1 **Characterization of sclerostin's response within white adipose tissue to an obesogenic diet at**
2 **rest and in response to acute exercise in male mice**
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15   **Abstract**

16   This study examined the effect of a high-fat diet (HFD) on sclerostin content within subcutaneous

17   inguinal visceral white adipose tissue (iWAT), and visceral epididymal WAT (eWAT) depots at rest

18   and in response to acute aerobic exercise. Male C57BL/6 mice (n=40, 18 weeks of age) underwent 10

19   weeks of either a low-fat diet (LFD) or HFD. Within each diet group, mice were assigned to either

20   remain sedentary (SED) or perform 2h of endurance treadmill exercise at 15 m·min^{-1} with 5° incline

21   (EX), creating 4 groups: LFD+SED (N=10), LFD+EX (N=10), HFD+SED (N=10), and HFD+EX

22   (N=10). Serum and WAT depots were collected 2h post-exercise. Serum sclerostin showed a diet-by-

23   exercise interaction, reflecting HFD+EX mice having higher concentration than HFD-SED (+31%,

24   p=0.03), and LFD mice being unresponsive to exercise. iWAT sclerostin content decreased post-

25   exercise in both 28 kDa (-31%, p=0.04) and 30 kDa bands (-36%, main effect for exercise, p=0.02).

26   iWAT β-catenin (+44%, p=0.03) and GSK3β content were elevated in HFD mice compared to LFD

27   (+128%, main effect for diet, p=0.005). Monomeric sclerostin content was abolished in eWAT of

28   HFD mice (-96%, main effect for diet, p<0.0001), was only detectable as a 30 kDa band in LFD mice

29   and was unresponsive to exercise. β-catenin and GSK3β were both unresponsive to diet and exercise

30   within eWAT. These results characterized sclerostin's mobilization to WAT depots in response to

31   acute exercise, which appears to be specific to a reduction in iWAT and identified a differential

32   regulation of sclerostin's form/post-translational modifications depending on diet and WAT depot.

33   **1   Introduction**

34   White adipocytes are a major storage site for lipids and play a key role in substrate mobilization (e.g.,

35   fatty acids) to tissues (e.g., skeletal muscle) during periods of high energy expenditure (Lundsgaard

36   et al., 2018). When energy intake is in excess to expenditure and is sustained, adipose tissue

37   expansion occurs through the hypertrophy and hyperplasia of adipocytes (Spalding et al., 2008;Arner

38   et al., 2013;Wang et al., 2013). Despite a growing understanding of the development of adipose

39   tissue depots, the mechanisms and signals regulating these responses according to changes in energy

40   needs is not fully understood.

41   Sclerostin is a glycoprotein that is mainly associated with the regulation of bone metabolism.

42   In the bone microenvironment, osteocytes secrete sclerostin in response to mechanical unloading,

43   which inhibits Wnt/β-catenin signaling within the bone forming cells, the osteoblasts. This leads to

44   the inhibition of bone formation and downstream to the upregulation of the activation of the bone

45   resorbing cells, the osteoclasts. While studies have shown lower expression by liver, kidney, both the

46   vascular smooth muscle (Zhu et al., 2011) and skeletal muscle (Magarò et al., 2021), testis, pyloric

47   sphincter, carotid arteries, and the cerebellum (Collette et al., 2013), the majority of circulating

48   sclerostin comes from bone (Kim et al., 2019;Kim et al., 2021). In contrast, adipose tissue does not

49   express sclerostin (Kim et al., 2017). However, sclerostin knockout mice or mice with peripheral

50   inhibition of sclerostin by a neutralizing antibody present with increased bone mass, as well as

51   reduced peripheral fat pad weights, increased insulin sensitivity, and fatty acid oxidation (Kim et al.,

52   2017;Kim et al., 2021). These findings suggest an endocrine role of bone derived sclerostin in

53   regulating adipose tissue mass and metabolism. Sclerostin's endocrine role in adipose tissue is likely

54   mediated in part by inhibiting Wnt signaling, a pathway known to inhibit adipogenesis (Cawthorn et

55   al., 2012). Indeed, when sclerostin content is increased both *in vivo* (Fulzele et al., 2017;Kim et al.,

56   2017;Fairfield et al., 2018) and *in vitro* (Ukita et al., 2016), Wnt signaling is inhibited, resulting in

57   increased adipogenesis and adipocyte cell size.

