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ORIGINAL ARTICLE

The effect of hyperinsulinaemic-euglycaemic clamp and exercise on bone remodeling markers in obese men

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Bone remodelling markers (BRMs) are suppressed following a glucose load and during glucose infusion. As exercise increases indices of bone health and improves glucose handling, we hypothesised that, at rest, hyperinsulinaemic-euglycaemic clamp will suppress BRMs in obese men and that exercise prior to the clamp will prevent this suppression. Eleven obese nondiabetic men (age 58.1 ± 2.2 years, body mass index = 33.1 ± 1.4 kg m^{-2} mean ± s.e.m.) had a hyperinsulinaemic-euglycaemic clamp (HEC) at rest (Control) and 60 min post exercise (four bouts × 4 min cycling at 95% of hazard ratio$_{peak}$). Blood samples were analysed for serum insulin, glucose, bone formation markers, total osteocalcin (tOC) and procollagen type 1 N-terminal propeptide (P1NP), and the bone resorption marker, β-isomerised C-terminal telopeptides (β-CTx). In the control trial (no exercise), tOC, P1NP and β-CTx decreased with HEC by >10% compared with baseline ($P < 0.05$). Fasting serum glucose, but not insulin, tended to correlate negatively with the BRMs (β range −0.57 to −0.66, p range 0.051–0.087). β-CTx, but not OC or P1NP, increased within 60 min post exercise (~16%, $P < 0.01$). During the post-exercise HEC, the glucose infusion rate was ~30% higher compared with the no exercise trial. Despite this, BRMs were only suppressed to a similar extent as in the control session (10%). HEC suppressed BRMs in obese men. Exercise did not prevent this suppression of BRMs by HEC but improved glucose handling during the trial. It remains to be tested whether an exercise intervention of longer duration may be able to prevent the effect of HEC on bone remodelling.

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Introduction

Bone remodelling markers (BRMs) are suppressed after a meal or following a glucose load[1,2] and correlate inversely with fasting plasma glucose levels as well as with 2 h glucose levels during an oral glucose tolerance test,[3] suggesting that glucose and insulin may affect osteoblast and osteoclast function. It is plausible that impairment of glucose handling might contribute to alterations in BRMs, thereby contributing to the increased fracture risk observed in obese people and those with type 2 diabetes. It has been suggested that the decrease in bone remodelling after feeding is mediated by gut peptides including glucagon-like peptide-2.[4] However, new evidence suggests that it is likely that the effects are independent of the effect of gut hormones as lower remodelling markers are also evident during intravenous (IV) glucose and insulin infusion.[5]

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Obesity is a major risk factor for insulin resistance and type 2 diabetes.[6,7] Lifestyle modifications, including increased physical activity, are important for the prevention and treatment of type 2 diabetes.[8] Exercise training increases skeletal muscle mass and insulin sensitivity and may reduce other risk factors independently of weight loss.[9] However, even a single bout of exercise increases glucose handling and insulin sensitivity for several hours after the cessation of exercise.[10] Acute exercise increases C-terminal telopeptides (CTx).[11,12] Acute exercise also increases OC and the undercarboxylated form of OC in obese men, and this increase is related to a reduction in serum glucose levels and higher insulin sensitivity as measured by the use of an hyperinsulinaemic-euglycaemic clamp (HEC).[13,14] Using these same patients, we now hypothesise that, (a) at rest, BRMs will be suppressed during a 2 h HEC in obese men, and (b) exercise prior to insulin clamping will increase BRMs and that in turn will prevent the predicted suppression of BRMs in response to the HEC. In the current study, we therefore use the HEC as a tool to deliver and then examine the effects of insulin and glucose on BRMs.

Results

Blood glucose, insulin, total osteocalcin (tOC), procollagen type 1 N-terminal propeptide (P1NP) and β-isomerised CTx (β-CTx) levels were similar at baseline in the resting (control) and exercise trials (**Table 1**). In a multivariable regression model, fasting blood glucose, but not insulin, tended to correlate negatively with tOC ($\beta = -0.66$, $P = 0.051$), P1NP ($\beta = -0.59$, $P = 0.075$) and β-CTx ($\beta = -0.58$, $P = 0.087$).

