Plasma adiponectin is increased in mice selectively bred for high wheel-running activity, but not by wheel running per se

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Abstract
Mice selectively bred for high wheel-running activity (S) have decreased fat content compared to mice from randomly bred control (C) lines. We explored whether this difference was associated with alterations in levels of circulating hormones involved in regulation of food intake and energy balance, and whether alterations were caused by the presence of a running wheel. Plasma levels of leptin, adiponectin, and corticosterone as well as body composition were analyzed in male S mice housed with (+) and without (-) access to running wheels at ages of 10 and 18 months. These levels were compared to those found in C+ mice. Plasma corticosterone did not differ among groups. While plasma leptin levels tended to be lower in S+ mice as compared to S- or C+ mice, these differences were largely attributable to differences in fat content. Adiponectin levels were increased in S mice (+60%) compared to C mice, irrespective of wheel access. High levels of this hormone may be a trait co-segregated in mice bred for high wheel-running activity.
INTRODUCTION

It is generally accepted that moderate physical activity has a positive influence on health and life expectancy (Holloszy, 1988; Navarro et al., 2004). From a clinical standpoint, the most beneficial effect of regular exercise is that it prevents or antagonizes increased adiposity and the adverse health risks (e.g. cardiovascular disease and metabolic syndrome, type-2 diabetes mellitus) that are associated with it (Holloszy, 1988; Novelli et al., 2004), for review see (Carroll and Dudfield, 2004). Indeed, physical activity promotes the breakdown of triglyceride stores inside adipose tissue and muscle, which in turn contributes to increased whole-body insulin action and improvement of tissue perfusion (Boden, 1997).

Besides having a direct effect on metabolic and vascular processes, it has been pointed out that physical activity could also affect endocrine activity, which in turn could influence body adiposity (McMurray and Hackney, 2005). In this respect, adipose tissue has received considerable attention because it secretes the adipocyte hormones leptin and adiponectin. These hormones are extremely important in the long-term maintenance of energy balance and fuel homeostasis (Caro et al., 1996; Halaas et al., 1995; McMurray and Hackney, 2005; Ryan et al., 2003) and thus have a strong influence on sustainable health. Circulating levels of leptin have been shown to correlate highly with indices of fat content in many species (Park et al., 2004), and as such may signal the available amount of body fat to the brain (Halaas et al., 1995). In this way, leptin regulates appetite and metabolism in a coordinated fashion (Caro et al., 1996; Van Dijk et al., 1999). Adiponectin, on the other hand, does not correlate positively with indices of fat content, but was found to be negatively correlated with fat content in humans (Cnop et al., 2003; Park et al., 2004; Ryan et al., 2003) or be unrelated (Ferguson et al., 2004). Adiponectin has been implicated to stimulate fat oxidation in metabolically active tissue (Berg et al., 2002; Bruce et al., 2005; Fruebis et al., 2001; Yamauchi et al., 2002) and peripheral insulin sensitivity (Baratta et al., 2004; Schondorf et al., 2005; Yamauchi et al., 2001). Circulating adiponectin levels are reduced and leptin levels are enhanced in obese humans compared with lean individuals (Arita et al., 1999; Havel, 2001). This might contribute to the insulin resistance that is observed in obese subjects (Gil-Campos et al., 2004).

Human studies on the interaction between physical activity and endocrine activity of adipose tissue are not conclusive, and outcomes may depend on the intensity of the exercise paradigm and the exercise capacity of subjects (Ferguson et al., 2004; Jurimae et al., 2005; Zoladz et al., 2005). In laboratory animals, however, leptin concentrations were found to be decreased in life-long voluntary exercising rats at 23 months of age (Novelli et al., 2004) and in hamsters that had been exercising voluntarily for 31 days (Coutinho et al., 2006), which may be consistent with a lowering of triglyceride stores in exercising animals. Mice selectively bred for high physical activity (Swallow et al., 1998) show a decreased body fat (Swallow et al., 2001) and a decreased leptin concentration in females at 3 month of age (Girard I., Rezende, E. L., and Garland, T., Jr., unpublished observation). The reduction in circulating
leptin level, however, was greater than could be explained by the reduced fat mass alone. To further investigate the relation between physical activity, circulating adipocyte hormones, and body composition, the present study assessed these relationships in selectively bred high-activity male mice with chronic access to running wheels, and compared these effects to those found in their random-bred controls at different ages. Because relatively low plasma leptin levels in the high activity-select ed females were associated with increased plasma corticosterone levels (Girard and Garland, Jr., 2002; Malisch et al., 2006) plasma levels of corticosterone were also assessed in the present study. To investigate the effects of wheel-running activity per sé on adipose and adrenal hormones and body composition, above-mentioned relations were also investigated in selectively-bred mice without the presence of a running wheel. These comparisons may shed light on the nature of changes that occur in the regulation of adipose and adrenal hormones; i.e., whether they are caused by high activity per se, or whether it is a trait that has genetically co-segregated with selection for high wheel-running activity.

