C-peptide Metabolism and the Liver

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SUMMARY

It is generally assumed that C-peptide is not degraded in the liver to any significant extent. This assumption is, however, based on indirect evidence. We therefore measured the hepatic extraction ratio (R) of insulin and C-peptide in anesthetized pigs with simultaneous sampling of portal and hepatic venous blood. Blood samples were taken at one-minute intervals for 10 minutes during basal (unstimulated) experimental conditions as well as after intravenous injection of 1 mg. glucagon into four normal-weight and four obese pigs. The hepatic extraction ratio of insulin or C-peptide was calculated as the portal concentration minus the hepatic concentration divided by the portal concentration. The average pre- and poststimulatory molar ratios of C-peptide to insulin were 1.5 ± 0.11 (S.E.M.) and 1.2 ± 0.06 in portal blood and 3.4 ± 0.21 and 2.2 ± 0.08 in hepatic blood. For all pigs the mean $R_{C\text{-peptide}}$ was 0.30 ± 0.03 and 0.33 ± 0.02 pre- and poststimulatory, respectively. The corresponding figures for $R_{insulin}$ (0.71 ± 0.02 and 0.61 ± 0.02) were both significantly higher ($p < 0.01$). The mean R-values for C-peptide and insulin were consistently higher in obese pigs than in normal-weight pigs ($p < 0.01$). Additional experiments in two normal-weight pigs showed that ligation of the hepatic artery elicited a significant fall of mean $R_{C\text{-peptide}}$, which, however, never became less than 0.12. These results suggest that besides the well-known hepatic extraction of insulin, the livers of anesthetized pigs also extract significant amounts of endogenous C-peptide. Hepatic extraction of C-peptide is, however, about 50 per cent lower than that of insulin. DIABETES *27* **(Suppl. 1):197-200, 1978.**

In the pancreatic beta cell, proinsulin is cleaved into insulin, the connecting peptide (C-peptide), and two pairs of basic amino acids.[447,451] Since the cleavage of one molecule of proinsulin yields one molecule of insulin and one molecule of C-peptide, these two peptides are secreted into the portal blood in equimolar amounts.[167,187,389] Before reaching the systemic circulation, insulin and C-peptide transverse the hepatic bed. Whereas it is well established that the liver extracts about half the amount of insulin presented to it,[55,114,207,280] it is generally believed that C-peptide is not extracted to any significant degree by the liver[460] but is metabolized mainly by the kidneys.[214] Simultaneous measurement of peripheral insulin and C-peptide levels should therefore be a more reliable indicator of insulin secretion than the measurement of plasma insulin alone.

The assumption that C-peptide is not taken up by the liver[460] is based on indirect evidence obtained in vitro by adding porcine C-peptide to an isolated rat liver perfusion system. The extensive interspecies variability in the primary structure of the connecting segment of the proinsulin molecule[450] requires that the metabolism of C-peptide from a given species be studied in a homologous system. For these reasons we studied the hepatic extraction of endogenous insulin and C-peptide in anesthetized catheterized pigs with specific porcine assays for both hormones.

MATERIAL AND METHODS

Experimental Procedure

In order to obtain a wide range of insulin secretion, both normal-weight (<100-kg.) and obese (>120-kg.) pigs (four of each kind) of the Danish Landrace were used for the study. The weight ranges of the normal-weight and obese pigs were 87-90 kg. and 155-165 kg., respectively. After a 12-hour fast with free access to drinking water, the pigs were anesthetized with halothane–nitrous-oxide–oxygen. The abdomen was opened by a midline incision and a Teflon catheter was introduced into the portal vein via a branch of the splenic vein. The tip of the catheter was placed approximately 2 cm. from the hepatic hilus. The hepatic vein was catheterized via a femoral vein, and , in order to avoid admixture of peripheral venous

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blood at the hepatic vein sampling site, the inferior cava was ligated just below the level of the hepatic veins. The position of catheters was checked by palpation. Catheters were also placed in a jugular vein (for infusions) and in a carotid artery (for monitoring of the arterial blood pressure). Portal and hepatic venous blood was drawn simultaneously and collected in chilled heparinized test tubes containing aprotinin (Trasylol, Bayer, Leverkusen) 500 K.I.U. per milliliter of blood. Plasma was separated by centrifugation at 4° C. and stored at −25° C. until analyzed. Blood samples were drawn at one-minute intervals for 10 minutes during basal (unstimulated) experimental conditions as well as after the intravenous injection of 1 mg. glucagon (Novo, Copenhagen). Since the first series of experiments indicated that the liver, contrary to expectation,[460] retained significant amounts of C-peptide, another two pigs of normal weight were investigated. These pigs were handled exactly as described above, with the exception that the hepatic artery was also dissected in order to make clamping of the vessel possible. Samples of portal and hepatic venous blood were drawn at two-minute intervals and the hepatic artery was clamped periodically for 10 minutes as indicated in figure 2. Plasma glucose was maintained at 15 mM during the first 22 minutes; thereafter it was suddenly elevated to and maintained at 25 mM (by square-wave technique). This procedure allowed an evaluation of the possible influence of the arterial supply of C-peptide to the liver on the hepatic C-peptide extraction ratio.

