Influences of Corticosteroids on Cardiac Glycogen Concentration in the Rat

By J. Charles Dow, Ph.D., Allan M. Lefer, Ph.D., and Robert M. Berne, M.D.

ABSTRACT

Bilateral adrenalectomy resulted in a decrease (4.97 to 2.79 mg/g) in cardiac glycogen concentration in pentobarbital-anesthetized rats. Similar differences were observed at 10 to 14 days and at 27 days after adrenalectomy. Sham-adrenalectomized rats had normal cardiac glycogen concentration (4.98 mg/g). Total phosphorylase and phosphorylase \( a \) activities and glucose-6-phosphate concentrations in hearts obtained from adrenalectomized rats were not significantly different from controls. Total glycogen transferase activity was decreased in adrenalectomized rats and for any given glycogen concentration, the percent of the glycogen transferase in the independent form (percent transferase \( I \)) was significantly lower in the adrenalectomized rats than in the intact controls. The glucocorticoid dexamethasone (40 \( \mu \)g/day) prevented the decreases in cardiac glycogen concentration and percent transferase \( I \) in adrenalectomized rats. In the intact rat dexamethasone (40 \( \mu \)g/day) increased cardiac glycogen concentration and total transferase activity to above control levels as did larger doses in both adrenalectomized and control rats. The mineralocorticoid deoxycorticosterone acetate (DOCA), had relatively little effect on cardiac glycogen concentration. Conclusion: glucocorticoids exert a regulatory role in cardiac glycogen metabolism.

ADDITIONAL KEY WORDS

glycogen transferase, glycogen synthetase, adrenalectomy, glucocorticoids, mineralocorticoids, deoxycorticosterone acetate (DOCA), dexamethasone, myocardial glycogen, heart glycogen, glucose-6-phosphate

In 1956, Russell and Bloom (1) reported that adrenalectomy does not alter the cardiac glycogen concentration in rats. However, Barta and Pavlovičová (2) recently demonstrated that cardiac glycogen is decreased in adrenalectomized rats. Furthermore, Suzuki (3) has shown that adrenalectomy markedly reduced the size and number of glycogen granules in myocardial cells in rats. In view of these recent developments, we undertook the present investigation with the following objectives: (1) to resolve this controversy, (2) to ascertain whether changes in the activities of two major enzymes of glycogen metabolism, glycogen transferase (synthetase) and glycogen phosphorylase, are involved in the metabolic mechanism responsible for any changes in cardiac glycogen concentration consequent to adrenalectomy or corticosteroid treatment, and (3) to determine whether the corticosteroids exert a regulatory function in cardiac glycogen metabolism.

Methods

Animals—Male, albino, Sprague-Dawley rats (210 to 350 g) were used. Rats were anesthetized with ether and bilaterally adrenalectomized via a dorsal lumbar approach. Rats with sham operations were similarly prepared except that the adrenals were not removed. The drinking water of all adrenalectomized rats was 0.9% NaCl. All rats were fed Purina rat chow ad libitum.

One group of adrenalectomized and one group of control rats with no operation were given 40, 200, or 1000 \( \mu \)g dexamethasone (Decadron,
activity was assayed by measuring the incorporation of the \(^1^4\)C-glucose moiety of \(^1^4\)C-glucose-labeled UDPglucose into glycogen primer as previously described (8), except that the incorporated radioactivity was determined by liquid scintillation spectrometry. The purified glycogen was dissolved in 1 ml H\(_2\)O and to it was added 10 ml of Triton-toluene 2:1, 2,5-diphenyloxazole (PPO) 5.5 g/L, 1,4-bis-(5-phenyloxazolyl)-benzene (POPOP) 150 mg/liter. The vials were chilled 1 hour and then counted immediately. Total transferase activity was determined in the presence of 7.22 mM glucose-6-P. Transferase I activity was regarded as the activity observed in the absence of added glucose-6-P.

**Phosphorylase.**—Total phosphorylase was determined in the presence of 1.5 mM AMP. Phosphorylase a activity was regarded as the activity of the Dowex-1-treated extract in the absence of added adenosine monophosphate as previously described (8).

