Glucocorticoid Uptake and Release by the Human Heart: Studies at Rest, during Prolonged Exercise, and during Nicotinic Acid Infusion

M. L. WAHLQVIST, L. KAIJSER, B. W. LASSERS,
H. LÖW & L. A. CARLSON
Dept. of Geriatrics, Uppsala University, Uppsala,
King Gustaf V Research Institute, Stockholm and
Depts. of Clinical Physiology and of Endocrinology,
Karolinska Hospital, Stockholm, Sweden

Wahlqvist, M. L., Kaijser, L., Lassers, B. W., Löw, H. & Carlson, L. A. Glucocorticoid Uptake and Release by the Human Heart: Studies at Rest, during Prolonged Exercise, and during Nicotinic Acid Infusion. *Scand. J. clin. Lab. Invest. 30*, 261–266, 1972.

Arterial and coronary sinus plasma glucocorticoid concentrations have been measured in 18 healthy men, 9 of whom received a nicotinic acid infusion to decrease arterial FFA concentration. At rest, the arterial-coronary sinus difference was correlated with the arterial concentration, so that above 10 μg/ml plasma glucocorticoid was taken up from, and below released into, the blood. To some extent, this was related to arterial insulin concentration. Although arterial glucocorticoid concentration was not significantly changed by nicotinic acid or exercise, significant release of glucocorticoid from the heart took place during prolonged exercise in those subjects receiving nicotinic acid. This release was associated with a rise in arterial growth hormone concentration. The release of glucocorticoid, here occurring in a situation with limited FFA availability, might facilitate the myocardial uptake of glucose although the concomitant growth hormone increase might tend to counteract this effect.

Key-words: [^{125}I]albumin; arterio-venous difference; coronary sinus; cortisol; exercise; glucocorticoid; growth hormone; insulin; myocardial metabolism; human; nicotinic acid

L. A. Carlson, M. D., Dept. of Geriatrics, University of Uppsala, Box 641, S-751 27 Uppsala 1, Sweden

Hormones affecting myocardial metabolism and function must, at various times, be taken up by the heart and may also be released again unchanged. There is evidence, for example, that catecholamines (3, 22) and thyroid hormones (12) can be removed from and released into the coronary circulation. We have recently reported release of insulin into the coronary circulation in man during prolonged exercise and also at rest during the infusion of nicotinic acid (28). In the present study, we have examined the possibility that plasma glucocorticoid might be taken up or released by the human heart, both in the fasting state when the main source of myocardial energy is lipid (16, 17) and also during the infusion of nicotinic acid when the main source is carbohydrate (18).

MATERIALS AND METHODS

Eighteen healthy men, aged between 21 and 42 years, were investigated without sedation after an overnight fast. Each subject had been given

oral iodine (Lugol's solution) and an intravenous injection of about 6 μC of [^{125}I]albumin (kindly provided by G. Birke and L.O. Plantin, King Gustaf V Research Institute, Stockholm, Sweden), as a tracer for plasma albumin, two days before the investigation. Teflon catheters were introduced into a brachial artery and the coronary sinus (17) so that blood samples could be drawn simultaneously from the two sites. Heparin was not administered. Blood samples were taken after 60 minutes at rest and again after between 65 and 125 minutes of exercise in the supine position on a cycle ergometer at a constant work load. The work load was 50 per cent of that which produced a heart rate of 170/minute after 6 minutes of exercise (W_{170}) (25, 30). It was intended that exercise should last 120 minutes, a duration tolerated by most healthy subjects at the load used (1). However, since the subjects had fasted and some of them received nicotinic acid infusions, 7 stopped before 120 minutes because of fatigue. The exercise sampling was made during the last 5 minutes of work in all subjects. In 9 of the 18 men a priming dose of 200 mg 5 per cent sodium nicotinate was given intravenously and then, 60 minutes before the first sampling, a continuous infusion of sodium nicotinate at a rate of 200 mg/hour in 3 and 400 mg/hour in 6 subjects was begun.