Laboratory Investigations and Calculations

Insulin immunoreactivity was measured in triplicate as described elsewhere.[233] Standards used were highly purified pork insulin obtained from Nove Research Institute, Copenhagen. The antibody used (4346) binds proinsulin and insulin with almost equimolar potency.[235] The sensitivity of the assay was 0.02 pmol per milliliter sample.

C-peptide immunoreactivity was measured in triplicate as described by Faber et al.[99] using porcine C-peptide standards, ^{125}I-tyrosylated pork C-peptide as tracer, and an antiporcine C-peptide antiserum raised in guinea pigs (M8717). The sensitivity of the assay was 0.03 pmol per milliliter sample.

The hepatic extraction ratio (R) of insulin or C-peptide was calculated as the portal concentration minus the hepatic concentration divided by the portal concentration.

Results were evaluated statistically by Student's *t*-test for unpaired observations, and differences resulting in p-values less than 0.05 were considered significant.

RESULTS

The Molar C-peptide:insulin Ratio in Portal and Hepatic Venous Blood (Table 1)

During basal (unstimulated) experimental conditions, the mean molar C-peptide:insulin ratio was 1.5 ± 0.11 (S.E.M.) in the portal vein, whereas after stimulation it fell to 1.2 ± 0.06 ($p < 0.05$). During the two 10-minute experimental periods variations in the molar C-peptide:insulin ratio of an individual animal never exceeded 20 per cent of the mean value of the period. At any experimental condition the molar ratio was significantly lower in portal venous blood than in hepatic venous blood, indicating a preferential hepatic uptake of insulin. There were no differences between the molar C-peptide:insulin ratios of the obese and the normal-weight pigs.

TABLE 1

Molar C-peptide:insulin ratios in the portal and hepatic venous blood of anesthetized pigs. The ratio was assessed during basal (unstimulated) experimental conditions and after stimulation of beta-cell function with intravenous administration of 1 mg. glucagon. Values shown are the means ± S.E.M. (N=8).

	Portal vein	Hepatic vein	p
Basal	1.5 ± 0.11	3.4 ± 0.21	< 0.05
Stimulated	1.2 ± 0.06	2.2 ± 0.08	< 0.01

Hepatic Extraction Ratio (R) of C-peptide and Insulin (Figure 1, Table 2)

Figure 1 shows the individual values of R for C-peptide and insulin, whereas table 2 gives the mean values ± S.E.M. For all pigs the mean $R_{C\text{-peptide}}$ was 0.30 ± 0.03 during basal conditions, and this value was not affected significantly by the larger portal supply of C-peptide to the liver that followed intravenous administration of glucagon. The mean $R_{C\text{-peptide}}$ values of the obese pigs were about two times higher than the corresponding values of the normal weight pigs.

The mean $R_{insulin}$ of all pigs was 0.71 ± 0.02 during basal experimental conditions, and again, R of the obese pigs was higher than R of the normal-weight pigs. All pigs exhibited a fall in $R_{insulin}$ after stimulation of insulin secretion with glucagon. During basal experimental conditions as well as after glucagon, the mean R-values of insulin were about two times higher than the corresponding R-values of C-peptide ($p < 0.01$).