**Glucose-6-Phosphate.**—Frozen powdered ventricle was ground for 3 min in a Teflon and glass tissue homogenizer with 9.25 volumes of cold (0°C) 0.6 N perchloric acid. The homogenate was centrifuged and a portion of the supernatant was neutralized with KOH. The neutralized extract was kept in the frozen state until assayed. The extracts were thawed and centrifuged to remove potassium perchlorate. Glucose-6-P was determined enzymatically with glucose-6-P dehydrogenase (Boehringer) in the presence of nicotinamide adenine dinucleotide phosphate (NADP) (9). The reduction of NADP was measured by the increase in optical density at 340 mg.

**Results**

In a preliminary study, the glycogen concentration of a series of papillary muscles was compared to that of the ventricles from which the papillary muscles had been excised. Papillary muscles and ventricles from adrenalectomized rats were compared with those of

**TABLE 1**

Glycogen Concentration of Rat Heart Tissue

<table>
<thead>
<tr>
<th></th>
<th>Glycogen concentration (mg/g)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole ventricle</td>
<td>4.79 ± 0.6</td>
<td>2.39 ± 0.4†</td>
<td></td>
</tr>
<tr>
<td>Papillary muscle</td>
<td>4.73 ± 0.4</td>
<td>2.30 ± 0.4‡</td>
<td></td>
</tr>
<tr>
<td>(from same hearts)</td>
<td>(5)</td>
<td>(6)</td>
<td></td>
</tr>
</tbody>
</table>

*Mean ± SEM; numbers in parentheses indicate number of hearts studied.† = P < 0.01, ‡ = P < 0.005.

*Circulation Research, Vol. XXII, May 1968*
CORTICOSTEROIDS AND CARDIAC GLYCOGEN

control rats (Table 1). The glycogen concentrations of ventricles and papillary muscles of control rats were significantly lower than those of adrenalectomized rats. However, the glycogen concentrations of ventricles and papillary muscles were not significantly different from each other within the intact control group or within the adrenalectomized group.

A more comprehensive study was undertaken to determine whether the decreased glycogen concentration in adrenalectomized rats was a real phenomenon or an artifact of

TABLE 2

<table>
<thead>
<tr>
<th>Condition</th>
<th>No.</th>
<th>Cardiac glycogen* (mg/g)</th>
<th>P value†</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pentobarbital Anesthesia</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>35</td>
<td>4.97 ± 0.17</td>
<td></td>
</tr>
<tr>
<td>Sham-adrenalectomized</td>
<td>7</td>
<td>4.98 ± 0.22</td>
<td>&gt; 0.5</td>
</tr>
<tr>
<td>Adrenalectomized (10-14 days)</td>
<td>24</td>
<td>3.25 ± 0.14</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Adrenalectomized (27 days)</td>
<td>8</td>
<td>2.97 ± 0.19</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td><strong>Ether Anesthesia</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>5</td>
<td>4.39 ± 0.26</td>
<td></td>
</tr>
<tr>
<td>Adrenalectomized (10-14 days)</td>
<td>6</td>
<td>2.91 ± 0.19</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

*All values are means ± SEM.
†Significance of difference from control.

Effect of corticosteroids on cardiac glycogen concentration in adrenalectomized rats. Cardiac glycogen concentration is expressed in mg/g of ventricular tissue. The dashed line is a reference line for nonadrenalectomized control rats (intact). The height of each column represents the means; the symbol at the top of each column represents ± 1 SEM. The number of rats studied is indicated by the numbers within the columns. Steroids were administered for 10 to 14 days starting on the day of adrenalectomy.

Circulation Research, Vol. XXII, May 1968
Effect of dexamethasone on cardiac glycogen concentration in intact rats. Cardiac glycogen is expressed in mg/g of ventricular tissue. The height of each column represents the means, and the symbol at the top of each column represents ± 1 SEM. The number of rats studied is indicated by the numbers within the column. Steroids were administered for 10 to 14 days immediately prior to collection of samples.

