

## Release of Immunoreactive Insulin from the Human Heart

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**Abstract.** Arterial-coronary sinus differences in plasma immunoreactive insulin concentration have been determined in 21 healthy men in the fasting state. Measurements were made both at rest and during prolonged exercise in all subjects and, in 9 of these, during an intravenous infusion of sodium nicotinate. Significant myocardial extraction of insulin could not be detected, but during prolonged exercise and also during the infusion of sodium nicotinate in resting subjects,

net release of insulin from the myocardium took place. There was a positive linear relationship between arterial-coronary sinus difference in insulin concentration and arterial insulin concentration. The most marked release of insulin from the heart occurred at the lowest arterial concentrations.

**Key words:** Human heart, insulin, arterial-coronary sinus difference, release, uptake.

### Introduction

Although much is known about uptake of substrates and release of metabolites by human tissues, there appears to be little information about the uptake and release of hormones [1, 2]. With sufficiently accurate methods, it should be possible to detect the extraction of a hormone by its target organ by measuring the arterio-venous concentration difference across the organ and to examine the relationship this bears to concentration in arterial blood under different physiological and pathological conditions. Furthermore, some hormones may undergo no catabolism at their site of action and be released again, under certain circumstances, physiologically active. There is evidence that tissue-bound labelled insulin can exchange with unlabelled insulin in the vascular compartment [3]. Also, insulin may be unaltered during binding to fat and liver cell membranes [4, 5].

We have studied myocardial extraction of immunoreactive insulin in healthy man both at rest and during exercise by obtaining simultaneous arterial and coronary sinus blood samples from fasting subjects and measuring the plasma insulin concentration. In addition, we have examined the effect on insulin extraction by the heart of altering the predominant myocardial energy source from lipid to carbohydrate by giving an intravenous infusion of the antilipolytic agent, sodium nicotinate [6–8].

### Methods

Twenty-one male subjects, aged 21 to 42 years, were investigated without sedation after an overnight fast. Teflon catheters were introduced into a brachial artery and the coronary sinus for blood sampling [9]. Heparin was not administered. Blood samples were taken after 60 minutes rest and again after 65 to 125 min. of exercise in the supine position on a cycle

ergometer at a constant, predetermined work load. The work load was 50% of that which produced a heart rate of 170/min. after 6 min. of exercise ( $W_{170}$ ) [10]. It was intended that exercise should last for 120 min., a duration tolerated by most healthy subjects at the load used. However, since the subjects were fasted and some of them infused with sodium nicotinate, 9 stopped earlier because of fatigue. The exercise sampling was made during the last 5 min. of work in all subjects. In 9 of the 21 subjects, a priming dose of 200 mg of 5% sodium nicotinate was given intravenously 60 min. before the first blood sampling. Then sodium nicotinate was infused at a rate of 200 mg/hour in 3 and 400 mg/hour in 6 subjects.

Each subject was given oral iodine (Lugol's solution) followed by an intravenous injection of about 6  $\mu$ C of  $^{125}$ I-albumin<sup>1</sup>, as a tracer for plasma albumin, two days before the investigation. This enabled estimation of any change in plasma protein concentration as an index of a shift of plasma water across the coronary circulation. Such shifts would affect estimates of arterial-coronary sinus differences in insulin concentration.  $^{125}$ I-radioactivity was determined on 10 replicates of each arterial and coronary sinus plasma sample [15]. The  $^{125}$ I did not interfere with the insulin assays, which depended on the use of  $^{125}$ I-insulin. This was because the  $^{125}$ I-albumin was present in small amounts compared to that of  $^{125}$ I-insulin, albumin was removed in the procedure for insulin determination and blank determinations were made without added  $^{125}$ I-insulin.

