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Changes in Insulin Immunoreactivity across the Coronary Circulation in Man during Infusions of Glucose and a Fat Emulsion

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Abstract. The extraction of immunoreactive insulin by the human heart has been examined using the technique of coronary sinus catheterisation. The subjects were 12 male volunteers. During the study infusions of a fat emulsion and glucose were given in an effort to create a "steady fed state". The higher arterial insulin immunoreactivities which were observed during the infusions were associated with greater arterial coronary sinus differences in insulin immunoreactivity. The possibility of insulin degradation by the heart is discussed. During prolonged exercise the process of insulin removal by the heart appeared more active.

Key words:

Introduction

It has been shown previously that in healthy fasting men immunoreactive insulin can be released from the heart and that this occurs most markedly at the lowest arterial concentration of immunoreactive insulin (10). The fed state, however, might constitute a circumstance in which immunoreactive insulin is extracted by the heart since arterial insulin concentrations are raised. In the present investigation, subjects were given glucose and a fat emulsion by constant intravenous infusion in order to simulate the fed state and achieve reasonably steady blood concentrations of myocardial substrates (1). The kind of "fed state" so achieved would resemble that where glucose absorption takes place during the phase of alimentary lipaemia. Under these conditions, the myocardial extraction of immunoreactive insulin has been examined.

Methods

Twelve male volunteers between the ages of 22 and 51 years were studied. They were of average physical fitness. Neither medical history nor examination suggested cardiovascular or metabolic disease. Each subject had a normal resting electrocardiogram and the exercise electrocardiograms for those who exercised were also normal. The subjects were investigated without sedation after an overnight fast. Teflon catheters were introduced into a brachial artery and the coronary sinus for blood sampling (6). Heparin was not administered. The tip of a Teflon catheter was placed in the superior vena cava for the infusion of 20 % glucose at 0.31 - 0.32 g/min. A cannula was inserted into an arm vein for the infusion of 10 % Intralipid®-S at a rate of 0.16 - 0.17 g triglyceride/min. after a priming dose of 0.1 g triglyceride/kg body weight. Intralipid®-S is a modified form of Intralipid® with 5 % w/v sorbitol instead of 2.5 % w/v glycerol (1). The actual composition of Intralipid®-S was soybean oil 10 g, egg yolk phospholipids 1.2 g, sorbitol 5 g, sterile water to 100 m†. One, two, three and four hours after the commencement of infusions simultaneous arterial and coronary sinus blood samples were drawn.

Five of the 12 subjects performed supine leg exercise at a fixed work load after the two-hour blood samples were obtained. The work load was 50 % of that which produced a heart rate of 170/min. after 6 min. of exercise (W_{170}) (5, 11). Three of the five exercised for 120 minutes, one for 110 minutes and one for 95 minutes. Simultaneous arterial and coronary sinus blood samples were taken one hour after the commencement of exercise and again during the final five minutes of exercise. Each subject was given oral iodine (Lugol's solution) followed by an intravenous injection of about 6μCi of ^{125}I-albumin††, as a tracer for plasma albumin,

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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 (10). Blood samples for determination of glucose (3), lactate (7), FFA (9), exogenous triglyceride [††] (1) and ^{125}I-albumin (10) were collected as previously described (1). There was no evidence from assays of Intralipid®-S and Intralipid® that sorbitol interfered with these assays. Plasma for insulin determination was frozen at -20°C until assayed. Insulin was assayed using a radioimmunsorbent technique with insulin antibodies chemically coupled to ultrafine Sephadex particles (12) [†††]. The presence of ^{125}I-albumin in no way interfered with this radioimmunoassay (10).

Each sample was assayed in three dilutions and each of these dilutions was assayed in triplicate. The curve for the sample was parallel to that of the standard for all measurements made. Corresponding arterial and coronary sinus samples were assayed at the same time, but they were allotted random numbers so that their identity was not known to the assayer.

[††]By "exogenous triglyceride" is mean that plasma triglyceride circulating as Intralipid emulsion. The technique of nephelometry was used for this assay.

[†††]Ultrafine Sephadex and ^{125}I-labelled insulin were kindley supplied by Pharmacia AB, Uppsala.

Results

The heart rate (beats/min.) prior to the infusions was 70 ± 2 (mean ± SEM), during infusions at rest 73 ± 2 and during infusions at the end of exercise 148 ± 7.