58   Evidence for a physiological role of sclerostin in regulating fat mass comes from assessment

59   of humans with higher adiposity (Amrein et al., 2012;Urano et al., 2012) and mice with increased

60   adiposity induced by a HFD, which both have higher circulating sclerostin compared to their normal

61   weight controls (Baek et al., 2014;Kim et al., 2017). Exercise differentially influences circulating

62   sclerostin levels; specifically, acute exercise induces a transient increase (Kouvelioti et al., 2019),

63   while resting levels decrease with long term exercise training (Ardawi et al., 2012). Additionally,

64   adolescent females appear to be protected from this increase while obese adolescent females are not

65   (Kurgan et al., 2020), providing evidence for adiposity dependent differential mobilization of

66   sclerostin in the circulation following acute exercise. Which may be related to sclerostin's association

67   with insulin sensitivity and glucose metabolism, which has been shown in humans (Urano et al.,

68   2012;Daniele et al., 2015) and *in vivo* murine models (Kim et al., 2017;Kim et al., 2019;Kim et al.,

69   2021). Taken together, these data suggest sclerostin's endocrine function may regulate adipose tissue

70   mass and metabolism in response to excess energy intake (e.g., HFD) and energy expenditure (e.g.,

71   exercise). Examining the mobilization of sclerostin to different WAT depots in response to acute

72   exercise or to chronic changes in energy availability (e.g., HFD) will provide physiological context to

73   sclerostin's endocrine function. The purpose of this study was to examine subcutaneous and visceral

74   WAT sclerostin content and Wnt/β-catenin signaling in response to a HFD and determine whether

75   the subsequent metabolic adaptations (reduced insulin sensitivity/dysregulated glucose homeostasis)

76   influence the post-exercise response in WAT sclerostin content.

77 **2   Materials and Methods**

78 **2.1   Animals**

79 Experimental protocols are in compliance with the Canadian Council on Animal Care and were

80 approved by the Brock University Animal Care Committee (File #17-06-02). This study utilized one

81 cohort of 40 male C57BL/6 mice (18 weeks of age; 31.9 ± 2.5 g) ordered from The Jackson

82 Laboratory (Bar Harbor, ME, United States) for a study originally designed to examine brain

83 signaling response to acute exercise (Baranowski et al., 2021). Upon arrival, the mice acclimatized

84 for 3d in the Brock University Comparative Biosciences Facility (St. Catharines, ON, Canada).

85 During acclimatization, mice were fed standard chow (2014 Teklad global 14% protein rodent

86 maintenance diet, Harlan Tekland, Mississauga, ON, Canada). All mice were housed in fours, were

87 kept on a 12 h light / 12 h dark cycle and had *ad libitum* access to food and water through the entirety

88 of the study.

89 **2.2   Experimental Design**

90 As previously described (Baranowski et al., 2021), mice were randomized into either a HFD ($n = 20$,

91 60% kcal fat, cat#D12492) or low-fat diet (LFD) group ($n = 20$, 10% kcal fat, cat#D12450B).

92 Baseline measurement of body mass of all mice ($n = 40$) was conducted immediately after

93 acclimatization and the mice remained on their respective diets for a 10-week period, with body mass

94 measurements taken weekly. Following the 10-week dietary intervention, LFD and HFD mice were

95 further randomized into either the sedentary or the acute exercise group, creating 4 groups:

96 LFD+Sedentary ($n = 10$), LFD+Exercise ($n = 10$), HFD+Sedentary ($n = 10$), or HFD+Exercise ($n =$

97 10). The sedentary groups did not exercise, while the exercise groups performed a single bout of

98 treadmill running for 2 h at 15 m·min^{-1} on a 5% incline as previously described (MacPherson et al.,

99 2015). Following a 2h recovery period, the mice were euthanized, and serum, as well as the inguinal

100 white adipose tissue (iWAT) and epididymal white adipose tissue (eWAT) depots, were collected.

101   Necropsy tissue masses were also measured prior snap freezing in liquid nitrogen and stored at -80°C

102   for future analysis.

103   **Glucose and Insulin Tolerance Testing**

104   Data from the glucose (GTT) and insulin tolerance testing (ITT) from a larger cohort of animals is

105   described and reported elsewhere (Baranowski et al., 2021). Briefly, intraperitoneal GTT were

106   performed on fasted (6 h), non-anesthetized mice during the 9th week of the dietary intervention. Tail

107   vein was sampled for blood glucose using an automated glucometer at baseline and at 15, 30, 45, 60,

108   90, and 120 min following an intraperitoneal injection of glucose (2 g·kg body mass^{-1}) and area

109   under the curve (AUC) of the glucose response over time is reported in Table 1 (Baranowski et al.,

110   2021).