**MATERIAL AND METHODS**

**Animals & housing**
The progenitors for the original selection experiment (Swallow et al., 1998) were 112 pairs of outbred, genetically variable Hsd:ICR mice obtained from Harlan-Sprague-Dawley in Indianapolis, Indiana, USA. After initial generations of random mating, the selection procedure then employed 8 separate lines, 4 in which breeders were chosen randomly within each line (control or C lines) and 4 in which the highest-running males and females from each family were used as breeders (preventing sib-matings, selected or S lines). At generation 31, 80 breeding pairs, representing all 8 lines, were shipped by air to the animal facility of the Biological Center in Haren, and a breeding colony was started. Male offspring from all 8 lines were used in the experiment described below. After weaning, mice were housed with their littermates until the age of 5 months when all animals were individually housed with or without a running wheel for the rest of their lives (Macrolon Type II, UNO Roestvaststaal BV, Zevenaar, NL; adapted to fit in a plastic running wheel with a 7 cm radius and 1 cm spacing between bars). Wood shavings were used as bedding material and all animals got a wooden stick. The animals had *ad libitum* food (Standard lab chow 2181, Hopefarms B.V., Woerden, NL) and water and were under a 12:12 light-dark cycle (lights on at 8:00). All procedures concerning animal care and treatment were in accordance with the regulations of the ethical committee for the use of experimental animals of the University of Groningen (DEC 2777(-1)).

**Experimental procedures**
Three experimental groups were created; group 1) Control mice housed with a running wheel (C+), group 2) Selected mice with a running wheel (S+), and group 3)
Selected mice without a running wheel (S-). Logistical constraints precluded inclusion of a fourth group, i.e., C- mice. For both C and S groups, mice from all four lines were represented, although not in equal proportions. Animals of two different ages were used, mice in the 10 month age group were 312±7 days old (mean±SD) and mice in the 18 month group were 559±10 days old. Every mouse was weighed once per month and left undisturbed except for the weekly cage cleaning. In the week prior to killing food intake was monitored over 3 consecutive days. In addition, wheel-running activity was assessed in the S+ and C+ groups over two weeks prior to sacrifice with a PC-based event recording system (ERS). Data on wheel-running activity was not available for all animals due to computer problems.

At different ages (10 and 18 months) 7–8 mice per cohort were briefly anaesthetized with CO₂ then killed by decapitation in the middle of the light phase. Mice were not fasted prior to blood sampling and all mice were asleep when they were taken for blood sampling. Trunk blood was collected in pre-chilled tubes with anticoagulant (EDTA) within 90 seconds from initial disturbance to the final drop of blood. Samples were spun down at 2600 g for 15 minutes at 4°C. Plasma was collected and stored at –80°C until later analysis for hormone levels. Corticosterone, leptin, and adiponectin levels were determined in duplicate with commercial radioimmunoassay kits (Linco Research, Nucli lab, The Netherlands).

After blood collection animals were dissected and fresh mass of different organs (heart, liver, kidney, lung, stomach, intestines, skin) and the remainder of the carcass were weighed to the nearest 0.0001 g. The fat content of all animals was determined by drying (ISO 6496-1983(E)) all separate tissues at 103°C followed by fat extraction using petroleum ether (Boom BV, Meppel, Netherlands) and subsequent drying.

