EFFECTS OF GROWTH AND OOPHORECTOMY ON CALCIUM BALANCE

Peter Damian O’Loughlin BSc(Hons)

Division of Clinical Biochemistry
Institute of Medical and Veterinary Science

and

Department of Physiology
University of Adelaide
Adelaide, South Australia

A thesis submitted for the degree of Doctor of Philosophy

to

The University of Adelaide

July 1996
<table>
<thead>
<tr>
<th>TABLE OF CONTENTS</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSTRACT</td>
<td>vi</td>
</tr>
<tr>
<td>DECLARATION</td>
<td>vii</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>viii</td>
</tr>
<tr>
<td>PUBLICATIONS AND PRESENTATIONS</td>
<td>ix</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>xi</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>xiii</td>
</tr>
<tr>
<td><strong>CHAPTER 1: CALCIUM HOMEOSTASIS AND CALCIUM BALANCE</strong></td>
<td></td>
</tr>
<tr>
<td>1.1 Introduction</td>
<td>1</td>
</tr>
<tr>
<td>1.2 Calcium</td>
<td>4</td>
</tr>
<tr>
<td>1.2.1 Physiology</td>
<td>4</td>
</tr>
<tr>
<td>1.2.2 Maintenance of circulating calcium</td>
<td>4</td>
</tr>
<tr>
<td>1.2.3 Nutritional requirement for calcium</td>
<td>5</td>
</tr>
<tr>
<td>1.3 Bone Tissue</td>
<td>6</td>
</tr>
<tr>
<td>1.3.1 Bone remodelling</td>
<td>7</td>
</tr>
<tr>
<td>1.4 Calcium balance and its components</td>
<td>7</td>
</tr>
<tr>
<td>1.4.1 Intestinal absorption</td>
<td>8</td>
</tr>
<tr>
<td>1.4.2 Excretion of calcium</td>
<td>12</td>
</tr>
<tr>
<td>1.5 Factors affecting calcium balance and its components</td>
<td>17</td>
</tr>
<tr>
<td>1.5.1 The calcitropic hormones</td>
<td>18</td>
</tr>
<tr>
<td>1.5.2 Non hormonal factors affecting calcium balance and absorption</td>
<td>31</td>
</tr>
<tr>
<td>1.5.3 Genetic factors affecting calcium balance</td>
<td>32</td>
</tr>
<tr>
<td>1.5.4 The effect of ageing on calcium balance</td>
<td>33</td>
</tr>
<tr>
<td>1.5.5 The effect of dietary calcium restriction on calcium balance</td>
<td>36</td>
</tr>
<tr>
<td>1.5.6 The effect of the menopause on calcium balance</td>
<td>38</td>
</tr>
<tr>
<td>1.6 The oophorectomised rat as a model for postmenopausal bone loss</td>
<td>43</td>
</tr>
<tr>
<td>1.7 Summary, Aims and Hypotheses</td>
<td>46</td>
</tr>
</tbody>
</table>
1.7.1 Calcium balance during growth and the effect of oophorectomy 46
1.7.2 The effect of oophorectomy on calcium balance in the adult rat 47
1.7.3 The effect of dietary calcium intake on calcium balance in sham and oophorectomised rats 47
1.7.4 The effect of treatment with 1,25 dihydroxyvitamin D and oestradiol on calcium balance 48

CHAPTER 2: MATERIALS AND METHODS

2.1 Background 50
2.2 Materials 52
2.3 Surgical procedures 52
2.4 Serum hormone analyses 54
   2.4.1 1,25 dihydroxyvitamin D 54
   2.4.2 Total serum oestradiol 55
   2.4.3 Intact Parathyroid hormone 55
2.5 Calcium balance method development 55
   2.5.1 Animals 55
   2.5.2 Housing 56
   2.5.3 Diet 56
   2.5.4 Diet preparation 56
   2.5.5 Non-absorbable dietary markers 60
   2.5.6 Estimation of endogenous faecal calcium 61
   2.5.7 The effect of duration of balance period on variance 62
   2.5.8 Performance of calcium balance studies 63
   2.5.9 Preparation of samples for analysis 64
   2.5.10 Analysis of urine, faeces and diet 66
   2.5.11 Calculation of calcium balance and components of balance 68
   2.5.12 Statistical analyses 69
2.5.13 Results 71
2.5.14 Discussion 80
2.6 Adopted protocol for calcium balance 92

