide improves adenosine-induced myocardial vasodilation in type 1 diabetes patients. Am. J. Physiol. Endocrinol. Metab. **286:** E14–E19
24 Etgen G., Fryburg D. and Gibbs E. (1997) Nitric oxide stimulates skeletal muscle glucose transport through a calcium/contraction- and phosphatidylininositol-3 kinase independent pathway. Diabetes **46:** 1915–1919
25 Young M., Radda G. and Leighton B. (1997) Nitric oxide stimulates glucose transport and metabolism in rat skeletal muscle in vitro. Biochem. J. **322:** 223–228
26 Ryo D., Perreault M. and Marette A. (1998) Insulin stimulation of glucose uptake in skeletal muscles and adipose tissues in vivo is NO dependent. Am. J. Physiol. **274:** E692–E699
27 Balon T. and Nadler J. (1997) Evidence that nitric oxide increases glucose transport in skeletal muscle. J. Appl. Physiol. **82:** 359–363
28 Forst T., Rave K., Pfüetzner A., Buchholz R., Pohlmann T. and Heinemann L. (2002) Effect of C-peptide on glucose metabolism in patients with type 1 diabetes. Diabetes Care **25:** 1096–1097
29 Melles E., Bergman T., Alvelius A., Jonsson A., Ekberg K., Wahren J. et al. (2003) Proinsulin C-peptide and its C-teminal pentapeptide: degradation in human serum and chiff base formation with subsequent CO2 incorporation. Cell. Mol. Life Sci. **60:** 1019–1025