The concentrations in arterial blood of four principal myocardial substrates are shown in Tables 1 and 2. For each subject, the infusions established

Table 1. Concentrations of myocardial substrates in blood (μmol/l) for each study at different times (hours)

	Plasma FFA					Plasma Exogenous TG				Blood Glucose					Blood Lactate	
	0	1	2	3	4	1	2	3	4	0	1	2	3	4	2	4
TL	710	960	1370	860	840	2670	2720	2120	2810	4600	9170	9900	9410	7900	990	1130
JB	380	1200	760	630	580	2010	1660	1840	2130	5070	8620	7570	7670	7650	1030	940
COH	580	590	620	770	740	3150	3270	2920	2680	4730	9100	9060	7530	8580	820	790
NEN	440	570	620	570	450	4070	4410	4520	5350	5400	9450	9510	9760	9220	1570	910
GL	430	430	530	620	580	2480	2580	2780	2920	4060	6280	6080	6390	6820	1130	1110
ID	570	530	580	600	630	1830	1800	1850	1890	3950	6660	7190	6910	7370	-	-
BN	470	710	730	600	550	2210	2110	1880	2290	4500	7700	9810	8710	10280	-	-
				EXERCISE				EXERCISE					EXERCISE		EXERCISE	
BM	910	670	720	750	1080	2696	2686	3150	3630	4990	8230	7950	5050	4530	1090	2890
LD	550	760	880	740	1110	1970	1730	1770	1960	4670	7570	7300	3670	3710	910	1320
GR	800	740	610	630	730	3660	4370	4360	5450	-	-	-	-	-	1020	1080
POF	1130	870	930	1350	1560	1100	1070	1410	1790	3700	7620	9320	3950	3960	730	1430
SFS	990	770	740	1680	2010	1880	1890	1970	2150	4390	8500	9780	3780	3910	890	1840

Table 2. Mean concentration of myocardial substrates in blood for the different experimental categories

	Fasting	During Infusions	
		Rest	Exercise
Plasma FFA	660±70 (12/12)	720±30 (36/12)	1080±170 (8/5)
Plasma Exogenous TG	0 (12/12)	2610±160 (36/12)	3030±480 (8/5)
Blood Glucose	4560±150 (11/11)	8200±190 (34/11)	4140±220 (6/4)
Blood Lactate	-	1000±50 (15/10)	1710±320 (5/5)

Concentrations are μmol/l. Mean ± SEM is shown with the corresponding number of observations followed by the number of subjects in parentheses below.

Table 3. Arterial (I_a) and arterial-coronary sinus differences (I_{a-cs}) in insulin immunoreactivity at different times during the study of each subject

Subject	0	1 hour		2 hours		3 hours		4 hours		Work Load kpm/min.
	I_a	I_a	I_{a-cs}	I_a	I_{a-cs}	I_a	I_{a-cs}	I_a	I_{a-cs}	
		Rest		Rest		Rest		Rest		
TL	15.0	35.0	0	76.0		89.0	-1.0	83.0	6.0	
JB	2.3	26.8	4.0	31.0	5.0	34.4	-2.1	22.1	-0.9	
COH	8.6	12.8	0.8	36.5	4.0	24.0	4.2	27.9	-1.6	
NEN	4.6	15.6	-1.7	24.7	-15.4	25.5	1.2	19.6	-2.3	
GL	7.7	9.5	-1.1	17.1	3.2	15.3	4.8	12.2	0.5	
ID	6.5	13.0	0.3	22.5	3.7	16.0	2.1	-	-	
BN	4.0	10.4	-1.1	12.6	0.1	19.7	-7.5	-	-	
						EXERCISE		EXERCISE		
BM	12.0	53.0	7.0	35.0	14.0	29.0	7.0	8.0	2.0	400
LD	3.2	20.4	3.0	15.0	-3.5	11.8	6.8	2.5	0.6	450
GR	4.3	8.3	0.5	19.9	-0.9	16.1	6.3	5.6	2.6	300
POE	7.0	20.5	-0.2	30.2	5.3	-	-	7.7	2.1	500
SES	7.5	10.5	-9.8	18.2	1.5	-	-	2.2	-1.0	700

LD exercised for 110 minutes and SES for 95 minutes, the last samples being taken during the final 5 minutes of exercise

Table 4. Arterial plasma insulin immunoreactivity (I_a) and arterial-coronary sinus difference (I_{a-cs}) in plasma insulin immunoreactivity

	Fasting (n = 12)	During Infusions Rest (n = 36)	Exercise (n = 8)
I_a	6.9±1.1	26.8±3.2	10.4±2.9
$I_{(a-cs)}$	-	1.2 ± 1.0^{ns}	$3.3\pm1.0^{\dagger}$

Units are μu/ml.
In each category mean ± SEM is shown.
Significance of the difference $I_{(a-cs)}$ from zero is shown by the superscript ns ($p > 0.05$) and † ($p < 0.05$).

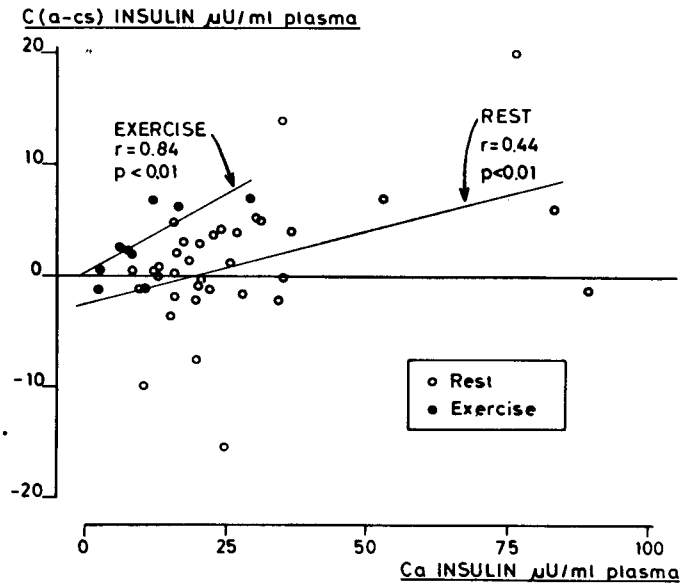

Fig. 1. Relationship between the arterial-coronary sinus difference in insulin immunoreactivity and arterial insulin immunoreactivity. Regression lines for observations made at rest and during prolonged exercises are shown separately