111   Intraperitoneal ITT were performed on non-anesthetized mice following 48 h of recovery

112   from the GTT. Tail vein was sample for blood glucose using an automated glucometer at baseline

113   and at 15, 30, 45, 60, 90, and 120 min following an intraperitoneal injection of insulin (0.75 U·kg

114   body mass^{-1}) and (AUC) of the glucose response over time is reported in Table 1 (Baranowski et al.,

115   2021).

116   **2.3   Blood sampling and ELISA**

117   At end point, blood samples were taken by cardiac puncture with 1 mL insulin syringes (cat#01ST;

118   ELIMEDICAL, Markham, Canada) and 25G needles (Cat#26403; EXCELINC International,Co.,

119   California, USA). Blood samples were left on ice to clot for 1 h before being centrifuged for 15 min at

120   1500 x g. Samples were aliquoted and stored at -80°C until analysis. Serum sclerostin was analysed in

121   duplicate using one Quantikine enzyme-linked immunoassay kits (cat#MSST00; R&D Systems,

122   Minneapolis, Minnesota, USA) according to manufacturer's instructions and had an intra assay

123   coefficient of variation of 4.8%.

124 **2.4 Adipose tissue homogenization**

125 iWAT and eWAT depots were tandemly homogenized (FastPrep®, MP Biomedicals, Santa Ana,

126 CA) on the same day in 3x volume per mg mass of tissue in NP40 Cell Lysis Buffer (Life

127 Technologies; cat# FNN0021) supplemented with 3x the recommended volume of

128 phenylmethylsulfonyl fluoride (PMSF) (3 mM) and 100x protease inhibitor (PI) cocktail (Sigma-

129 Aldrich; cat#7626 and cat#P8340, respectively). Following homogenization, samples were placed on

130 ice for 30 min then centrifuged at 4°C for 5 min at 5,000 x g. The infranatant was then collected and

131 protein concentration was determined using a Bicinchoninic acid assay (cat#B9643; Sigma-Aldrich,

132 St. Louis, Missouri, USA; copper (II) sulfate pentahydrate, cat#BDH9312, VWR, Radnor,

133 Pennsylvania, USA). The samples were prepared to contain equal concentrations ($1 \ \mu g \cdot \mu l^{-1}$) of

134 protein in 4x Laemmli buffer (diluted to 1x; cat#1610747, Bio-Rad, Hercules, California, USA) and

135 placed in a heating block at 100°C for 10 min, allowed to cool, and then stored at -80°C for future

136 analysis.

137 **2.5 Immunoblotting**

138 20 μg of protein were loaded and resolved on 10% TGX fast cast gels (cat# 1610173, Bio-Rad,

139 Hercules, California, USA) for 30 min at 250 V. Proteins were then semi-dry transferred onto a

140 polyvinylidene difluoride membrane at 1.3 A and 25 V for 7 min (Trans-Blot® Turbo™ Transfer

141 System, Bio-Rad, Hercules, California, USA). Membranes were blocked in Tris buffered saline/0.1%

142 Tween 20 (TBST) with 5% non-fat powdered milk for 1 h at room temperature. Primary antibody

143 (1:500-2000 ratio dilution in 5% milk) was then applied and left to incubate on a shaker at 4°C for

144 ~16 h. Membranes were then washed with TBST 3 x 5 min and then incubated with the

145 corresponding secondary antibody conjugated with horseradish peroxidase (anti rabbit (cat#HAF008)

146 and anti-goat (cat#CAF109), R&D Systems, Minneapolis, Minnesota, USA – 1:2000 dilution in 5%

147 milk) for 1h at room temperature. Signals were detected using either Clarity™ Western

148   chemiluminescent substrate (cat#170-5061, Bio-Rad, Hercules, California, USA), or SuperSignal™

149   West Femto maximum sensitivity chemiluminescent substrate (cat#34095, ThermoFisher Scientific,

150   Waltham, Massachusetts, USA) and were imaged using a Bio-Rad ChemiDoc™ Imaging System

151   (Hercules, California, USA). Each membrane was stained with Ponceau S to normalize to total

152   protein. Densitometry analysis was done using ImageLab Software (Bio-Rad, Hercules, California,

153   USA) and compared to sedentary LFD mice protein content (e.g., 100%). Antibodies against total

154   GSK3β (cat#12456S), serine 9 phospho-GSK3β (cat#9336S), total β-catenin (cat#8480S) were

155   purchased from Cell Signalling (Danvers, USA). Sclerostin antibody was purchased from R&D

156   Systems, Inc. (cat#MAB1406, R&D Systems, Minneapolis, Minnesota, USA), and was confirmed by

157   immunoblotting femur homogenates and recombinant sclerostin (cat# 1406, R&D Systems,

158   Minneapolis, Minnesota, USA) (Supplemental Figure 1).

159   **2.6   Statistics**

160   Baseline comparisons between diet groups were done using independent *t*-tests. Comparisons of all

161   proteins responses to diet and exercise were done with a 2-way ANOVA (diet by exercise groups)

162   and significant interactions were followed up with Tukey *post hoc* analysis using GraphPad Prism 9.