Blood samples for determination of glucose [11] and lactate [12] were deproteinised immediately with perchloric acid. Samples for plasma free fatty acids (FFA),  $^{125}$ I-albumin and insulin determinations were immediately transferred from the collection syringes

<sup>1</sup> Kindly provided by G. Birke and L. O. Plantin, King Gustaf V Research Institute, Stockholm, Sweden.

to heparinised test tubes, placed in iced water and centrifuged at 4 °C within 30 min. Plasma FFA were measured according to Trout *et al.* [13]; the heptane phase was washed twice with 0.05% H<sub>2</sub>SO<sub>4</sub>. Plasma samples for insulin determination were stored at -20 °C and allotted random numbers so that their origin would not be known at the time of determination. Plasma insulin was determined in duplicate using an insulin immunoassay kit (Radiochemical Centre, Amersham, England) which resembles the double antibody method described by Hales and Randle [14]. The coefficient of variation for the standards with which the samples were run, so that batch to batch variation was included, was 14.4% ( $n=15$ ). However, since plasma samples for insulin determination from the entire series were randomised, the sources of error for the standards have been taken into account in the S.E.M.s for the plasma samples.

### Results

The heart rate (beats/min.) at rest was  $72 \pm 3$  (mean  $\pm$  S.E.M.) without and  $75 \pm 4$  with nicotinate infusion. At the end of exercise it was  $143 \pm 5$  without and  $146 \pm 6$  with nicotinate infusion.

The concentrations in arterial blood of three of the principal substrates for myocardial metabolism are shown in Table 1 for the four experimental categories. During prolonged exercise, plasma FFA and blood lactate concentrations increased about two-fold but the blood glucose concentration fell. The infusion of

nicotinate led to marked decreases in FFA concentration both at rest and during prolonged exercise but to little change in blood glucose and lactate concentrations.

The corresponding arterial plasma concentrations of insulin are shown in Table 2. Nicotinate had no significant effect on the arterial concentration of insulin. During prolonged exercise the insulin concentration fell, irrespective of the infusion of nicotinate.

Both at rest with nicotinate and during prolonged exercise without nicotinate there were significant negative arterial-coronary sinus differences in insulin concentration indicating net efflux of insulin into coronary sinus blood in these two categories (Table 2).

The <sup>125</sup>I-albumin data (Table 3) show that the observed insulin releases cannot be accounted for by a shift of plasma water. Even in the category "prolonged exercise with nicotinate infusion" where there was a significant change in plasma protein concentration this amounted to only 1%. However, the coronary sinus insulin concentrations were about 15 to 20% higher than the arterial concentrations.

There was a significant linear relationship between the arterial-coronary sinus difference in insulin concentration and arterial insulin concentration with the most marked release of insulin occurring at the lowest arterial concentrations (Fig. 1). This applied to the resting and exercise observations considered separately ( $r=0.52$ ,  $P<0.05$ ;  $r=0.54$ ,  $P<0.05$ ) as well as to the combined observations (Fig. 1).

Table 1. Concentrations of myocardial substrates in arterial blood in the four experimental categories

	Rest		Exercise	
	Without nicotinate ( $n=12$ )	Nicotinate infusion ( $n=9$ )	Without nicotinate ( $n=12$ )	Nicotinate infusion ( $n=9$ )
Plasma FFA	650 $\pm$ 50	250 $\pm$ 20	1320 $\pm$ 110	260 $\pm$ 40
Blood Glucose	4150 $\pm$ 150	4230 $\pm$ 90	3430 $\pm$ 140	3340 $\pm$ 270
Blood Lactate	590 $\pm$ 60	860 $\pm$ 80	1200 $\pm$ 130	1480 $\pm$ 230

Concentrations are  $\mu\text{mol/l}$ . In each category Mean  $\pm$  SEM is shown.