relatively constant blood concentrations of exogenous triglyceride, about twice those found in alimentary lipaemia (1). Glucose concentrations achieved during infusion were comparable with those that are found post-prandially. During prolonged exercise plasma FFA and blood lactate concentrations rose and blood glucose concentrations returned to the fasting range.

Arterial plasma insulin immunoreactivities increased from fasting values during the infusions at rest (Table 3, 4). During prolonged exercise, they were not significantly different from pre-in-

Table 5. Percentage change in plasma ^{125}I-albumin radioactivity during passage through the coronary circulation

Rest	Exercise
0.1 ± 0.5^{ns}	-0.3 ± 0.6^{ns}
(16/10)	(5/5)

Mean ± SEM is shown with the number of observations followed by the number of subjects in parenthesis below. Significance is indicated by ns ($p>0.05$).

fusion values (Table 3, 4). For the combined exercise observations, but not for those at rest, there was a significant myocardial extraction of immunoreactive insulin i.e. arterial-coronary sinus difference (I_{a-cs}) (Table 4).

$I_{(a-cs)}$ was significantly correlated with arterial insulin immunoreactivity (I_a) in both experimental categories (Figure 1). The same relationships would apply to the correlation, insulin uptake ($I_{(a-cs)}$ x flow) with insulin flow (I_a x flow), since flow is common to both.

Discussion

Our recent observations, at rest during nicotinate infusion and during exercise, of the cardiac release of immunoreactive insulin in relation to arterial insulin immunoreactivity were made at low or fasting insulin concentrations (10). Extrapolation of the regression lines suggested that at higher arterial insulin concentrations, insulin might be extracted by the heart. Parenteral feeding at rest in the present investigation led to arterial insulin values higher than the fasting values and to more positive $I_{(a-cs)}$ values than in the fasting investigation (10). One interpretation of these findings is that the phenomenon insulin + receptor $\rightleftharpoons$ insulin-receptor complex is occurring and obeying the law of mass action. This seems the most likely explanation for the release of insulin from the heart (10). It does imply, however, that a new steady state would in due course be reached, after altering I_a, when $I_{(a-cs)}$ would again be zero. The observations of $I_{(a-cs)}$ in the present investigation were made at 1, 2, 3 and 4 hours during parenteral feeding and although pooled, they appear to fit a common relationship for either the resting or exercise observations (Table 3, Fig. 1). This suggests that a phenomenon whereby insulin is constantly removed, for example degradation, might also contribute to $I_{(a-cs)}$. For liver, there is evidence that binding of insulin to receptors and degradation both occur and that they are independent (2).

During prolonged exercise there was a significant extraction of immunoreactive insulin for the group of observations. According to the regression equations, a given $I_{(a-cs)}$ occurred at a lower I_a during prolonged exercise than at rest. If ($I_{(a-cs)}$ x coronary plasma flow) rather than $I_{(a-cs)}$ should be related to I_a insulin then this would accentuate the difference in regression equations since coronary blood flow during exercise is probably 2 or 3 times that at rest (4). However, since I_a was lower for the exercise observations than for the resting observations by a factor of about 2.5, the actual amount of insulin presenting to a receptor population per unit time would have been similar during exercise and at rest. If there is a constant removal of insulin by the heart by degradation, then this process would be more active during exercise than at rest. It would be worthwhile in a future investigation to look for insulin degradation products in effluent blood.

Some estimate can be made of the contribution the heart would make to the peripheral removal of insulin during exercise in the fed state. The fractional turnover rate of insulin is about 10 %/min. (10) and, if the plasma insulin concentration is 10 μu/ml, the plasma pool will be about 30,000μu. Thus the total turnover rate of insulin will be about 3,000μu/min. With a coronary plasma flow 2-3 times resting flow i.e. about 400 ml/min. and a cardiac insulin extraction of 3μu/ml, the cardiac removal of insulin would be about 1,200μu/min. or about 30 % of the total turnover rate of insulin. The depression of arterial insulin concentrations during exercise is well recognized and generally attributed to sympathetic regulation of insulin release from the pancreatic ß-cell (8). It is conceivable that part of the fall in arterial insulin during exercise is due to increased peripheral removal. It is also possible that insulin removal by the heart has a role in myocardial glucose extraction during exercise in the fed state. However, as previously discussed (10), it cannot be asserted that the fate of extracted immunoreactive insulin is the myocardial muscle cell.

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