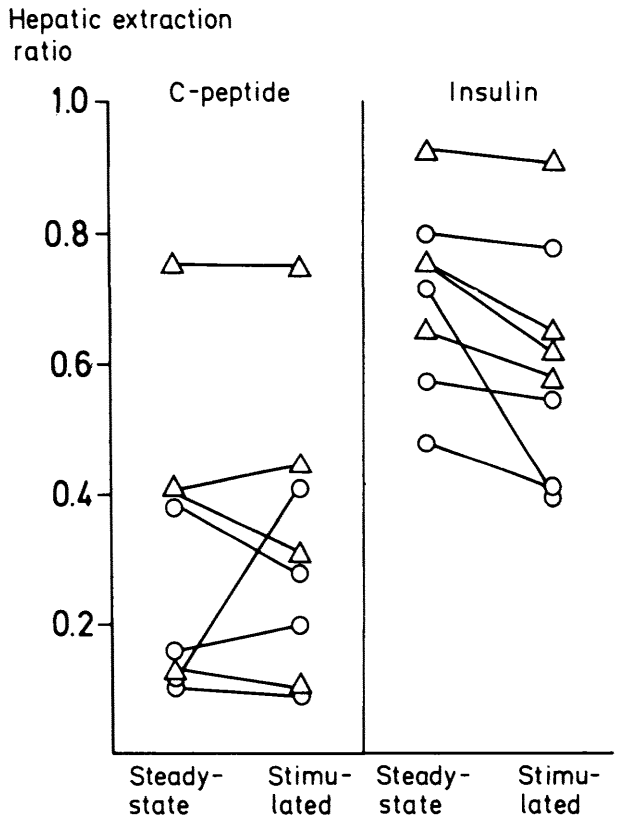

FIG. 1. Individual hepatic extraction ratios of C-peptide (left panel) and insulin (right panel) in four normal-weight (o) and four obese (Δ) anesthetized pigs. The ratios were assessed during basal (unstimulated) experimental conditions and after intravenous injection of 1 mg. glucagon. Each point represents the mean of 10 determinations.

Effect of Clamping of the Hepatic Artery on the Hepatic Extraction Ratio (R) of C-peptide (Figure 2)

As the results obtained were identical in the two pigs, figure 2 shows only the data obtained in one of the pigs. At the beginning of the experiment, mean $R_{C\text{-peptide}}$ was 0.32 ± 0.07. After clamping the hepatic artery, the R-value fell to 0.12 ± 0.04 ($p < 0.05$). When the arterial clamp was removed and blood glucose levels were elevated to 25 mM, the mean $R_{C\text{-peptide}}$ increased to 0.24 ± 0.04 ($p < 0.05$). Reclamping of the artery resulted in an insignificant drop of $R_{C\text{-peptide}}$ to 0.19 ± 0.03. The final removal of the clamp was not associated with significant changes in the R-value.

DISCUSSION

The present study has shown that significant amounts of endogenous C-peptide are extracted by the liver of anesthetized pigs. Thus, about one third of the amount of C-peptide present in the portal vein was extracted (table 2). Since the arterial supply of C-peptide to the liver was not taken into account in the formula used for calculation, the hepatic extraction ratio might, to some extent, be overestimated. Accordingly, additional experiments designed to elucidate this problem were carried out, and the results showed that the hepatic extraction ratio of

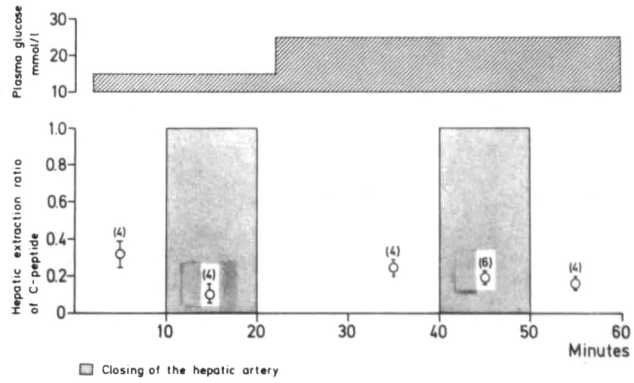

FIG. 2. Effect of transient closure of the hepatic artery on the hepatic extraction ratio of C-peptide in an anesthetized normal-weight pig. The determinations were performed at two different levels of plasma glucose, as indicated in the top of the figure. The mean values ± S.E.M. are shown. Number of individual determinations is given in parenthesis.

TABLE 2

Corresponding values of the hepatic extraction ratio (R) of C-peptide and insulin in anesthetized pigs. R was assessed during basal (unstimulated) experimental conditions and after stimulation of the beta-cell function with intravenous administration of 1 mg. glucagon. Values shown are the means ± S.E.M.