**Data analysis**

To test for effects of treatment and/or age we applied ANCOVA models in the MIXED procedure in SAS for Windows (version 9.1). Group, age, and the group x age interaction were fixed effects. The main interest of this study was to determine effects of selective breeding and effects of the presence of a running wheel; therefore, a priori, we tested for differences between group 1 and group 2 (C+ versus S+), and between group 2 and group 3 (S+ versus S-), by adding contrasts to the model. Because we did not maintain a C- group and because sample sizes per group x age combination were relatively small (see Table 4.1), we did not include line as a random effect nested within linetype. Covariates were included as appropriate. For instance, leptin and adiponectin are produced by fat cells and have been shown to correlate with total fat content. Therefore, fat content was added into the model as a covariate for both hormone levels. Data were log₁₀-transformed as necessary to improve normality and linearity of relations with covariates. Based on previous findings, one-tailed tests could be used for some traits; however, for simplicity two-tailed p-values are given for all variables. The significance level of \( p \leq 0.05 \) was used. Pearson correlations were used to explore relations between traits of interest, considering each group separately.
RESULTS

Body composition, food intake, and wheel-running activity

Table 4.1 shows the main characteristics of the three experimental groups. As expected from previous studies, selected mice with a running wheel (S+) tended to have lower body mass ($F_{1,40}=17.4$, $p<0.001$) and higher food intake than control mice with wheels (C+) (not significant: $F_{1,40}=3.72$, 2-tailed $p=0.061$). Food intake also did not differ significantly between S+ and S- mice, but body mass of S+ mice was lower than S- mice ($F_{1,40}=5.4$, $p=0.025$). Adding body mass to the model as a covariate did not alter these effects for food intake.

Absolute fat content was approximately 50% lower in S+ mice compared to C+ and S- mice ($F_{1,40}=12.2$, $p=0.001$ and $F_{1,40}=11.3$, $p=0.002$, respectively) and absolute dry lean mass was higher in controls than both selected groups ($F_{1,40}=12.1$, $p<0.001$). Both fat and dry lean mass strongly correlated with total wet body mass (see Figure 4.1) and therefore body mass was added to ANOVA models as a covariate. This analysis showed that fat mass was significantly lower in S+ mice compared to S- mice ($F_{1,39}=6.1$, $p=0.02$) and that age did not affect fat mass. Dry lean mass did not differ significantly anymore between the groups or with age once wet body mass was included as a covariate.

Table 4.1. Main characteristics of mice from control lines housed with a running wheel (C+) and of mice from selected lines housed either with (S+) or without a running wheel (S-).

<table>
<thead>
<tr>
<th></th>
<th>Age</th>
<th>C+</th>
<th>S+</th>
<th>S-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass (g)</td>
<td>10</td>
<td>42.5a</td>
<td>36.7</td>
<td>39.6b</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>44.4</td>
<td>33.4</td>
<td>39.5</td>
</tr>
<tr>
<td>Food intake (g d⁻¹)</td>
<td>10</td>
<td>4.3</td>
<td>6.8</td>
<td>5.3</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>6.2</td>
<td>6.4</td>
<td>5.9</td>
</tr>
<tr>
<td>Fat mass (g)</td>
<td>10</td>
<td>8.8a</td>
<td>5.3</td>
<td>10.7b</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>10.5</td>
<td>4.7</td>
<td>8.9</td>
</tr>
<tr>
<td>Dry lean mass (g)</td>
<td>10</td>
<td>9.4a</td>
<td>8.3</td>
<td>8.3</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>9.7</td>
<td>8.1</td>
<td>8.8</td>
</tr>
<tr>
<td>Wheel-running activity (km d⁻¹)</td>
<td>10c</td>
<td>14.0</td>
<td>18.2</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>7.4</td>
<td>9.4</td>
<td>-</td>
</tr>
</tbody>
</table>

Values are simple means (SEM). Two-way ANOVA was used to test for differences between groups and with age (months). Data were log-transformed as necessary to improve normality.

a Indicates a significant difference between C+ and S+ mice ($p<0.05$). b indicates a significant difference between S+ and S- mice ($p<0.05$). c indicates a significant effect of age ($p<0.05$). Sample size was 8 per group, but in the S+ and S- groups it was 7 at 18 months.
Wheel-running activity assessed over the two weeks prior to sacrifice was calculated in km per day. As expected, wheel-running activity was higher in the selected mice and decreased with age. On average selected mice ran approximately 30% more than controls at both ages and wheel-running activity decreased approximately 50% in both groups between 10 and 18 months. The group effect on wheel-running activity was not significant, but the age effect was (F1,21=4.6, p=0.043).

Hormone concentrations
Figure 4.2 shows leptin, adiponectin, and corticosterone levels in plasma for the different experimental groups and Table 4.2 gives an overview of the statistical analysis. Leptin levels were slightly lower in S+ mice compared with C+ and S- mice. This effect of group was statistically significant when comparing S+ with S- mice. No effect of age on leptin levels was shown. When total fat mass was added to the model as a covariate, no significant effects of age or group remained and total body fat was a significant predictors of leptin levels in the model.