163   Data are presented as means ± standard deviation (SD) with significance assumed at an $\alpha = 0.05$.

164   **3   Results**

165   **3.1   Diet induced changes to body mass and insulin sensitivity**

166   Table 1 presents data from a smaller cohort of previously published data (Baranowski et al., 2021).

167   The difference in sample size required us to perform analysis on these variables again to characterize

168   the difference between groups in this specific study. Mice on HFD had higher body mass (mean

169   difference = +16.2g or +1.5-fold, $p < 0.0001$), necropsy iWAT mass (mean difference = +1.5g or

170   +4.8-fold, $p < 0.0001$), necropsy eWAT mass (mean difference = +0.8g or +2-fold, $p = 0.0003$), and

171   necropsy liver mass (mean difference = +1.3g or +2.2-fold, $p < 0.0001$) compared to mice on a LFD

172   (Table 1). Mice on HFD also had higher area under the curves (AUC) of blood glucose in response to

173   ITT (mean difference = +435.2 mmol·L^{-1}·min^{-1} or +1.8-fold, $p < 0.0001$) and GTT (mean difference

174   = +758.2 mmol·L^{-1}·min^{-1} or +1.6-fold, $p < 0.0001$) compared to a LFD. These results provide

175   evidence that our HFD resulted in fat mass expansion and perturbed insulin sensitivity\glucose

176   metabolism.

177   **INSERT TABLE 1**

178   **3.1   Circulating sclerostin increases acutely following exercise only in HFD mice**

179   Circulating sclerostin content showed no main effects for exercise ($p = 0.1$) or diet ($p = 0.3$), but

180   there was a diet by exercise interaction ($p = 0.02$), indicating that the sclerostin response to acute

181   exercise was different between diets. Pairwise comparisons identified that HE mice had higher serum

182   sclerostin compared to HS (mean difference = +36.9 pg·ml^{-1}, $p = 0.03$) and trended to be

183   significantly higher than LE mice (mean difference = +30.3 pg·ml^{-1}, $p = 0.08$), while there were no

184   differences between LS and LE groups. These findings reflect similar results observed in humans

185   with obesity compared to those with normal weight (Kurgan et al., 2020).

186   **INSERT FIGURE 1**

187   **3.2   Obesogenic diet increases GSK3β and β-catenin content and acute exercise reduces**

188   **sclerostin content within iWAT**

189   When immunoblotting for sclerostin within iWAT, a distinct banding pattern occurred at 28 and 30

190   kDa, which represents glycosylation at N-linked sites (N175) (Krause et al., 2010;Holdsworth et al.,

191   2012;Kim et al., 2020). There was also a band at ~56 kDa, that we believe to be a sclerostin dimer

192   (Hernandez et al., 2014). This band was haloing when the monomer was detectable, which could

193   indicate a saturated signal and thus a higher abundance than the monomer (Supplemental Figure 3).

194   Nevertheless, the monomeric form of sclerostin has been shown to be functional and interact

195   specifically with the Wnt coreceptor (LRP6) inhibiting its action (Kim et al., 2020). Thus, we report

196   here analysis only on the monomer of sclerostin, which was accomplished by analyzing membranes

197   that had been cut below 56 kDa to only detect monomeric sclerostin without the haloing 56 kDa band

198   (e.g., Supplemental Figure 2). Examination of iWAT sclerostin showed a main effect for exercise

199   (difference in predicted means from pre- to post-exercise = -34.7%, p = 0.04), with no main effect for

200   diet (difference in predicted means of LFD and HFD = +3.0%, p = 0.9) and no interaction (p = 0.5)

201   for sclerostin's 28 kDa band (Figure 2a). There was a main effect for exercise (difference in predicted

202   means from pre- to post-exercise = -35.3%, p = 0.02), but no main effect for diet (difference in

203   predicted means of HFD and LFD = +27.4%, p = 0.08;) and no interaction (p = 0.8) for sclerostin's

204   30 kDa band within iWAT (Figure 2a). There was a main effect for diet (difference in predicted

205   means of LFD and HFD = +173.7%, p = 0.004), but no main effect for exercise (difference in

206   predicted means from pre- to post-exercise = +7.1%; p = 0.9) and no interaction (p = 0.9) for serine9