The experimental design. Therefore, adrenalectomized rats were studied at different postoperative times, under different anesthetic agents, and were compared with sham-operated animals (Table 2). These data clearly indicate that there is a significant reduction of cardiac glycogen at 10 to 14 and at 27 days after adrenalectomy. The values at 27 days are not significantly different from the 10 to 14 day values. Similar results were obtained in the rats anesthetized with ether.

To determine whether corticosteroids could prevent the effects of adrenalectomy, dexamethasone, a potent synthetic glucocorticoid having very little mineralocorticoid activity, and deoxycorticosterone acetate (DOCA), a potent mineralocorticoid with very little glucocorticoid activity, were administered. Dexamethasone at a dose of 40 µg/day prevented the decrease in cardiac glycogen concentration observed in adrenalectomized rats (Fig. 1). Higher doses of dexamethasone in the pharmacological range (200 to 1000 µg/day) elevated the cardiac glycogen concentration to above control levels. These values were significantly higher (P < 0.005 and P < 0.001, respectively) than those of control animals. In contrast, DOCA at a daily dose of 1000 µg, was ineffective in preventing the decrease in cardiac glycogen concentration of adrenalectomized rats.

Dexamethasone, 40 and 200 µg/day, significantly elevated cardiac glycogen in control rats (P < 0.005 and P < 0.001, respectively) (Fig. 2). Although the larger dose of dexamethasone produced a higher cardiac glycogen concentration than did the smaller dose, the difference in glycogen concentration attained is not statistically significant. Thus, in the control rats a plateau was reached at a daily dose of 40 µg, whereas a progressive increase was obtained at higher doses in the adrenalectomized rats.

In an attempt to determine the role of the

<table>
<thead>
<tr>
<th>Group</th>
<th>Total phosphorylase activity*</th>
<th>Phosphorylase (%)</th>
<th>Glucose-6-P (µmoles/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2282 ± 174 (8)</td>
<td>3.73 ± 0.89 (8)</td>
<td>0.19 ± 0.021 (7)</td>
</tr>
<tr>
<td>Adrenalectomized</td>
<td>2107 ± 222 (8)</td>
<td>2.67 ± 0.49 (8)</td>
<td>0.18 ± 0.018 (7)</td>
</tr>
<tr>
<td>Significance (t)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

All values are means ± SEM. Numbers of rats in parentheses.

*Phosphorylase activity expressed as µmoles P/G wet weight/hour.
CORTICOSTEROIDS AND CARDIAC GLYCOPEN

TABLE 4

<table>
<thead>
<tr>
<th>Group</th>
<th>Total transfase activity (μmoles UDPG/g/hr)</th>
<th>Transfase I activity (μmoles UDPG/g/hr)</th>
<th>Percent transfase I</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (15)</td>
<td>77.4 ± 1.8</td>
<td>24.0 ± 1.9</td>
<td>30.9 ± 2.3</td>
</tr>
<tr>
<td>Control + dexamethasone 40 μg/day (6)</td>
<td>90.4 ± 3.3a</td>
<td>25.3 ± 4.8</td>
<td>27.8 ± 4.5</td>
</tr>
<tr>
<td>Control + dexamethasone 200 μg/day (6)</td>
<td>97.8 ± 1.9a</td>
<td>16.5 ± 3.5</td>
<td>16.9 ± 3.5c</td>
</tr>
<tr>
<td>Adrenalectomized (12)</td>
<td>62.7 ± 1.9a</td>
<td>12.0 ± 1.1d</td>
<td>19.2 ± 1.6d</td>
</tr>
<tr>
<td>Adrenalectomized + dexamethasone 40 μg/day (6)</td>
<td>74.3 ± 5.0e</td>
<td>22.7 ± 3.0e</td>
<td>30.6 ± 3.2e</td>
</tr>
<tr>
<td>Adrenalectomized + dexamethasone 200 μg/day (6)</td>
<td>80.8 ± 3.7b</td>
<td>14.2 ± 2.6a</td>
<td>17.5 ± 2.7c</td>
</tr>
<tr>
<td>Adrenalectomized + dexamethasone 1000 μg/day (6)</td>
<td>86.2 ± 2.1bh</td>
<td>9.6 ± 1.5l</td>
<td>11.2 ± 1.8lf</td>
</tr>
<tr>
<td>Adrenalectomized + DOCA 1000 μg/day (6)</td>
<td>74.8 ± 2.6e</td>
<td>15.3 ± 1.0a</td>
<td>20.5 ± 1.1a</td>
</tr>
</tbody>
</table>