Circulating blood glucose levels in the final 30 min of the clamp were similar between the two sessions, and insulin concentrations in the last 30 min of both sessions were elevated compared with baseline with no differences between the two trials. In addition, the glucose infusion rate in the last 30 min of the clamp was higher post exercise by ~30% ($P < 0.05$).[14]

Total OC and P1NP were not elevated post exercise ($P > 0.05$); however, β-CTx was elevated (~16%, $P < 0.01$, **Figure 1**). Changes in BRMs in response to the HEC both at rest (control trial) and post exercise are displayed in **Figure 1**. OC, β-CTx and P1NP were all reduced (10–20%, $P < 0.05$) during both HEC, and there were no differences between the rest (control) and exercise trials.

Discussion

These data indicate that acute infusion of glucose and insulin suppresses BRMs in obese men and that fasting glucose, but not insulin, tended to correlate negatively with BRMs. Exercise increased β-CTx but did not prevent the suppressive effect of HEC on BRMs such that they were reduced to the same level after the clamp, despite, at least in the case of β-CTx, increasing after exercise.

BRMs are suppressed post-prandially as well as following an oral glucose load,[1,2] possibly via a mechanism that includes the release of gut peptides.[4] New research, however, indicates that the suppression of BRMs can be independent of these gut peptides, with IV glucose also resulting in reduced BRMs. The exact mechanism(s) responsible for the effect of IV glucose infusion remains to be determined; however, it has previously been shown that the suppressive effect is related to the glucose infusion rather than the hyperinsulinaemic state.[5] There are theoretical reasons for an increase in BRMs following hyperinsulinaemia. Insulin is an anabolic hormone, which is known to promote osteoblast proliferation and differentiation via the MAPK and PI3K pathways and upregulation of osterix and insulin-like growth factor-1.[15] Insulin also increases alkaline phosphatase activity, a bone formation marker, and the expression and secretion of collagen type I, and OC expression.[15] In addition, insulin increases bone resorption via the suppression of osteoprotegrin, the decoy receptor and thus inhibitor of receptor activator of nuclear factor-κB ligand,[16] and as such it is plausible that β-CTx, a marker of bone resorption, may also be increased with hyperinsulinaemia. Our current data, however, show that the hyperinsulinaemic state does not overcome the suppressive effect of glucose on BRMs.

Previous reports indicate that glucose, not insulin, affects BRMs. For example, altering blood glucose levels (HEC versus hyperinsulinaemic-hypoglycaemic clamp) results in different BRMs, despite comparable insulin levels.[5] In addition, patients with type 2 diabetes and polycystic ovarian syndrome are characterised by hyperinsulinaemia; yet, they have low circulating levels of OC, P1NP and β-CTx.[2,3] These studies all suggest that glucose may affect osteoblast function. In the current study, the circulating glucose levels were maintained around 5 mmol l^{-1}; however, glucose was constantly infused to achieve this targeted blood glucose level. This constant infusion of glucose would be taken up by both skeletal muscle and osteoblasts following the hyperinsulinaemic state and may in fact impair osteoblastic function. Furthermore, it is possible that, in the current study, the inclusion of obese men who are at a higher risk for insulin resistance may explain the inability of the hyperinsulinaemia to overcome the suppressive effect of glucose on BRMs, suggesting that the osteoblastic response to insulin in obese men may be reduced. Indeed, the euglycemia produced in the obese men was comparable to that achieved in a previous study in non-obese men; yet, in the non-obese men, euglycemia did not suppress BRMs.[5] Further *in vitro* studies aimed at investigating the direct effects of glucose and/or insulin on bone cells are thus required.

In the current study, the reduction in BRMs was also evident when the HEC was commenced 60 min post exercise. This was evident despite the observed post-exercise increase in BRMs. This finding was in contrast to our hypothesis that exercise would prevent the glucose-induced suppression of BTMs. However, it is important to note that the exercise session may offer some protection for BRMs from the adverse effects of glucose. In the current study, the glucose infusion rate during the last 30 min of the insulin clamp that was commenced post exercise was around 30% higher compared with the control session; yet, in both sessions, BRMs were suppressed to a similar extent, compared with baseline levels. As exercise has the capacity to increase the insulin sensitivity and thus the flux of glucose into muscle cells,[14] this would reduce the proportion of infused glucose to which the bone cells are exposed. Thus, despite receiving more glucose to maintain the constant blood glucose levels post exercise, the bone is not exposed to all of this extra glucose. Furthermore, it is not clear whether the effects of glucose on BRMs are due to the local glucose flux into the osteoblasts or to the total blood glucose levels.