Blood samples for hormone and [^{125}I]albumin determinations were immediately transferred from the collection syringes to heparinized test tubes, placed in iced water, and centrifuged at 4 °C within 30 minutes. ^{125}I radioactivity was determined on 10 replicates of each arterial and coronary sinus sample (2). Plasma for hormone analysis was stored at −20 °C. Each sample was allotted a random number, and this was its only identification at the time of hormone determination. Plasma glucocorticoid was measured in duplicate, using the fluorometric method of DeMoor et al. (7), as modified by S. Laurell (personal communication). The use of chloroform instead of methylene chloride to extract glucocorticoid from the glass fibre papers (used as supporting medium for plasma in the Laurell modification) resulted in lower blank values. The method measures total unconjugated plasma cortisol and corticosterone. The addition of nicotinic acid in concentrations up to 50 μg/ml did not alter plasma glucocorticoid values. Plasma insulin was determined in duplicate, using an insulin immunoassay kit (Radiochemical Centre, Amersham, England) based on the double antibody method described by Hales & Randle (11). Plasma growth hormone was determined in duplicate using the double antibody radioimmunoassay of Cerasi et al. (6). The [^{125}I] albumin did not interfere with the insulin and growth hormone determinations (28). The coefficients of variation for the standards with which the samples were run, so that batch to batch variation is included, were 17.0 per cent (n = 20) for glucocorticoid, 14.4 per cent

Table I. Concentrations in arterial plasma (C_a) and extractions from coronary plasma (C_{a-cs}) of glucocorticoid in the four experimental categories

	Rest		Exercise	
	Without nicotinate (n = 9)	Nicotinate infusion (n = 9)	Without nicotinate (n = 9)	Nicotinate infusion (n = 9)
C_a (μg/100 ml)	12.5 ± 1.9	13.4 ± 2.7^{ns}	11.6 ± 2.1^{ns}	17.7 ± 2.9^{ns}
C_{a-cs} (μg/100 ml)	0.6 ± 1.4^{ns}	1.3 ± 1.8^{ns}	0.1 ± 1.6^{ns}	−7.1 ± 1.8**

In each category, mean ± S.E.M. is shown.
Significance of difference in concentration between the categories with and without nicotinate infusion, assessed by a t-test of the difference between two means, or significance of extraction, assessed by a t-test of paired differences, is indicated by ns ($P > 0.05$) or ** ($P < 0.01$) as a superscript. Significance of difference in concentration between the categories rest and exercise has also been assessed by a t-test of paired differences, but significance is indicated by ns ($P > 0.05$) as a subscript.

Table II. Percentage change in plasma [^{125}I]albumin radioactivity (c.p.m./ml) from artery to coronary sinus and corresponding glucocorticoid extractions, adjusted accordingly

	Rest		Exercise	
C_{a-cs}	Without nicotinate (n = 8)	Nicotinate infusion (n = 9)	Without nicotinate (n = 8)	Nicotinate infusion (n = 9)
[^{125}I]albumin (%)	-0.5 ± 0.3^{ns}	0.1 ± 0.4^{ns}	-0.5 ± 0.4^{ns}	$-1.0 \pm 0.2^{**}$
Glucocorticoid (μg/100 ml)	1.7 ± 1.4^{ns}	1.2 ± 1.8^{ns}	0 ± 1.8^{ns}	$-6.9 \pm 1.8^{**}$

In each category, mean ± S.E.M. is shown.
C_{a-cs} indicates difference between arterial and coronary sinus plasma.
Significance, assessed by a t-test of difference between two means [^{125}I]albumin or by a t-test of paired differences (Glucocorticoid), is indicated by ns ($P > 0.05$) or ** ($P < 0.01$).

(n = 15) for insulin, and 12.0 per cent (n = 15) for growth hormone. However, since plasma samples for hormone determinations from the entire series were randomized, the sources of error for the standards have been taken into account in the S.E.M.s for the plasma samples.