Adiponectin levels were increased by approximately 60% in both groups of selected mice compared with control mice at both ages (10 and 18 months) and decreased significantly with age. This group effect remained when fat content was added to the model as a covariate. Fat content was not a significant predictor of adiponectin levels in the model. Basal corticosterone levels did not significantly differ between control and selected mice with or without a wheel (See Figure 4.2 and Table 4.2) and did not change with age.

Correlations
As shown in Table 4.3, body mass and fat content were negative predictors of wheel-running activity in control mice. In selected mice, a similar trend was visible
Adiponectin in mice bred for high activity

Figure 4.2. Leptin, adiponectin, and corticosterone concentrations in C+ (white bars), S+ (dark grey bars) and S- (light grey bars) mice. Values represent simple means ± SEM. Sample size is 8 in C+ mice at all ages. In S+ and S- mice the sample size was 8 and 7 at 10 and 18 months respectively.

Table 4.2. Results of two-way nested ANCOVA of leptin, adiponectin, and corticosterone plasma levels in control (C) and selected mice (S) housed with (+) or without (-) a running wheel.

<table>
<thead>
<tr>
<th>Variable</th>
<th>N</th>
<th>P</th>
<th>Age</th>
<th>P</th>
<th>C+ vs S+</th>
<th>S+ vs S-</th>
<th>Covariate</th>
<th>P</th>
<th>Covariate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leptin (ng ml⁻¹)</td>
<td>45</td>
<td></td>
<td>0.346</td>
<td>0.091</td>
<td>0.029</td>
<td></td>
<td>none</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leptin (ng ml⁻¹)</td>
<td>45</td>
<td></td>
<td>0.154</td>
<td>0.154</td>
<td>0.641</td>
<td></td>
<td>FAT</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Adiponectin (ng ml⁻¹)</td>
<td>46</td>
<td></td>
<td>0.035</td>
<td>0.001</td>
<td>0.673</td>
<td></td>
<td>FAT</td>
<td></td>
<td>0.534</td>
</tr>
<tr>
<td>Adiponectin (ng ml⁻¹)</td>
<td>46</td>
<td></td>
<td>0.037</td>
<td>0.003</td>
<td>0.939</td>
<td></td>
<td>FAT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corticosterone (ng ml⁻¹)</td>
<td>46</td>
<td></td>
<td>0.309</td>
<td>0.203</td>
<td>0.805</td>
<td></td>
<td>none</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Two-way ANOVA were performed with age, group, and group × age as fixed factors. A priori we tested for differences between C+ and S+ or S+ and S- mice. All data were log-transformed to improve normality. P-values of age effects and group (C+ vs. S+ and S+ vs. S-) are given in the table. No significant interaction effects were found and therefore p-values for interaction effects (age × group) are not shown. Fat mass was added into the model as a covariate for leptin and adiponectin. Total sample size (N) and degrees of freedom (d.f.) are given for all groups. Sample size was 8 per group, except for S+ and S- group at 18 months where n was 7. One leptin plasma sample gave a leptin concentration of 0 ng ml⁻¹, so this sample was not used in the analyses (S+ group at 10 months).
but probably due to the small sample size (n=9), the correlations were not significant. Overall, lighter and leaner animals ran more than heavier and fatter animals. Food intake did not correlate with any of the measures of body composition. Plasma leptin levels positively correlated with fat in all groups and correlated with dry lean mass and body mass in C+ and S- mice, but not S- mice. Leptin did not correlate with food intake or wheel-running activity. Plasma adiponectin and corticosterone did not correlate with any of the measures of body composition nor with wheel-running activity and food intake in any of the groups. No significant correlations between the different hormones measured were found in selected mice, but in control mice corticosterone significantly correlated with adiponectin. Figure 4.3 shows the relationships between hormones and two measures of body composition, total fat, and dry lean mass.