All values are means ± SEM. Number of hearts assayed is in parentheses. a, b, c, d, e = Significantly different from control group. e, f, g, h = Significantly different from adrenalectomized group. a and e = P < 0.025; b and f = P < 0.01; c and g = P < 0.005; d and h = P < 0.001.

major regulatory enzymes of glycogen metabolism in these changes in cardiac glycogen, the activities of glycogen transferase and glycogen phosphorylase and the tissue concentration of glucose-6-P were determined.

No significant differences were found between hearts of control and adrenalectomized rats with respect to the total phosphorylase or phosphorylase a activities. The tissue concentration of glucose-6-P was also not significantly altered by adrenalectomy (Table 3). Thus, the changes in cardiac glycogen seen in adrenalectomized rats cannot be attributed to alterations in glycogen phosphorylase activity, or in reduced cofactor activation of transferase D.

Table 4 summarizes the influence of adrenalectomy and corticosteroid replacement on the glycogen transferase activities in rat hearts. Adrenalectomy decreased total transferase activity and transferase I activity, with a resultant decrease in percent transferase I. Dexamethasone (40 μg/day) maintained total transferase activity, transferase I activity, and percent transferase I of adrenalectomized rats at control values, whereas higher doses elicited an increase in total transferase activity but reduced transferase I activity and percent transferase I. In control rats, dexamethasone (40 and 200 μg/day) significantly elevated total transferase activity above control values. Dexamethasone (200 μg/day) did not significantly decrease transferase I activity (P > 0.05); however, percent transferase I was significantly decreased (P < 0.005). Although DOCA prevented the decrease in total transferase activity of adrenalectomized rats, it did not maintain transferase I activity and percent transferase I at pre-adrenalectomy levels.

Discussion

The present study clearly shows that the concentration of glycogen in the heart is decreased following adrenalectomy. These results contradict the findings of Russell and...
Bloom (1) and Bray and Morrison (10). Russell and Bloom reported no change in cardiac glycogen 14 to 28 days after adrenalectomy. The methods that Russell and Bloom used are very similar to ours and the small differences in technique cannot account for the difference in results. In the case of Bray and Morrison (10) who failed to see a decrease in cardiac glycogen 6 days after adrenalectomy in rats fasted for 24 hours, the normal glycogen values may have been due to the effects of fasting. It is possible that fasting of their rats increased the cardiac glycogen more in adrenalectomized rats than in intact rats, which would obscure the difference between the two groups.

The present finding of a decrease in cardiac glycogen after adrenalectomy is consistent with that of others (2, 3, 11, 12). Britton and associates (11) observed a decrease in cardiac glycogen of New World white-faced monkeys 1 to 18 days after adrenalectomy, but failed to see a similar change in night monkeys or marmosets. Barta and Pavlovičuvá (2) found a decreased cardiac glycogen in rabbits 18 to 21 days after adrenalectomy and fasted for 24 hours prior to study. Fizel and Fizelová (12) also found a decreased cardiac glycogen in rabbits 7 to 12 days after adrenalectomy. With electron microscopy, Suzuki (3) observed a decrease in the size and number of glycogen granules in the hearts of rats 5 to 13 days after adrenalectomy.