RESULTS

Heart rates

The heart rate (beats/min) at rest was 73 ± 4 (Mean ± S.E.M.) without and 75 ± 4 with nicotinic acid infusion; at the end of exercise it

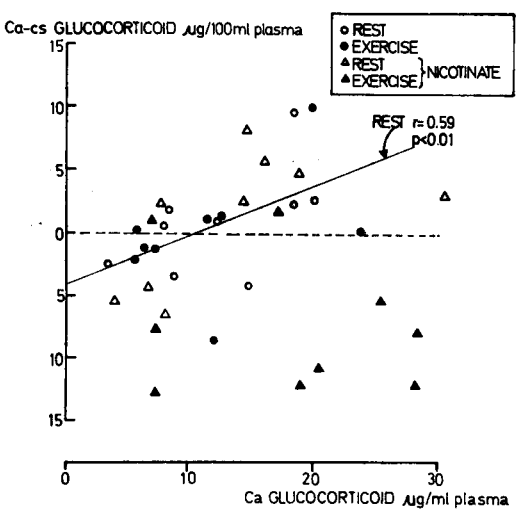

Fig. 1. Relationship between myocardial extraction of glucocorticoid and plasma glucocorticoid concentration in 18 subjects, 9 of whom received an infusion of nicotinic acid.

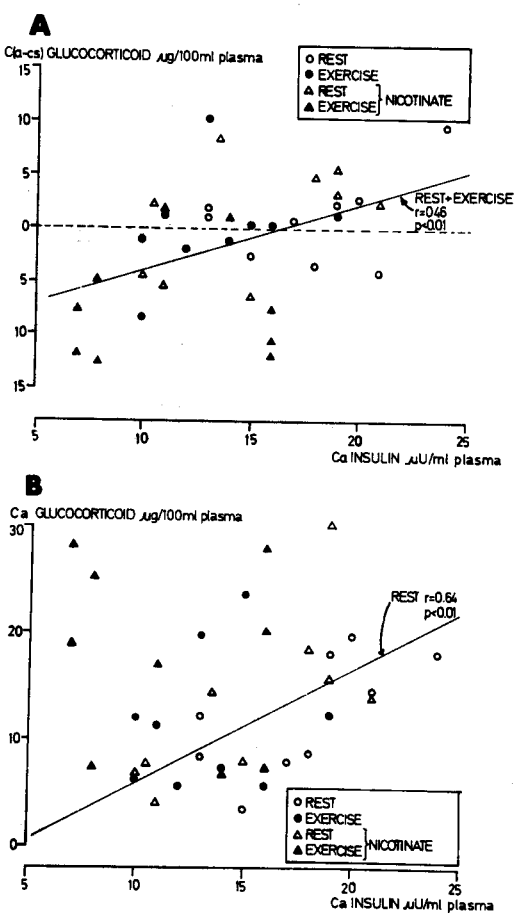

Fig. 2. A. Relationship between myocardial extraction of glucocorticoid and plasma insulin concentration.
B. Relationship between arterial plasma concentrations of glucocorticoid and insulin.