**DISCUSSION**

Mice selectively bred for high wheel-running activity (S) generally have decreased body mass and fat content compared to control (C) mice (Dumke *et al.*, 2001; Swallow *et al.*, 2001). We explored whether these differences are associated with alterations in plasma levels of leptin, adiponectin, and corticosterone, and whether they depend on age, the actual levels of physical activity or other traits. Consistent with our expectations was the observation that wheel-running activity of mice was
a negative predictor of body fat content, and fat content in turn correlated positively with circulating levels of leptin. The levels of leptin were better predicted by fat content than by physical activity, which suggests that the leptin-reducing effects of physical activity may be mediated primarily through an effect on body fat content. In contrast to previous studies on these mice, at younger ages (Koteja et al., 1999; Swallow et al., 2001), we did not find a positive relationship between wheel-running activity and food intake in control or activity-selected mice. Food intake was

Figure 4.3. Correlations between hormones (leptin, adiponectin, and corticosterone) and body composition (fat mass and dry lean mass). Linear regressions were performed to examine significant relations. White dots represent C+ mice, black dots represent S+ mice and grey dots represent S- mice. Regression lines are shown in graphs when relations were significant (dotted line for C+, solid line for S+, dashed line for S-).
increased in S+ mice compared to C+ mice in the present study, and sample sizes may have been too small to show effects of wheel running on food intake at the individual level.

Leptin levels were found to be significantly decreased in S+ mice compared to S- mice (not corrected for fat mass), but not compared to C+ mice. The latter is at odds with an unpublished observations of decreased plasma leptin levels in young female S mice compared to C mice, even after correction for fat mass (Girard I., Rezende, E. L., and Garland, T., Jr., unpublished observation). The difference in outcome between this study and the one mentioned above may reflect a partial sex-dependent difference in the regulation of leptin levels. Indeed, women are known to have higher leptin levels than men with a similar fat content (Havel, 2001; Hickey et al., 1996). Leptin levels are known to decrease in voluntarily exercising rodents compared to sedentary controls (rats; (Novelli et al., 2004) and hamsters; (Coutinho et al., 2006)) and this was also shown in the present study when comparing S+ and S- mice. Obviously differences in activity were largest between the sedentary housed S- mice and the S+ mice than between the S+ and C+ mice and this might explain why leptin levels were not decreased in the S+ mice compared to C+ mice.

The most important finding in the present study was the observation that plasma adiponectin levels were significantly increased in S mice compared to the levels found in their random-bred controls. This increase was found in S mice irrespective of the availability of running wheels and occurred in all of the separate selection lines (results not shown), which suggests that this increase is a genetically co-segregated trait with selection for increased wheel-running activity, instead of being mediated via increased physical activity per se. At present, we do not know whether this increased adiponectin is of high or low molecular weight (active or inactive form; (Fruebis et al., 2001)), or whether it is a cause of altered release or clearance. Future work is necessary to study this and to determine whether the increased adiponectin levels in activity-selected mice are associated with increased insulin sensitiviy. Also, if the increased levels of adiponectin are responsible for their low fat content, does this or the high adiponectin levels protect them against diet-induced obesity?

There a number of reasons to suggest that increased circulating adiponectin levels might contribute to the different phenotypes seen in S mice compared to C mice. For example, Fruebis et al (Fruebis et al., 2001) found that chronic administration of gAcrp30 (i.e., adiponectin) caused weight loss in mice despite the fact that food consumption was unaffected. This is a phenotype which appears homologous to the one found in selected animals in this and previous studies (Swallow et al., 1999; Swallow et al., 2001). Fruebis et al. attributed the effect of adiponectin on body mass to increased fat oxidation, specifically in liver and muscle (Fruebis et al., 2001), and this was confirmed in subsequent studies (Berg et al., 2002; Bruce et al., 2005; Yamauchi et al., 2002). Although not directly assessed in the present study, we recently observed a decrease in the respiratory quotient (RQ) in S mice compared to C mice measured over a period of 24 hours in non-fasted animals (see
Chapter 5), which indeed indicates higher levels of fat oxidation in selected mice. It might be speculated that an increased capacity to down-grade lipids in muscular tissue contributes the increased physical activity displayed by activity-selected mice. The effects of adiponectin on fat oxidation are believed to arise through stimulation of AMP-activated protein kinase (AMPK) (Berg et al., 2001; Yamauchi et al., 2002). Zhang et al. have shown increased levels of phosphorylated AMPK in aorta of male activity-selected mice compared with controls (Zhang et al., 2006), which is consistent with the observed elevated levels of adiponectin in this study. At present we do not know whether there is a positive relationship between adiponectin and AMPK at the individual level in the activity-selected mice.