The decrease in cardiac glycogen after adrenalectomy in the present study was prevented by glucocorticoid replacement therapy but was unaffected by a massive dose of mineralocorticoid. Furthermore, larger doses of glucocorticoids increased the cardiac glycogen above the concentrations observed in intact rats. Bray and Morrison (10) also observed an increase in the cardiac glycogen of adrenalectomized, fasted rats 24 hours after a single injection of cortisone acetate (1.0 mg/100 g body weight), and Fizel and Fizelová (12) reported an increase of cardiac glycogen in the left ventricles of intact rabbits injected with 15 mg/kg hydrocortisone and 4 mg/kg DOCA twice daily for 4 days.

However, Illingworth and Russell (13) failed to see any significant effect of aqueous adrenocortical extract on the cardiac glycogen of glucose-fed adrenalectomized rats, a finding which may be due to the low steroid content of this substance. Thus, the steady-state cardiac glycogen level appears to be a function of the amount of glucocorticoid present in the rat, indicating that the glucocorticoids exert more than a permissive action with respect to maintenance of the levels of cardiac glycogen.

The present study also demonstrates that the glycogen concentration of the left ventricular papillary muscles is representative of the whole ventricle. This is consistent with the previous observation of Hazelwood and Ullrick (14) that the glycogen concentration of left ventricular trabeculae carnea muscle is comparable to that of the left ventricle.

There are two major possibilities for direct enzymatic mechanisms accounting for changes in tissue glycogen. These are changes in the activities of the regulatory enzymes of glycogen metabolism, glycogen transferase (synthetase), and glycogen phosphorylase. Tissue glycogen could be increased or decreased by changes in total enzyme activity and/or by changes in the proportion of enzyme in the cofactor-independent ("active") form (transferase I and phosphorylase a) and/or by changes of the activators, inhibitors, cofactors and substrates (glucose-6-P, AMP, ATP, UDPG, glycogen, P1, glucose-1-P).

Total phosphorylase and phosphorylase a activities were unchanged after adrenalectomy. The phosphorylase a activity, consistent with numerous other reports (15, 16), was low, on the order of 2 to 4% of the total activity. Hence, an increase in neither total phosphorylase nor phosphorylase a activity can account for the decrease in cardiac glycogen found in adrenalectomized rats.

The decrease in cardiac glycogen concentration after adrenalectomy was also accounted for by changes in cardiac glucose-6-P concentration, since the levels in adrenalectomized animals were not significantly different from those of control animals. Thus, the...
observed decrease in cardiac glycogen concentration after adrenalectomy is probably not due to an alteration of glucose and fatty acid metabolism with decreased intracellular glucose-6-P (17), unless there are glucose-6-P changes in some circumscribed intracellular pool. The possible contribution of changes in other carbohydrate intermediates, nucleotides and nucleotide sugars has not been investigated.

Significant effects were observed in transferase activities. Total transferase activity was lowest in the hearts of adrenalectomized rats, and highest in the hearts of rats given large doses of glucocorticoids (Table 4). Furthermore, total transferase activity was increased at higher cardiac glycogen concentrations (Figs. 1 and 2, and Table 4). Therefore, it seems likely that glucocorticoids induce the formation of glycogen transferase in the heart, as they do in the liver (18). Because glycogen transferase is unstable in the absence of adequate concentrations of glycogen (19, 20), it seemed possible that this correlation was an artifact caused by the lower glycogen concentration in extracts from hearts with less glycogen. This possibility was tested by the addition of glycogen to the extraction medium. Hearts of adrenalectomized rats with initial glycogen concentrations of about 1.8 to 2.9 mg/g were extracted in the presence of an additional 5 mg/g of purified glycogen. Transferase I and total transferase activities were then assayed as described under Methods. No change in transferase activities was observed. Thus, the correlation of transferase activity with the cardiac glycogen concentration does not appear to be an artifact of the extraction method, unless added glycogen cannot replace intracellular glycogen.