207   phosphorylation of GSK3β within iWAT (Figure 2b). Likewise, there was a main effect for diet

208   (difference in predicted means of LFD and HFD = +141.5%, p = 0.005), but no main effect for

209   exercise (difference in predicted means from pre- to post-exercise = +7.3%, p = 0.8) and no

210   interaction (p = 0.7) for total GSK3β content within iWAT (Figure 2c). Together, these responses

211   resulted in no main effects for either exercise (difference in predicted means from pre- to post-

212   exercise = -8.1%, p = 0.4) or diet (difference in predicted means of LFD and HFD = +10.9%, p = 0.4)

213   and interaction (p = 0.5) for serine9 phosphorylation/total GSK3β ratio within iWAT (Figure 2d).

214   There was a main effect for diet (difference in predicted means of LFD and HFD = +65.8%, p = 0.03)

215   with no main effect for exercise (difference in predicted means from pre- to post-exercise = +55.4%,

216   p = 0.07) and no interaction (p = 0.7) for total β-catenin content within iWAT (Figure 2e).

217   **INSERT FIGURE 2**

218   **3.3   Obesogenic diet abolishes sclerostin content within eWAT and results in a differential**

219   **response to acute exercise in inhibitory GSK3β serine9 phosphorylation status**

220   In contrast to the iWAT doublet banding pattern at 28 and 30 kDa when sclerostin was

221   immunoblotted, eWAT only had 1 band at 30 kDa (there were 2/20 samples with detection of the 28

222   kDa band). Thus, only analysis of the 30 kDa band is presented. There was a main effect for diet

223   (difference in predicted means of LFD and HFD = -114.9%, p = <0.0001), while there was no main

224   effect for exercise (difference in predicted means from pre- to post-exercise = +17.7%, p = 0.2) and

225   no interaction (p = 0.1) in the 30 kDa band of sclerostin within eWAT. The main effect for diet was a

226   result of the 30 kDa monomer of sclerostin being nearly undetectable in eWAT of HFD fed mice

227   (Figure 3a). It is important to note that there were detectable bands at 30 kDa in recombinant

228   sclerostin when compared to eWAT samples (Supplemental Figure 2) and at sclerostin's dimer

229   predicted molecular weight of 56 kDa (Supplemental Figure 3).

230   There were no main effects for either exercise (difference in predicted means from pre- to

231   post-exercise = +5.2%, p = 0.8) or diet (difference in predicted means of LFD and HFD = +12.9%, p

232   = 0.6) and no interaction (p = 0.3) for total GSK3β content within eWAT (Figure 3b). Likewise,

233   there were no main effects for either exercise (difference in predicted means from pre- to post-

234   exercise = -6.3%, p = 0.7) or diet (difference in predicted means of LFD and HFD = +17.8%, p = 0.2)

235   and no interaction (p = 0.3) for serine9 phosphorylation of GSK3β within eWAT (Figure 3c). There

236   were no significant main effects for either exercise (difference in predicted means from pre- to post-

237   exercise = -0.1%, p = 0.9) or diet (difference in predicted means of LFD and HFD = -1.5%, p = 0.9)

238   and no interaction (p = 0.07) for serine9 phosphorylation over total GSK3β within eWAT (Figure

239   3d). There were no main effects for either exercise (difference in predicted means from pre- to post-

240   exercise = -2.2%, p = 0.5) or diet (difference in predicted means of LFD and HFD = +12.9%, p = 0.9)

241   and no interaction (p = 0.7) was found for total β-catenin content within eWAT (Figure 3e).

242   **INSERT FIGURE 3**

243   **4.   Discussion**

244   In this study, we show new evidence that sclerostin is elevated in the circulation during recovery

245   from acute exercise only in HFD induced obese and insulin resistant mice. When looking at the

246   adipose tissue specific responses, we found that in iWAT, sclerostin decreased following exercise,

247   independent of diet; however, eWAT sclerostin content was not responsive to exercise and was no

248   longer detected after the HFD. Moreover, revealing downstream markers of the Wnt signaling

249   pathway (GSK3β and β-catenin content) identified differential responses to HFD between iWAT and

250   eWAT. Together, these results provide novel context to sclerostin's endocrine role, which appears to

251   be responsive to acute exercise (iWAT) and diet induced obesity/insulin resistance (eWAT)

252   depending on the depot. We also present differences in molecular weights of sclerostin between

253   WAT depots and diets, providing support for tissue specific and diet dependent regulation of

254   sclerostin's post-translational modifications.