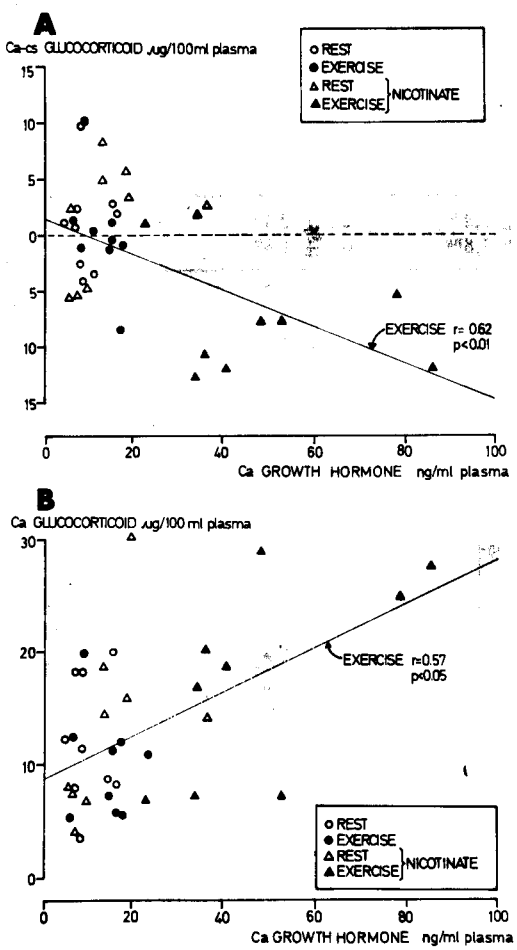

Fig. 3. A. Relationship between myocardial extraction of glucocorticoid and plasma growth hormone concentration.
B. Relationship between plasma glucocorticoid and growth hormone concentrations.

was 141 ± 5 without and 146 ± 6 with infusion.

Arterial-coronary sinus difference and arterial concentration of glucocorticoid

The arterial plasma glucocorticoid concentration was the same at rest and during prolonged exercise, and infusion of nicotinic and acid at rest or during exercise did not significantly alter the arterial concentration (Table I). However, since diurnal rhythm of glucocorticoid concentration is well-recognized (5), this cannot be taken as evidence that exercise does not affect plasma glucocorticoid concentration; in the present investigation, exercise observations were made two hours later in the morning than resting observations.

During prolonged exerise in the presence of a nicotinate infusion, a significant release of glucocorticoid into the coronary circulation was observed (Table I). This release, which amounted to 40 per cent of the arterial plasma concentration, could not be due to haemoconcentration, since the coronary sinus [^{125}I]albumin concentration was in no situation more than 1 per cent greater than that of the artery (Table II). In the other three experimental categories, no significant mean release or uptake was found (Table II).

However, when the resting observations for all 18 subjects were considered, a significant and positive linear correlation was found between the arterial-coronary sinus difference in glucocorticoid concentration and the arterial plasma glucocorticoid conentration (Fig. 1). But after eliminating the effect of insulin concentration by partial correlation analysis (26), the relationship was no longer significant ($r = 0.47$, $p > 0.05$). This is because both glucocorticoid extraction ($r = 0.51$, $p < 0.05$) and glucocorticoid concentration ($r = 0.64$, $p < 0.001$) are correlated with insulin concentration.

Arterial-coronary sinus difference of glucocorticoid and arterial insulin and growth hormone concentrations

The arterial-coronary sinus glucocorticoid difference was significantly and positively correlated with the arterial plasma insulin concentration at rest and for the combined resting and exercise observations (Fig. 2A). It was significantly and negatively correlated with the arterial plasma growth hormone concentration during exercise (Fig. 3A).

DISCUSSION

Glucocorticoid extraction

The present observations at rest have shown a positive relationship between the arterial-

coronary sinus glucocorticoid difference and arterial plasma glucocorticoid concentration at rest, glucocorticoid being extracted by the myocardium at plasma concentrations above 10 μg/ml plasma and released below this concentration. A concentration-dependent uptake of cortisol has also been shown for isolated cells (10).

The reason why the relationship mentioned is influenced by plasma insulin concentration is not clear, and there is little relevant information in the literature. Insulin might alter the binding of glucocorticoid to plasma proteins, although structurally the two hormones are quite dissimilar. Another possibility is that glucocorticoid, in its association with the cell membrane (20), might interact with insulin. This could apply even though part of the action of glucocorticoid appears to be at a nuclear level (9, 15, 20). If the uptake of glucocorticoid by myocardial cells were energy dependent, insulin, by increasing the availability of glucose, might facilitate glucocorticoid uptake, but available evidence indicates that only the outward transport of cortisol from cells is glucose dependent (10). However, the statistical correlation between glucocorticoid uptake and insulin concentration does not imply a causal relationship.