		I. Basal	II. Stimulated	p (I vs. II)
$R_{C\text{-peptide}}$	1. Normal-weight pigs (4)	0.19 ± 0.02	0.24 ± 0.02	N.S.
	2. Obese pigs (4)	0.42 ± 0.04	0.42 ± 0.04	N.S.
	3. All pigs (8)	0.30 ± 0.03	0.33 ± 0.02	N.S.
	p (2 vs. 1)	< 0.01	< 0.01	
$R_{insulin}$	1. Normal-weight pigs (4)	0.64 ± 0.03	0.53 ± 0.03	< 0.05
	2. Obese pigs (4)	0.77 ± 0.01	0.68 ± 0.02	< 0.05
	3. All pigs (8)	0.71 ± 0.02	0.61 ± 0.02	< 0.01
	p (2 vs. 1)	< 0.01	< 0.01	

Numbers of individual experiments are given in parentheses.

C-peptide was significantly diminished by clamping of the hepatic artery (figure 2). However, the ratio never became less than 0.12, indicating that at least 12 per cent of the amount of C-peptide reaching the liver was removed. Moreover, variations in the arterial contribution to liver blood flow may account for the differences between the individual hepatic extraction ratios of C-peptide and seen in the experiments without clamping of the hepatic artery (figure 1).

The decline of the hepatic extraction ratio of C-peptide following clamping of the hepatic artery might also be a consequence of the diminished supply of blood to the liver, leading to a degree of liver cell damage. However, since the hepatic artery contributes only about 20 per cent to liver blood flow, this explanation is not likely to be true. In any event, the problem needs further investigation, such as by means of an isolated perfused porcine liver model.

In accordance with previous results in pigs,[234] we found that the liver extracted about half of the amount of insulin presented to it by the portal vein (table 2).

In general the liver extracted twice as much insulin as C-peptide (table 2), but the mean hepatic extraction ratios of C-peptide and insulin were both significantly higher in obese than in normal-weight pigs. It has been shown that, within physiologic portal vein concentrations of insulin, the hepatic extraction ratio increases with increasing portal vein insulin concentrations.[114,207,316] For this reason the enhanced hepatic extraction ratio of insulin observed in the obese pigs might be ascribed to the higher concentrations of insulin in their portal plasma. The finding that the hepatic extraction ratio of insulin decreased in both groups of pigs after stimulation of insulin secretion with glucagon (figure 1, table 2) does not necessarily contradict this interpretation since glucagon, besides its effect on insulin secretion, is also believed to influence splanchnic hemodynamics. Administration of glucagon increases the total liver blood flow in all species investigated.[228,231,261,431] In the pig, the increased hepatic blood flow after glucagon is the result of an increase in hepatic arterial blood flow, whereas the portal blood flow is not altered.[261] It thus follows that the enhanced concentrations of insulin in the portal vein after glucagon administration may be counterbalanced by the increased flow of arterial blood to the liver, which contains lower concentrations of insulin.

In contrast to our finding of an increased hepatic extraction ratio of insulin in obese pigs, Karakash et al.[211] recently reported that perfused livers of obese hyperglycemic mice removed less insulin than the livers of lean mice. The discrepancy between this finding and our remains unexplained but may depend on differences in the experimental design or the species investigated.

Using an isolated rat liver perfusion system, Stroll et al.[460] were unable to demonstrate a significant clearance of porcine C-peptide. These authors added physiologic amounts of porcine C-peptide to the perfusion medium and subsequently measured the concentration of C-peptide in the perfusate with a porcine C-peptide immunoassay. The detection limit of this assay was not specified. The use of porcine C-peptide in a rat liver perfusion system might account for the differences between the findings of Stoll et al.[460] and ours, but this explanation is, of course, unproved. No definite biologic effect has been attributed to C-peptide other than its role in the biosynthesis of insulin.[160,221,518] Teleologically, therefore, there should be no metabolic need for the liver to bind and/or remove C-peptide from the circulation. The possibility exists, however, that C-peptide is trapped nonspecifically on its passage through the liver. Studies designed to elucidate this possibility are in progress in our laboratory.

The fact that the molar C-peptide:insulin ratio was higher in hepatic venous blood than in portal venous blood suggests preferential hepatic uptake of insulin (table 1). In contrast to the finding by Heding et al.[167] of equimolar concentrations of C-peptide and insulin in the venae pancreaticoduodenales of a pig, we found an average molar ratio of 1.5 in the portal vein during basal, unstimulated experimental conditions. The molar C-peptide:insulin ratio in peripheral venous blood is far above unity.[101,167,171,172,187] Therefore, the high C-peptide concentration in the portal vein might be attributed to the admixture of blood from the superior mesenteric vein. The observation that the average molar C-peptide:insulin ratio decreased from 1.5 to 1.2 after stimulation of beta-cell function with glucagon supports this interpretation.

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