The reason(s) for the inability of exercise to fully prevent the suppression of BRMs is not yet understood, but it is plausible

Table 1 Baseline variables between the Control (rest) and exercise trials

	Control (rest)	*Exercise*
Glucose (mmol l^{-1})	5.2 ± 0.2	5.3 ± 0.3
Insulin (μU ml^{-1})	13.1 ± 2.4	13.5 ± 2.0
tOC (ng ml^{-1})	17.5 ± 1.4	18.2 ± 1.4
P1NP (μ l^{-1})	36.3 ± 1.8	36.1 ± 1.3
β-CTx (μ l^{-1})	269.8 ± 36.0	306.5 ± 41.0

Abbreviations: P1NP, procollagen I N-terminal propeptide; tOC, total osteocalcin; β-CTx, β-isomerised C-terminal telopeptides. Data presented as mean ± s.e.m. All comparisons $P > 0.05$.

that the effect of glucose on osteoblasts, and therefore indirectly on osteoclasts, is more profound compared with the effect of exercise. A second possibility is related to the flux of glucose into the muscle cells—whereby the exercise chosen was of high intensity, and, in future tests may require a load bearing exercise that shifts the relative difference of bone and muscle need for glucose, possibly increasing the glucose availability at the local bone environment.

The clinical implications of these findings are also not yet clear; however, an extrapolation of the data would indicate that those with insulin resistance and/or elevated glucose levels, including patients with type 2 diabetes, may need to be engaged in exercise on a regular basis (chronic exposure) to minimise the glucose effect on BRMs. A potential limitation of the study is the small sample size. The small sample size was due to the invasiveness of the study. This study was part of a larger study that included several muscle biopsies.[14] The study was, however, powered to detect changes in the examined BRMs.

In conclusion, an acute HEC suppresses BRMs in obese men in whom the fasting glucose, but not insulin, is negatively correlated with BRMs. This is not consistent with the previous findings in non-obese men, indicating that obese men are at higher risk of the deleterious effects of glucose infusion on bone. The effect of the HEC on BRMs was not significantly different after exercise, although under these conditions far more glucose was infused. It remains to be tested whether an exercise intervention of longer duration or chronic exercise training may be able to minimise the effect of an HEC on bone remodelling.

Materials and Methods

Participants

Eleven middle-aged (58.1 ± 2.2 years mean ± s.e.m., range 40–70 years), obese (body mass index = 33.1 ± 1.4 kg m^{-2}), nondiabetic men (fasting glucose 5.3 ± 0.2 mmol l^{-1} and $HbA1_C = 5.6 \pm 0.1\%$) participated in the study. Power calculation was based on the expected 6–10% change in BRMs following exercise.[13] Bone mineral density for all subjects was considered to be within the normal range (*T*-score 1.1 ± 0.3). Exclusion criteria were as follows: men with bone diseases, men taking anti-hyperglycaemic medications or medications known to affect bone metabolism, insulin secretion or insulin sensitivity, men with musculoskeletal or other conditions that prevent daily activity and men with symptomatic or uncontrolled metabolic or cardiovascular disease or receiving warfarin or vitamin K supplementation.