Table 2. Arterial blood plasma concentrations ( $C_a$ ) and arterial-coronary sinus concentration differences ( $C_{(a-cs)}$ ) in plasma insulin in the four experimental categories

	Rest		Exercise	
	A Without nicotinate ( $n=12$ )	B Nicotinate infusion ( $n=9$ )	C Without nicotinate ( $n=12$ )	D Nicotinate infusion ( $n=9$ )
$C_a$	17.9 $\pm$ 0.9	15.2 $\pm$ 1.4	13.8 $\pm$ 0.8	11.4 $\pm$ 1.4
$C_{(a-cs)}$	-1.1 $\pm$ 0.8 <sup>ns</sup>	-2.3 $\pm$ 0.6 <sup>**</sup>	-2.8 $\pm$ 0.9 <sup>**</sup>	-1.9 $\pm$ 1.2 <sup>ns</sup>

Units are  $\mu\text{Units/ml}$ .

In each category Mean  $\pm$  SEM is shown.

P values for  $C_a$ : A vs. B = ns; C vs. D < 0.01; B vs. D < 0.001. Significance of the difference  $C_{(a-cs)}$  from zero is shown by superscripts: \*\* $P < 0.01$ .

Table 3. Percentage change in plasma  $^{125}\text{I}$ -albumin radioactivity in the four experimental categories during passage through the coronary circulation

Rest		Exercise	
Without nicotinate	Nicotinate infusion	Without nicotinate	Nicotinate infusion
$-0.7 \pm 0.3^{\text{ns}}$ (11)	$0.1 \pm 0.4^{\text{ns}}$ (9)	$-0.8 \pm 0.5^{\text{ns}}$ (11)	$-1.0 \pm 0.2^{**}$ (9)

In each category Mean  $\pm$  SEM and number of observations in parentheses are shown. Significance is indicated by ns ( $P > 0.05$ ) and \*\* ( $P < 0.01$ ).

### Discussion

To our knowledge, the present work is the first demonstration in man of the release of immunoreactive insulin during passage of blood across the heart. Release of immunoreactive insulin from exercising forearm muscle has been shown in man during glucose infusion [16]. However, it must be emphasised that it is not known whether arterio-venous differences of immunoreactive insulin across an organ represent the true difference in the biologically active hormone. In our study, the  $^{125}\text{I}$ -albumin measurements indicate that if  $^{125}\text{I}$ -albumin is a valid indicator of plasma water shifts, then increases in plasma insulin concentration across the coronary circulation of the magnitude found cannot be accounted for by haemoconcentration.

The conditions of the present studies, fasting and fasting-exercise, are known to be associated with low arterial concentrations of insulin [17]. In spite of the narrow range of arterial insulin, 7–24  $\mu\text{U}/\text{ml}$ , it was possible to demonstrate a positive relationship between the arterial-coronary sinus difference in insulin concentration and the arterial concentration. If we use the regression line for this relationship (Fig. 1), insulin is taken up by the heart above an arterial concentration of 20  $\mu\text{U}/\text{ml}$  and released below that concentration. This would be in accordance with the view that interaction of insulin with its receptors on cell membranes follows the law of mass action [18]. Caution must be exercised, however, in the quantitative interpretation of the relationship between the insulin concentration in arterial plasma and its uptake/release by the heart because time, among other things, must be important for this relationship. For any given plasma insulin concentration, time will be required for an equilibrium to be reached between insulin concentration in plasma and at the receptor sites (whether at the capillary or in the tissues) so that release/uptake does not occur.

In the isolated perfused rat heart it has been shown that insulin-like activity is present at the beginning of a perfusion and that this activity decreases over the first 20 to 30 min. of perfusion. This effect has been attributed to the presence of insulin bound to the myocardium and subsequently released into the