CHAPTER 3: THE EFFECT OF OOPHORECTOMY ON CALCIUM BALANCE IN YOUNG GROWING RATS

3.1 Background 94
3.2 Protocol 96
  3.2.1 Animals 96
  3.2.2 Statistical analyses 97
3.3 Results 97
  3.3.1 Body weight 97
  3.3.2 Calcium balance 100
  3.3.3 Calcium absorption 100
  3.3.4 Body weight as a determinant of calcium balance and true calcium absorption 103
  3.3.5 Calcium excretion 103
3.4 Discussion 109

CHAPTER 4: SHORT TERM EFFECTS OF OOPHORECTOMY ON CALCIUM BALANCE IN THE ADULT RAT

4.1 Background 117
4.2 Protocol 119
  4.2.1 Animals 119
  4.2.2 Statistical analyses 119
4.3 Results 120
  4.3.1 Body weight 120
  4.3.2 Calcium balance and calcium absorption 120
  4.3.3 Calcium excretion 125
4.4 Discussion 125
CHAPTER 5: EFFECT OF DIETARY CALCIUM RESTRICTION, 1,25 DIHYDROXYVITAMIN D ADMINISTRATION AND OESTRADIOL REPLACEMENT ON CALCIUM BALANCE IN OVARY-INTACT AND OOPHORECTOMISED ADULT RATS.

5.1 Background

5.2 Protocol

5.2.1 Animals

5.2.2 Adaptation to dietary calcium restriction

5.2.3 Calcium balance at varying dietary calcium levels

5.2.4 1,25 dihydroxyvitamin D administration and oestradiol replacement

5.2.5 Statistical analyses

5.3 Results

5.3.1 Adaptation to dietary calcium restriction

5.3.2 Calcium balance at varying dietary calcium levels

5.3.3 1,25 dihydroxyvitamin D administration and oestradiol replacement

5.4 Discussion

CHAPTER 6: CONCLUSIONS

6.1 Relationship between calcium balance and intestinal calcium absorption

6.2 The effect of oophorectomy on calcium balance and its components

BIBLIOGRAPHY

APPENDICES
ABSTRACT

This thesis assesses calcium balance and its components, measured by metabolic balance studies, in ovary-intact (sham) and oophorectomised (oophx) young (1.5-4 month) and adult (7-14 month) rats.

In the young rat, calcium balance diminished with age as rats approached full size. The major factor leading to reduced balance with age was reduced true calcium absorption. The age-related reduction in calcium balance was more rapid in oophx rats, due mainly to a transient rise in intestinal calcium secretion and partly to a fall in true calcium absorption.

Oophorectomy in adult rats led to reduced calcium balance resulting from transient rises in intestinal calcium secretion and urine calcium excretion in the short term and in the long term reduced calcium balance was sustained by reduced intestinal calcium absorption. Although calcium balance and intestinal calcium absorption were lower in oophx rats, the magnitude of adaptation in balance and absorption to dietary calcium restriction (0.02% Ca) was unaffected by oophorectomy. Oophorectomy did not affect the slopes for the relationships between calcium consumption with calcium balance and intestinal calcium absorption, but the intercepts for both relationships were lower in oophx rats. However, circulating 1,25 dihydroxyvitamin D was not reduced in oophx rats. Administered 1,25 dihydroxyvitamin D stimulated intestinal calcium absorption in both sham and oophx rats but did not significantly increase calcium balance, whereas oestradiol stimulated intestinal calcium absorption in oophx rats (without affecting circulating 1,25 dihydroxyvitamin D) and increased calcium balance.

It is concluded from the findings of the study that oophorectomy reduces calcium balance regardless of age by transient rises in calcium excretion and prolonged reduction in intestinal calcium absorption. In adult rats the impairment to intestinal calcium absorption is not the result of reduced circulating 1,25 dihydroxyvitamin D or reduced intestinal responsiveness to 1,25 dihydroxyvitamin D. Oestradiol stimulates intestinal calcium absorption probably by a direct effect on the intestine in addition to its effects on bone.