255   Circulating sclerostin's response to acute and chronic exercise has been extensively studied in

256   humans, while there is no evidence in mice. In young adult humans (~30 years of age), circulating

257   sclerostin increases immediately following an acute bout of exercise, independent of mechanical

258   loading in both male and female young adults (Kouvelioti et al., 2019). Older adults with

259   osteoporosis also have an acute increase in sclerostin in response to walking/resistance exercise

260   (Gombos et al., 2016). However, postmenopausal women without osteoporosis showed no acute

261   sclerostin response to plyometric exercise but have higher levels of resting sclerostin compared to

262   young adult women (Nelson et al., 2020). Our lab has also shown that normal weight healthy

263   children have no response of circulating sclerostin to acute exercise, while those with obesity have an

264   adult-like, transient post-exercise increase (Dekker et al., 2017;Kurgan et al., 2020). Taken together,

265   there appears to be protection from an increase in circulating sclerostin in healthy children (likely a

266   result of increased bone formation) and healthy older adults (inability to mobilize bone turnover),

267   which is lost in individuals with perturbed metabolism or bone status. This is consistent with our

268   present findings, where HFD mice had increased levels of sclerostin 2 h post-exercise, while LFD

269   mice were unresponsive to acute exercise. There were no differences in sclerostin at rest in HFD

270   mice compared to LFD mice, which is in contrast to previous studies that have shown that a HFD

271 increases bone sclerostin expression (Baek et al., 2014) leading to increased circulating sclerostin

272 (Kim et al., 2017). Since we used the same strain and sex of mice, these differences may be due to

273 age or the length of the HFD, as these studies used 8 weeks of HFD with mice ~16 weeks of age,

274 while we used 10 weeks of a HFD with same strain mice 18 weeks of age (24 vs. 28 weeks of age at

275 analysis).

276 The identification of sclerostin's endocrine role in regulating fat mass (Kim et al., 2017;Kim

277 et al., 2019;Kim et al., 2021) and HFD mice circulating sclerostin increasing following acute exercise

278 led us to examine the influence of fat mass expansion and perturbed insulin sensitivity (HFD vs. LFD

279 fed mice) on the response/mobilization of sclerostin within adipose tissue at rest and in response to

280 acute exercise. In contrast to peripheral sclerostin, there appears to be a reduction in monomeric

281 sclerostin content (both 28 and 30 kDa bands) within iWAT following acute exercise independent of

282 diet. While there was no significant effect of acute exercise on iWAT GSK3β serine9

283 phosphorylation, there was a trend ($p = 0.07$) for an increase in β-catenin content, which is expected

284 with less sclerostin and increased Wnt signalling. This increase in Wnt signaling could represent an

285 attempt to use fat stores for energy rather than store them (e.g., adipogenesis). The timing of

286 sampling is likely why we only observed a trend ($p = 0.07$) for an increase in β-catenin content

287 following acute exercise. This response is likely temporal, as our study examined the response 2 h

288 post-exercise and previous studies have shown Wnt/β-catenin signaling within adipose tissue

289 increases immediately following exercise (Shen et al., 2016).

290 It is interesting to note that iWAT total GSK3β and β-catenin content were both higher in

291 HFD mice compared to LFD mice. With its role in the Wnt signalling pathway, an increase in

292 GSK3β would theoretically lead to reduced levels of β-catenin. However, our data suggests

293 otherwise, where HFD enhanced Wnt signalling independent of changes in total GSK3β. This is

294 likely a compensatory response to changes in the regulation of Wnt signaling with HFD feeding

295   aimed at enhancing lipolysis in the face of excessive adiposity. This compensation is likely regulated

296   by other components of Wnt signaling (e.g., Wnt's co-receptors LRP 5/6). In addition, it has been

297   shown that HFD feeding in adipocyte specific β-catenin knockout mice can compensate for this

298   inhibited Wnt signaling by increasing β-catenin expression by stromal-vascular cells within adipose

299   tissue, which can be used to rescue Wnt signaling within adipocytes (Bagchi et al., 2020).

300   Furthermore, an increase in GSK3β content is not entirely surprising given its role in adipogenesis

301   and metabolism where it has been shown that GSK3β can inhibit expression of the thermogenic gene

302   program (Markussen et al., 2018), and increase inflammation (Wang et al., 2018) and adipogenesis

303   (Benjamin et al., 1994;Ross et al., 1999;Zaragosi et al., 2008). We propose that GSK3β increases

304   content in iWAT in response to HFD feeding to increase adipogenesis, and acute exercise can

305   counteract this by increasing Wnt signaling (trend for increased β-catenin content), which may be

306   regulated in part by reduced sclerostin.