Previous studies have shown an increase in resting corticosterone levels in 2-month old male and female S mice (Girard and Garland, Jr., 2002; Malisch et al., 2006), without a change in the absolute corticosterone concentration in response to 40 min. restraint stress (Malisch et al., 2006). In the present study we investigated whether these differences were present in older animals. No statistically significant differences were found in corticosterone levels between C and S males at 10 or 18 months of age. Glucocorticoids increase in response to exercise (Droste et al., 2003) and can affect leptin and adiponectin concentrations (Droste et al., 2003; Fallo et al., 2004; Van Dijk et al., 1997). Nevertheless, we did not show any relations between wheel-running activity, plasma adiponectin or leptin and corticosterone levels in C+ and S- mice. In S+ mice, however, a negative relationship between corticosterone and adiponectin was found.

In conclusion, mice from lines that have been selectively bred for high wheel-running activity over many generations show several endocrine alterations. Male activity-selected animals have higher adiponectin levels than their randomly-bred controls, independent of the presence of a running wheel. Leptin and corticosterone levels were unchanged in selected mice of 10 months and older, although a decrease in leptin and an increase in corticosterone were shown previously in younger females. These effects might be driven mainly by differences in wheel-running activity between the groups at young ages. Selected mice have been shown to be leaner than control mice (Dumke et al., 2001; Swallow et al., 2001) and together with the observed increase in adiponectin concentration this might render the mice less prone to develop insulin resistance and other aspects of the metabolic syndrome. This would make them a suitable model to study whether “physical activity genes” exist, and whether these could influence mechanisms underlying proneness for diet-induced obesity and related diseases.

Acknowledgements
The authors thank Gerard Overkamp for expert technical assistance and Berber de Jong for help with the experimental procedures. Jan Bruggink is thanked for performing analytical procedures. This work was supported primarily by a Career Development Grant from the Dutch Diabetes Association (to GvDijk). Additional funding was provided by grants from the U.S. National Science Foundation to T.G., most recently IOB-0543429.
Hormone levels of leptin, adiponectin and corticosterone were determined in plasma of male C57BL mice exposed to 10°C (CC) or 22°C throughout life (WW), and in mice exposed to 10°C till the age of 15 months and at 22°C afterwards (CW; for a detailed description of the experimental protocol see Chapter 8). Trunk blood was collected in tubes with anticoagulant (EDTA) at 3, 11, 19 and 27 months of age, centrifuged at 4°C for 15 min at 2600 g. Then plasma was collected and stored at −80°C for later hormone analyses (with RIA, Linco kits). Plasma samples at 27 months of age were not analyzed for leptin and adiponectin levels. Corticosterone measurements at 19 months were left out of statistical analysis: results were unreliable because the cold mice were probably disturbed prior to the measurements resulting in very high corticosterone values at this age in these mice.

Figure 4.4 shows the results. Both, levels of leptin and adiponectin were decreased in mice exposed to the CC compared to WW mice (Two-way ANOVA with group and age as fixed factors (excluding CW mice); Effect of group: Leptin; F1,48=41.8, p<0.001, Adiponectin; F1,47=7.8, p<0.01). Age did not affect adiponectin levels significantly but leptin levels were significantly affected by age (Effect of age: Leptin; F2,48=21.4, p<0.001) and there was a significant interaction between group and age (GroupxAge interaction: Leptin; F2,48=10.1, p<0.001). Both hormones, leptin and adiponectin are produced by adipose tissue and specifically leptin is known to correlate strongly with fat content. Therefore, we also applied ANCOVA models with fat as a covariate. In these models there was still a significant effect of group on leptin levels (F1,47=14.9, p<0.001), but adiponectin was no longer significantly different between groups. In both cases fat content was significantly correlated to the hormone levels (p<0.001 and p=0.01 respectively for leptin and adiponectin). There was no longer a significant effect of age on leptin levels, but the group x age interaction remained significant (F2,47=5.5, p<0.01), indicating that leptin levels increased more with age in the WW mice than in the CC mice. We also tested for differences in leptin and adiponectin levels between CW, CC and WW mice using one-way ANOVA with a factor group. No significant effect of group on adiponectin level was found, but leptin did differ significantly between groups (p<0.05), and the CW and WW mice had significantly higher levels of leptin relative to CC mice (see figure 4.4). Corticosteron levels did not differ between groups and were not affected by age.

These results agree with several other studies (Puerta et al., 2002; Imai et al., 2006), and may indicate a role for leptin and adiponectin in the extensive metabolic adaptations to cold.
Figure 4.4. Leptin, adiponectin, and corticosterone concentrations in WW (white bars), CC (dark grey bars) and CW (light grey bars) mice. Values represent simple means ± SEM. Sample size is 5-8 in all groups.