Glucocorticoid release

Glucocorticoid released from the myocardium during prolonged exercise in the presence of nicotinate could be derived from an intracellular site (10, 27), from cell membranes (20), or from interstitial binding proteins such as transcortin or albumin (5, 24) or even from connective tissue (19). Presumably this glucocorticoid is physiologically active, since the method of analysis does not measure conjugated derivations. However, the possibility that the hormone which is released is a non-conjugated derivate which has been produced by, for example, myocardial fibroblasts (8) cannot be excluded.

If one assumes a myocardial blood flow of 400–500 ml/min (13, 14, 16), an efflux of about 30 μg glucocorticoid/min from the heart would have occurred during prolonged work in the presence of nicotinate. Since the secretion rate of cortisol from the adrenals is about 10 μg/min (5), the observed myocardial release could not occur except during short periods. There is no evidence in the literature that the myocardium could synthesize corticoid.

The question still remains as to why glucocorticoid should have been released during prolonged exercise only when nicotinic acid was given. Whether nicotinic acid has a direct or an indirect effect, for example via growth hormone or FFA, on glucocorticoid release cannot be answered from the present investigation.

Metabolic implications

There is evidence that glucocorticoid can decrease myocardial utilization of glucose (21, 23). Thus uptake or release of glucocorticoid could be expected to alter myocardial glucose metabolism. The action of insulin to increase glucose uptake (29) may be partly offset if insulin facilitates myocardial uptake of glucocorticoid. However, a release of glucocorticoid, which may tend to increase glucose uptake, is seen in a situation (prolonged exercise, nicotinate) where FFA availability is low, although it is associated with high growth hormone concentrations which may again tend to reduce glucose uptake (29).

ACKNOWLEDGEMENTS

This work was supported by a grant from the Swedish Medical Research Council (19x–204). M.L Wahlqvist was recipient of a Life Insurance Medical Research Fund of Australia and New Zealand Overseas Fellowship and B.W. Lassers of a Wellcome Swedish travelling fellowship.

REFERENCES

1. Ahlborg, B. Capacity for prolonged physical exercise in relation to some anthropometric and other data. *Försvarsmedicin 2,* 194, 1966.
2. Boberg, J., Carlson, L. A. & Freyschuss, U. Determination of splanchnic secretion rate of plasma triglycerides and of total and splanchnic turnover of plasma free fatty acids in man. *Europ. J. clin. Invest, 2,* 123, 1972.