307   Since sclerostin knockout models are protected against an obesogenic diet in both

308   subcutaneous and visceral fat pads by inhibiting adipogenesis and increasing insulin sensitivity and

309   fat oxidation (Kim et al., 2017), we hypothesized that a HFD would increase sclerostin content in

310   both WAT depots due to reduced insulin sensitivity. In contrast, we found monomeric sclerostin

311   content was unresponsive in iWAT and undetectable in eWAT of HFD mice compared to LFD fed

312   mice at rest. However, this does not mean sclerostin was not present in eWAT, as there was a strong

313   band at ~56 kDa (Supplemental Figure 3), representing a sclerostin dimer (Hernandez et al., 2014).

314   We suspect that there is either a push to preferentially form dimers and reduce monomeric sclerostin

315   content or there is a reduction in the mobilization of monomeric sclerostin to visceral WAT while

316   subcutaneous WAT is not impacted. There are no crystallized structure images on protein data bank

317   (Berman et al., 2000) of the sclerostin dimer, which prevents speculation on the influence of changes

318   in glycosylation or dimerization on protein function. Imaging sclerostin's dimer structure or

319   predicting its structure through computational methods (Brini et al., 2019) would provide insight into

320   the influence dimerization would have on sclerostin's ability to interact with its LRP6 epitope (Kim

321   et al., 2020) and enable comparison of dimeric sclerostin's inhibition of Wnt function compared to

322   monomeric sclerostin. It is important to note that eWAT sclerostin and components of Wnt/β-catenin

323   signaling were unresponsive to acute exercise in this study, suggesting sclerostin may not influence

324   eWAT's long term adaptations to diet or exercise, which may be due to alterations in its sensitivity to

325   sclerostin action (e.g., receptor content – LRP4 and 6 (Kim et al., 2019;Kim et al., 2020)). The

326   importance of these changes to sclerostin's content and post-translational modifications with exercise

327   and HFD feeding are critical in furthering our understanding of its role in regulating bone (Poole et

328   al., 2005), adipose tissue (Kim et al., 2017), and skeletal muscle (Magarò et al., 2021) growth and

329   metabolism, which we plan to directly examine in future studies.

330   There are several limitations to the present study design, that limits our analysis and

331   interpretation to correlations in the response of sclerostin and Wnt signaling. Thus, our conclusions

332   lack mechanistic insight or causative effects of changes in sclerostin content and Wnt signaling

333   within adipose tissue and their influence on growth and metabolism. Rather, we present responses in

334   proteins with known functions in these tissues providing preliminary evidence for their role in

335   adaptations to diet and exercise and rationale for further examination. Additionally, assessment of

336   more post-exercise time points, particularly immediately post-exercise, is needed to fully understand

337   the temporal behavior of sclerostin and Wnt signaling acutely post-exercise. WAT is also comprised

338   of mature adipocytes, preadipocytes, endothelial, stromal, and immune cells, thus it is important to

339   consider the changes we are observing may be a result of changes occurring in any of these cell types

340   (Bagchi et al., 2020). Cell specific responses to high fat feeding and acute exercise is important to

341   elucidate which cell types are contributing to these responses. Importantly, since our data include

342   whole tissue lysate, we cannot answer the question whether sclerostin is bound to receptors on

343   adipocytes or whether is expressed in other cell types that are present in adipose tissue. We also only

344   report responses in male mice, which neglects sex specific differences. Lastly, an important caveat is

345   that GSK3β activity is not solely regulated by serine9 phosphorylation status or content and Wnt

346   signaling does not require its phosphorylation to inhibit its kinase activity for β-catenin as its

347   substrate (Piao et al., 2008). Although phosphorylation of GSK3β can occur in tandem, increased β-

348   catenin content is an indicator of canonical Wnt signal transduction.

349   Given sclerostin's association with fat oxidation and insulin sensitivity, future studies are

350   needed that examine sclerostin's response to changes in adipose tissue lipolysis (e.g., induced by

351   catecholamines or models of inhibited WAT lipolysis) or insulin stimulated glucose uptake. These

352   studies will provide mechanistic insight into sclerostin's mobilization with exercise and the

353   differential response between fat pads. Assessment of the importance of sclerostin reduction within

354   iWAT with multiple bouts of acute exercise (i.e., exercise training) is also needed to understand its

355   role in regulating adipose tissue mass and adipocyte cell size. Lastly, assessing eWAT and iWAT *ex*

356   *vivo* explants differences in Wnt signaling activation to varying doses of Wnt ligands (e.g., Wnt 3a)

357   and sclerostin will allow for further understanding of depot specific sensitivity to Wnt signaling and

358   sclerostin action (e.g., receptor content).