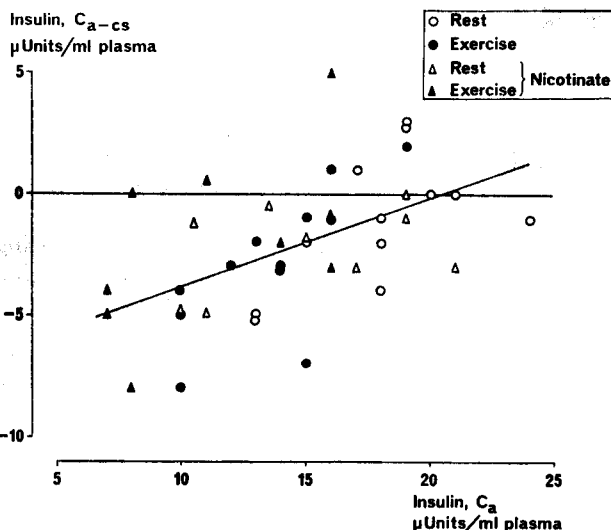


Fig. 1. Relationship between extraction from coronary blood plasma ( $C_{(a-cs)}$ ) and arterial plasma concentration ( $C_a$ ) of insulin in 12 subjects not receiving nicotinate and 9 subjects receiving a continuous intravenous infusion of nicotinate. Regression line drawn is for the resting and exercise observations combined ( $r = 0.53$ ,  $p < 0.001$ )

perfusate [19, 20]. Human forearm tissues at rest do fix insulin in relation to plasma concentration [1, 2, 16].

On the basis of the present data it is not possible to be certain that the insulin appearing in coronary sinus blood is coming from the heart although the rat heart data referred to above would support this. An increase in insulin immunoreactivity would accompany the transformation of proinsulin to insulin [21]. However, it seems unlikely that proinsulin is converted to insulin to any significant extent in the vascular bed of an organ [21]. It is also unlikely that blood cells are the source of insulin [3].

There is evidence from studies of plasma and thoracic duct lymph insulin that there is a partial capillary barrier to insulin in man [22]. In the dog hindlimb, tissue-bound  $^{125}\text{I}$ -insulin moves readily into venous blood, but not into lymph [3, 22]. Thus the myocardial vasculature could be the immediate source of the arterio-sinus increment in insulin concentration in the present investigation.

Nevertheless, a concentration gradient from capillaries to muscle or fat cells presumably exists so that these cells would also release insulin.

The question arises as to how much insulin can be released by the myocardium. At rest with a coronary plasma flow of about 150 ml/min., about 350  $\mu\text{U}$  insulin/min. would be released from the myocardium. This would correspond to about 1  $\mu\text{U}/\text{min.}/\text{g}$  myocardium. In one hour of a nicotinate infusion, assuming a steady-state insulin release, about 20000  $\mu\text{U}$  insulin would be released. This can be compared with the total turnover rate of insulin. If the biological half-life of insulin is 7 min. [22], its fractional turnover rate

will be 10%/min., and if the plasma insulin concentration is 15  $\mu$ U/ml, the plasma pool will be about 45000  $\mu$ U. Thus the total turnover rate of insulin is about 4500  $\mu$ U/min. and the heart could account for up to 8% of this during a nicotinate infusion.

Insulin release into the coronary circulation appeared to occur at the lower plasma concentrations. Both epinephrine and norepinephrine can inhibit insulin secretion by the pancreas, thus lowering plasma insulin concentration [17]. During the infusion of nicotinate there may be increased sympathetic drive to the pancreas along with that to the cardiovascular system [23]. Together with an increased output of epinephrine following nicotinate administration [24], such a sympathetic effect on the pancreas might overcome the direct effect which nicotinate has on islet tissue of stimulating insulin secretion [25] and therefore reduce insulin concentration. Low insulin concentrations produced in this way may result, therefore, in insulin release from the heart. The insulin release across the coronary circulation during prolonged exercise might also be related to a fall in plasma insulin concentration, as a result of a similar inhibition of pancreatic insulin secretion due to the increased sympathetic activity and concentration of circulating epinephrine [17].

It is known that both infusion of nicotinate [26] and prolonged exercise [27] increase myocardial glucose uptake, yet in both circumstances insulin release from the myocardium takes place. This suggests that either insulin is released from a site such as the capillary [22] where it does not affect myocardial metabolism or other factors, such as fatty acids [26] and exercise [27-29] play an important role in determining glucose extraction by the myocardium under these circumstances. In severe heart failure, insulin secretion by the pancreas is apparently reduced [30] and the availability of insulin to the myocardium may be decreased disproportionately through the myocardial release of insulin.

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