3. Braunwald, E., Harrison, D. C. & Chidsey, C. A. The heart as an endocrine organ. *Amer. J. Med. 36,* 1, 1964.
4. Catt, K. J. ABC of endocrinology III-Growth hormone. *Lancet I,* 933, 1970.
5. Catt, K. J. ABC of endocrinology V-Adrenal cortex. *Lancet I,* 1275, 1970.
6. Cerasi, E., Della Casa, L., Luft, R. & Roovete, A. Determination of human growth hormone (HGH) in plasma by a double antibody radioimmunoassay. *Acta endocr. (Kbh) 53,* 101, 1960.
7. De Moor, P., Steeno, O., Raskin, M. & Hendrikx, A. Fluorimetric determination of free plasma 11-hydroxycorticosteroids in man. *Acta endocr. (Kbh.) 33,* 297, 1960.
8. Dougherty, T. F. & Berliner, D. L. Some ways by which ACTH and cortisol influence functions of connective tissue. pp. 143–166 in Page, I. H. (ed.) *Connective Tissue, Thrombosis & Atherosclerosis* Academic Press, New York, 1959.
9. Fain, J. N., Kovacev, V. P. & Scow, R. O. Effect of growth hormone and dexamethasone on lipolysis and metabolism in isolated fat cells of the rat. *J. biol. Chem. 240,* 3522, 1965.
10. Gross, S. R., Aronow, L. & Pratt, W. B. The outward transport of cortisol by mammalian cells in vitro. *J. Cell Biol. 44,* 103, 1970.
11. Hales, C. N. & Randle, P. J. Immunoassay of insulin with insulin-antibody precipitate. *Biochem. J. 88,* 137, 1963.
12. Hillier, A. P. The uptake and release of thyroxine and tri-iodothyronine by the perfused rat heart. *J. Physiol. (Lond.) 199,* 151, 1968.
13. Holmberg, S., Serzysko, W. & Varnauskas, E. Coronary circulation during heavy exercise in control subjects and patients with coronary heart disease. *Acta med. scand. 190,* 465, 1971.
14. Jörgensen, C. R., Kitamura, K., Gobel, F. L., Taylor, H. L. & Wang, Y. Long-term precision of the N_2O method for coronary flow during heavy upright exercise. *J. appl. Physiol. 30,* 338, 1971.
15. Jost, J.-P. & Rickenberg, H. V. Cyclic AMP. *Ann. Rev. of Biochem. 40,* 741, 1971.
16. Kaijser, L., Lassers, B. W., Wahlqvist, M. L. & Carlson, L. A. Myocardial lipid and carbohydrate metabolism in healthy fasting men during prolonged exercise. *J. appl. Physiol, 32,* 847, 1972.
17. Lassers, B. W., Kaijser, L. & Carlson, L. A. Myocardial lipid and carbohydrate metabolism in healthy, fasting men at rest: Studies during continuous infusion of ^{3}H-palmitate. *Europ. J. clin. Invest. 2,* 348, 1972.
18. Lassers, B. W., Wahlqvist, M. L., Kaijser, L. & Carlson, L. A. The effect of nicotinic acid on myocardial lipid and carbohydrate metabolism in healthy fasting men at rest and during prolonged exercise. *J. appl. Physiol. 33,* 72, 1972.
19. Levin, M. E., Daughaday, W. H. & Bremer, R. Tissue binding of hydrocortisone. *J. Lab. clin. Med. 45,* 833, 1955.
20. Munck, A. The effects of hormones at the cellular level. pp. 139–179 in V. H. T. James (ed.) *Recent Advances in Endocrinology, 8th Edition.* J & A Churchill, London, 1968.
21. Newsholme, E. A. & Randle, P. J. Regulation of glucose uptake by muscle 7. Effects of fatty acids, ketone bodies and pyruvate, and of alloxan diabetes, starvation, hypophysectomy and adrenalectomy, on the concentrations of hexose phosphates, nucleotides and inorganic phosphates in perfused rat heart. *Biochem. J. 93,* 641, 1964.
22. Opie, L. H. Metabolism of the heart in health and disease. Part II. *Amer. Heart J. 77,* 100, 1969.
23. Regen, D. M., Davis, W. W., Morgan H. E. & Park, C. R. The regulation of hexokinase and phosphofructokinase activity in heart muscle. Effects of alloxan diabetes, growth hormone, cortisol and anoxia. *J. biol. Chem. 239,* 43, 1964.
24. Sandberg, A. A., Woodruff, M., Rosenthal, H., Nienhouse, N. & Slaunwhite, W. R. Transcortin: A corticosteroid-binding protein of plasma. VII. Half-life in normal and estrogen-treated subjects. *J. clin. Invest. 43,* 461, 1964.
25. Sjöstrand, T. Changes in the respiratory organs of workmen at an ore smelting works. *Acta med. scand.* Suppl. *196,* 687, 1947.
26. Snedecor, G. W. *Statistical Methods.* Iowa State University Press, Iowa, 1956.
27. Stumpf, W. E. Estrogen, androgen and adrenal hormone attracting neurons in the periventricular brain. *Fed. Proc.* Abstract 654, 1971.
28. Wahlqvist, M. L., Kaijser, L., Lassers, B. W., Löw, H. & Carlson, L. A. Release of insulin from the human heart (in preparation).
29. Wahlqvist, M. L., Kaijser, L., Lassers, B. W., Löw, H. & Carlson, L. A. The role of fatty acid and of hormones in the determination of myocardial carbohydrate metabolism in healthy fasting men (in preparation).
30. Wahlund, H. Determination of the physical working capacity. *Acta med. scand.* Suppl. *215,* 1948.

Received 29 March 1972
Accepted 13 July 1972