359   **4.1   Conclusion**

360   Herein we have characterized the monomeric sclerostin response to acute exercise as well as the

361   influence of HFD feeding on this response. While HFD and LFD mice have no difference in resting

362   levels, HFD augments circulating sclerostin response to acute exercise, which remains unresponsive

363   in LFD mice. Within visceral eWAT, monomeric sclerostin content is abolished and is unchanged in

364   iWAT in HFD mice compared to LFD mice, while acute exercise does not influence eWAT

365   sclerostin it leads to a reduction in iWAT independent of diet. Thus, sclerostin is responsive to acute

366   exercise (iWAT) and fat mass expansion (eWAT), conferring a contribution of this bone derived

367   endocrine factor in regulating WAT growth and metabolism that requires further examination.

368 **Conflict of Interest**

369 The authors declare that the research was conducted in the absence of any commercial or financial
370 relationships that could be construed as a potential conflict of interest.

371 **Author Contributions**

372 NK, AM, VF, RM and PK conceived and designed the study. PK obtained funding. RM was
373 responsible for the surgeries. NK, BB and JS collected the samples and performed the experiments.
374 All authors contributed to data analysis and interpretation of the results. NK drafted the manuscript
375 and did the subsequent revisions. All authors edited and revised the manuscript and approved the
376 final version submitted for publication.

377 **Funding**

381 **Data Availability Statement**

382 The datasets for this study can be found in the [NAME OF REPOSITORY] [LINK].

383 **Figure legends**

384 **Figure 1.** Serum sclerostin response to HFD and acute exercise. Light grey bars represent sedentary
385 mice and dark grey bars represent mice sampled 2 h following 2 h of acute endurance exercise and
386 are grouped by diet; LFD = low fat diet; HFD = high fat diet. Data are presented as means±SD. A
387 two-way factorial ANOVA was used to examine main effects for diet and exercise as well their
388 interaction. *Tukey* correction was used for multiple pairwise comparisons.

389

390 **Figure 2.** Response of iWAT sclerostin content (a), serine9 phosphorylation status (b and d) and total
391 GSK3b content (c), and total b-catenin content (e) to HFD and acute exercise. Light grey bars
392 represent sedentary mice and dark grey bars represent mice sampled 2 h following 2 h of acute
393 endurance exercise and are grouped by diet; LFD = low fat diet; HFD = high fat diet. Proteins are
394 corrected for by the house keeping protein vinculin and are all relative to LFD sedentary mice, data
395 are presented as means±SD and main effects are presented on graphs only if they are either close to
396 or are significant. A two-way factorial ANOVA was used to examine main effects for diet and
397 exercise as well their interaction. *Tukey* correction was used for multiple pairwise comparisons.

398

399 **Figure 3.** Response of eWAT sclerostin content (a), serine9 phosphorylation status (b and d) and
400 total GSK3b content (c), and total b-catenin content (e) to HFD and acute exercise. Light grey bars
401 represent sedentary mice and dark grey bars represent mice sampled 2 h following 2 h of acute
402 endurance exercise and are grouped by diet; LFD = low fat diet; HFD = high fat diet. Proteins are
403 corrected for by the house keeping protein vinculin and are all relative to LFD sedentary mice, data
404 are presented as means±SD and main effects are presented on graphs only if they are either close to
405 or are significant. A two-way factorial ANOVA was used to examine main effects for diet and
406 exercise as well their interaction. *Tukey* correction was used for multiple pairwise comparisons.

407 **Table 1.** Group differences in body mass, tissue necropsy, and insulin sensitivity measures following
408 8 weeks of dietary intervention.

	LFD	HFD	p-value
Body Mass (g)	32.2±3.2	48.4±5.2	<0.0001
iWAT Mass (g)	0.4±0.4	1.9±0.5	<0.0001
eWAT Mass (g)	0.8±0.4	1.6±0.3	0.0003
Liver Mass (g)	1.1±0.2	2.4±0.6	<0.0001
ITT AUC (mmol·L^{-1}·min^{-1})	520.4±80.3	955.6±84.6	<0.0001
GTT AUC (mmol·L^{-1}·min^{-1})	1299.1±159.6	2057.3±286.5	<0.0001

409 Data are means±SD. Independent sample *t*-tests were used to examine group differences. LFD = low fat diet; HFD = high
410 fat diet; iWAT = inguinal white adipose tissue; eWAT = epididymal white adipose tissue; ITT = insulin tolerance test;
411 AUC = area under the curve; GTT = glucose tolerance test. Data is a cohort of previously published data (Baranowski et
412 al., 2021).