A second effect of adrenalectomy and glucocorticoids on cardiac glycogen transferase activity was an alteration in the relationship between percent transferase I and tissue glycogen concentration. Both glycogen concentration and transferase I activity were decreased in adrenalectomized rats. In contrast, large doses of dexamethasone increased the cardiac glycogen concentration, but decreased the transferase I activity or percent transferase I of both intact and adrenalectomized rats. Skeletal muscle glycogen is known to be inversely correlated with percent transferase I, presumably because of inhibition of transferase phosphatase at high concentrations of glycogen (21, 22). Several experimental conditions have been studied in skeletal muscle in which the feedback inhibition of transferase phosphatase by glycogen and the inverse relationship between percent transferase I and glycogen are altered, thereby resulting in a modified tissue glycogen concentration (21). Our findings indicate an inverse relationship between glycogen and transferase I activity or percent transferase I in intact and adrenalectomized rats when cardiac glycogen is increased by exogenous glucocorticoids. However, in glucocorticoid-insufficient rats, transferase I activity and percent transferase I have a different relationship to glycogen than in the other groups in that they are lower for any given glycogen concentration. An inverse relationship between transferase I activity and myocardial glycogen has been previously reported, but a shift in this relationship has not been previously described for the heart (8, 23).

The reduction in cardiac transferase I and total transferase activities after adrenalectomy and the increase in total transferase activity with the administration of glucocorticoids are consistent with control of cardiac glycogen concentration by glucocorticoids. These changes in enzyme activities would result in decreased glycogen synthesis in hearts of adrenalectomized rats and could therefore account for the observed reduction in glycogen concentration in these hearts. The increased cardiac glycogen concentration in glucocorticoid-treated rats can be largely explained by the increased total transferase activity with increasing doses of glucocorticoids. The biphasic response of transferase I activity is attributable to a restoration of normal values by replacement therapy with dexamethasone and a subsequent depression of activity by the high concentrations of cardiac glycogen. The role played by possible...
alterations of the in vivo concentrations of activators, inhibitors, substrates, and products of glycogen synthesis other than glucose-6-P have not been evaluated in this study.

In some species, adrenalectomy results in a severe depression of cardiac function with resulting hypotension (24, 25). It seemed possible that the results seen in adrenalectomized animals could be partially or wholly attributed to inadequate coronary perfusion. The mean arterial blood pressure was measured directly under pentobarbital anesthesia in 30 adrenalectomized (14 days post-adrenalectomy) and 10 control rats. Neither of these groups was used for metabolic studies. The mean arterial blood pressure was significantly reduced in adrenalectomized rats compared with control rats (112 ± 4.7 SEM VS. 142 ± 3.3 mm Hg, \( P < 0.001 \)). However, this degree of reduction in blood pressure is not sufficient to significantly impair coronary perfusion because of autoregulation of coronary blood flow (28).

It is also possible that the observed changes in cardiac glycogen are secondary to changes in blood glucose concentration. The consensus is that blood glucose does not decrease in adrenalectomized rats fed \textit{ad libitum} and maintained on NaCl in the drinking water for 10 to 14 days after adrenalectomy (27, 28). However, some investigators have reported a decrease (124 to 97 mg/100 ml blood) in blood glucose concentration in adrenalectomized rats (29). In fasted nonadrenalectomized rats, glucocorticoids elicit only a slight increase in blood glucose concentration (30). Thus, changes in blood glucose concentration are relatively minor at different levels of available glucocorticoid, and probably cannot account for the alterations in cardiac glycogen reported in this study. The observed changes in cardiac glycogen concentration also cannot be accounted for by changes in tissue water content since cardiac water content is unaltered in adrenalectomized rats maintained on 1% NaCl solution (31).

Acknowledgments

The authors gratefully acknowledge the assistance of Mr. Thomas F. Inge, Jr., Mr. James Sipes, and Mrs. Luvenia Wigginton. The authors also express their appreciation to Merck, Sharp and Dohme for their generous supply of dexamethasone.

References

Influences of Corticosteroids on Cardiac Glycogen Concentration in the Rat

J. CHARLES DAW, Allan M. Lefer and Robert M. Berne

doi: 10.1161/01.RES.22.5.639

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1968 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/22/5/639

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/