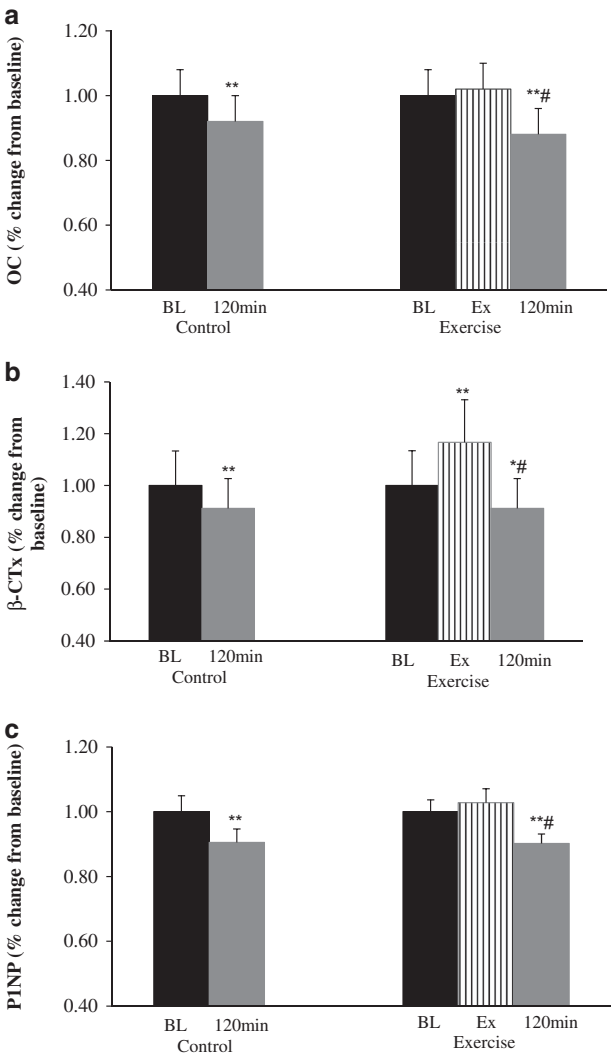

Figure 1 ■ baseline, ■ post-2 h of insulin clamp, ▥ post exercise. OC (osteocalcin, **a**), β-CTx (β-isomerised C-terminal telopeptides, **b**) and P1NP (procollagen 1 N-terminal propeptide, **c**) were all suppressed post resting insulin clamp. Exercise significantly increased β-CTx but could not prevent the reduction in those markers after insulin clamp. * indicates $P < 0.05$ compared with baseline. ** indicates $P < 0.01$ compared with baseline. # indicates $P < 0.05$ compared with exercise.

Each participant was given written and verbal explanations about the study before signing an informed consent form. The study protocol was approved by the Human Research Ethics Committee, Victoria University.

Study protocol

This study was part of a larger project, and the detailed protocol has previously been reported.[14] Participants underwent anthropometric measurements, assessment of their aerobic power (VO_{2peak}) and a fasting blood test to exclude type 2 diabetes. Participants also completed two sessions of 2 h HEC, once at rest (Control) and the second commenced 60 min after an acute bout of exercise (see below; post-exercise).

A blood sample was collected following an overnight fast. Blood was analysed at Austin Health (Melbourne, VIC, Australia) pathology using standard hospital assay protocols for glucose, HbA1c and insulin. Weight was measured using a calibrated scale (TANITA, Tanita Corporation, Tokyo, Japan). Dual-energy

X-ray absorptiometry (DXA; GE Lunar Prodigy, Software version 9.1, Madison, WI, USA) was used to assess the *T*-score. DXA measurements were performed at the Bone Density Unit, Austin Health. Aerobic power (VO_{2peak}) was assessed during a sign and symptom-limited graded exercise test as was previously described.[14,17]

Experimental trials

Participants attended our laboratory twice for the experimental trials. Both sessions were performed in the morning and in fasting conditions (fasting for 10 h). The two experimental trials (control or exercise) were conducted 3–5 weeks apart.

Control trial. HEC was performed at rest as we previously reported.[14] Briefly, regular venous blood samples (10 ml) were collected prior and during each session. Insulin (Actrapid; Novo Nordisk, Bagsvaerd, Denmark) was infused at 40 mU m^{-2} per min for 120 min generating an elevated, stable insulin concentration in the last 30 min of both sessions. During the HEC, exogenous glucose (21% sterile glucose solution, Baxter Healthcare, NSW, Australia) was variably infused to achieve the target blood glucose of $\sim$5mmol l^{-1} for the duration of the HEC, using variable infusion. Glucose was assessed every 5 min during the clamp (YSI2300 STAT Plus Glucose and Lactate Analyser, Melbourne, Victoria, Australia).

In the second trial, the HEC was performed following an acute high-intensity bout of exercise. Participants were initially supine and a resting blood sample was taken. As described previously,[14] following the initial blood sampling, participants performed 30 min of high-intensity interval exercise that included a warm up of 4 min of exercise at approximately 50–60% of HR_{peak} followed by four bouts of high-intensity cycling: 4 min each at 90–95% of HR_{peak} interspersed by 2 min of 'active' recovery (cycling at a lower intensity: 50–60% of HR_{peak}). Blood samples were obtained immediately post exercise and at 30 and 60 min post exercise to identify the peak change in BRMs. The HEC was then commenced and was performed as described in the control session.

BRMs assays

Serum OC. Total serum OC was measured using an automated immunoassay (Elecsys 170; Roche Diagnostics, Indianapolis, IN, USA). This assay has a sensitivity of 0.5 μg l^{-1}, with an intra-assay precision of 1.3%. The tOC assay was performed at PathWest QEII Medical Centre, Perth.

Insulin, β-CTx (bone resorption marker) and P1NP (a bone formation marker) were all analysed at Austin pathology, Melbourne, using a Roche Hitachi Cobas e602 immunoassay analyser, according to the manufacturer's guidelines.

Statistical analyses

Changes in bone remodelling markers and circulating insulin across the two insulin clamp sessions were assessed using repeated-measures analysis of variance followed by post-hoc analysis using LSD. Multi-linear regression model with age, body mass index, fasting glucose and fasting insulin was used to determine associations with BRMs. All data are reported as mean $\pm$ s.e.m., and all statistical analyses were conducted at the 95% level of significance ($P \leqslant 0.05$).

Conflict of Interest

The authors declare no conflict of interest.

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References

1. Clowes JA, Hannon RA, Yap TS, Hoyle NR, Blumsohn A, Eastell R. Effect of feeding on bone turnover markers and its impact on biological variability of measurements. *Bone* 2002; **30**: 886–890.
2. Schwetz V, Lerchbaum E, Schweighofer N, Hacker N, Trummer O, Borel O *et al.* Osteocalcin levels on oral glucose load in women being investigated for polycystic ovary syndrome. *Endocr Pract* 2014; **20**: 5–14.
3. Iglesias P, Arrieta F, Pinera M, Botella-Carretero JI, Balsa JA, Zamarron I *et al.* Serum concentrations of osteocalcin, procollagen type 1 N-terminal propeptide and beta-CrossLaps in obese subjects with varying degrees of glucose tolerance. *Clin Endocrinol* 2011; **75**: 184–188.
4. Henriksen DB, Alexandersen P, Bjarnason NH, Vilsboll T, Hartmann B, Henriksen EE *et al.* Role of gastrointestinal hormones in postprandial reduction of bone resorption. *J Bone Miner Res* 2003; **18**: 2180–2189.
5. Clowes JA, Robinson RT, Heller SR, Eastell R, Blumsohn A. Acute changes of bone turnover and PTH induced by insulin and glucose: euglycemic and hypoglycemic hyperinsulinemic clamp studies. *J Clin Endocrinol Metab* 2002; **87**: 3324–3329.
6. Chan JM, Rimm EB, Colditz GA, Stampfer MJ, Willett WC. Obesity, fat distribution, and weight gain as risk factors for clinical diabetes in men. *Diabetes Care* 1994; **17**: 961–969.
7. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care* 2004; **27**: 1047–1053.
8. Lumb A. Diabetes and exercise. *Clin Med* 2014; **14**: 673–676.
9. Duncan GE, Perri MG, Theriaque DW, Hutson AD, Eckel RH, Stacpoole PW. Exercise training, without weight loss, increases insulin sensitivity and postheparin plasma lipase activity in previously sedentary adults. *Diabetes Care* 2003; **26**: 557–562.
10. Holloszy JO. Exercise-induced increase in muscle insulin sensitivity. *J Appl Physiol* 2005; **99**: 338–343.
11. Guillemant J, Accarie C, Peres G, Guillemant S. Acute effects of an oral calcium load on markers of bone metabolism during endurance cycling exercise in male athletes. *Calcif Tissue Int* 2004; **74**: 407–414.
12. Scott JP, Sale C, Greeves JP, Casey A, Dutton J, Fraser WD. The effect of training status on the metabolic response of bone to an acute bout of exhaustive treadmill running. *J Clin Endocrinol Metab* 2010; **95**: 3918–3925.
13. Levinger I, Zebaze R, Jerums G, Hare DL, Selig S, Seeman E. The effect of acute exercise on undercarboxylated osteocalcin in obese men. *Osteoporos Int* 2011; **22**: 1621–1626.
14. Levinger I, Jerums G, Stepto NK, Parker L, Serpiello FR, McConell GK *et al.* The effect of acute exercise on undercarboxylated osteocalcin and insulin sensitivity in obese men. *J Bone Miner Res* 2014; **29**: 2571–2576.
15. Yang J, Zhang X, Wang W, Liu J. Insulin stimulates osteoblast proliferation and differentiation through ERK and PI3K in MG-63 cells. *Cell Biochem Funct* 2010; **28**: 334–341.
16. Clemens TL, Karsenty G. The osteoblast: an insulin target cell controlling glucose homeostasis. *J Bone Miner Res* 2011; **26**: 677–680.
17. Levinger I, Goodman C, Hare DL, Jerums G, Selig S. The effect of resistance training on functional capacity and quality of life in individuals with high and low numbers of metabolic risk factors. *Diabetes Care* 2007; **30**: 2205–2210.