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Detection, Analysis and Interactions of Plasma Ghrelin, Leptin and Growth Hormone in the Mink (*Mustela vison*)

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ABSTRACT—The aim of this study was to obtain basic knowledge of the plasma concentrations and interactions of weight regulatory hormones in juvenile minks (*Mustela vison*). Ghrelin, leptin, and growth hormone (GH) levels were validated and determined by radioimmunoassay methods from the plasma of 30 female and 30 male minks. The female minks had higher plasma ghrelin and GH levels than the males. The plasma ghrelin concentrations of the females correlated positively with their body masses (BMs). The plasma leptin levels did not differ between sexes, but there was a positive correlation between the plasma leptin concentrations and BMs in the male minks. When the data from the male and female minks were combined, the correlation between the leptin levels and the BMs was still clear, but this was not observed in the females alone. In the male minks, the plasma GH levels correlated positively with the BMs and with the plasma leptin concentrations. However, there was no correlation between the plasma ghrelin and GH or leptin concentrations. The hormone concentrations were quite similar to earlier measurements in other carnivores.

Keywords: ghrelin, growth hormone, leptin, mink, *Mustela vison*

INTRODUCTION

Ghrelin is a 28-amino acid peptide hormone expressed mainly in the stomach (Kojima *et al.*, 1999). Substantially lower ghrelin levels are found in the small and large intestine, in α cells of the pancreatic islets (Date *et al.*, 2002), the hypothalamus (Kojima *et al.*, 2001), the pituitary (Korbonits *et al.*, 2001), the kidney (Mori *et al.*, 2000), and the placenta (Gualillo *et al.*, 2001). Ghrelin stimulates growth hormone (GH) secretion (Kojima *et al.*, 1999) and has important roles in the regulation of energy homeostasis, body mass (BM) and food intake by activating hypothalamic neuropeptide Y (NPY) neurons (Shintani *et al.*, 2001). It also reduces fat utilisation in rodents (Tschöp *et al.*, 2000).

Leptin is a protein secreted mostly by the white adipose tissue (Zhang *et al.*, 1994; Cinti *et al.*, 1997). It controls food intake and body weight homeostasis (Friedman and Halaas, 1998). Plasma leptin concentrations correlate positively with the body fat content in humans and rodents (Maffei *et al.*, 1995; Friedman and Halaas, 1998) and with the BM of the European brown bear (*Ursus a. arctos*, Hissa *et al.*, 1998)

and the mink (*Mustela vison*, Tauson and Forsberg, 2002). However, during some phases of the seasonal cycles of the mink this correlation has been found to be absent (Mustonen *et al.*, 2000). Furthermore, plasma leptin levels are decoupled from body adiposity in many other carnivores, such as the raccoon dog (*Nyctereutes procyonoides*, Nieminen *et al.*, 2001, 2002a) and the Antarctic fur seal (*Arctocephalus gazella*, Arnould *et al.*, 2001), as well as in a chiropteran, the little brown bat (*Myotis lucifugus*, Kronfeld-Schor *et al.*, 2000), and a rodent, the Syrian hamster, (*Mesocricetus auratus*, Schneider *et al.*, 2000). Leptin inhibits and ghrelin stimulates appetite in male rats probably by modulating NPY signalling in the hypothalamus (Date *et al.*, 2000; Shintani *et al.*, 2001). Leptin also stimulates GH secretion by inhibiting the action of NPY on GH release (Vuagnat *et al.*, 1998).

The mink (*Mustela vison*, Schreber 1777) is a semi-aquatic carnivore (Stubbe, 1993). It was introduced for fur farming and released in many parts of Europe in the early 20th century. At present, the mink is common in most parts of Europe inhabiting forested areas in close proximity to water. Its diet usually includes small mammals, fish, amphibians, waterfowl, and aquatic invertebrates. Adult female minks are substantially smaller than males. Because

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the mink is a top predator, it is often used as an indicator species in environmental contaminant exposure and ecosystem health studies (Aulerich *et al.*, 1987). It has been also used in studies focusing on seasonal physiological adaptations of carnivores (Mustonen *et al.*, 2000).

Recently, it has been shown that there are interactions between ghrelin, leptin and GH in the raccoon dog (Nieminen *et al.*, 2002a). It has also been reported that the plasma leptin concentrations of the mink have seasonal variations (Mustonen *et al.*, 2000). The aim of the present study was to obtain basic knowledge of the average circulating concentrations of ghrelin, leptin and GH and their interactions in juvenile minks of both sexes. The results of this study can be used as reference values and starting points for future experiments.

MATERIALS AND METHODS

Sixty farmed juvenile male ($n=30$) and female ($n=30$) wild-type minks born in May 2002 were selected for the study. The animals were paired and housed in standard wire cages ($85 \times 31 \times 45$ cm) with wooden den boxes ($27 \times 31 \times 38$ cm). The cages were suspended above the ground in a shed at the Juankoski Research Station of the University of Kuopio (63°N ; 28°E). The animals were kept in natural photoperiod and temperature, but the effects of wind were absent. They had free access to water and food (4 355 kcal/kg dry matter; 35.9% protein, 48.5% fat, and 15.6% carbohydrate) was offered twice a day.

The animals were weighed and blood samples were taken at 9–12 o'clock on August 13th 2002 before the animals were fed. The samples were taken from a hind leg nail cut with scissors, collected to test tubes containing 5% EDTA and centrifuged at 3000 rpm for 10 min. The plasma samples were stored at -40°C . All the procedures conformed to the Helsinki Convention.

The plasma ghrelin levels were measured with the Ghrelin (Human) Radioimmunoassay (RIA) kit (Phoenix Pharmaceuticals, Belmont, CA, USA; intra- and interassay variations <5 and $<14\%$ CV, respectively). The plasma leptin levels were determined with the Multi-Species Leptin RIA kit (Linco Research, St Charles, MO, USA; 2.8–3.6 and 6.5–8.7% CV) and the plasma GH levels using the hGH Double Antibody Human Growth Hormone kit (Diagnostic Products Corporation, Los Angeles, CA, USA; 1.5–5.9 and 1.8–8.3% CV). The mink GH shares a 66% amino acid homology with the human GH (Shoji *et al.*, 1990), whereas the homologies between the human and mink leptin and ghrelin molecules are unknown. The plasma ghrelin, leptin and GH assays were validated such that serial dilutions of the mink plasma showed linear changes in B/B_0 values that were parallel with the standard curves produced with human standards (Fig. 1a–c). The plasma leptin assay has been also previously validated for the mink (Mustonen *et al.*, 2000; Tauson and Forsberg, 2002). A gamma counter (Wizard 1480, Wallac, Turku, Finland) was used for the actual measurements.

Statistical analysis was performed using the SPSS 11.0 software (SPSS Inc., Chicago, IL, USA). Differences between the sexes were determined using the Student's *t*-test. Correlations were calculated by the Spearman's Correlation Coefficient analysis. Results are presented as the mean \pm SE. $P < 0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION

The male minks had a higher mean BM than the

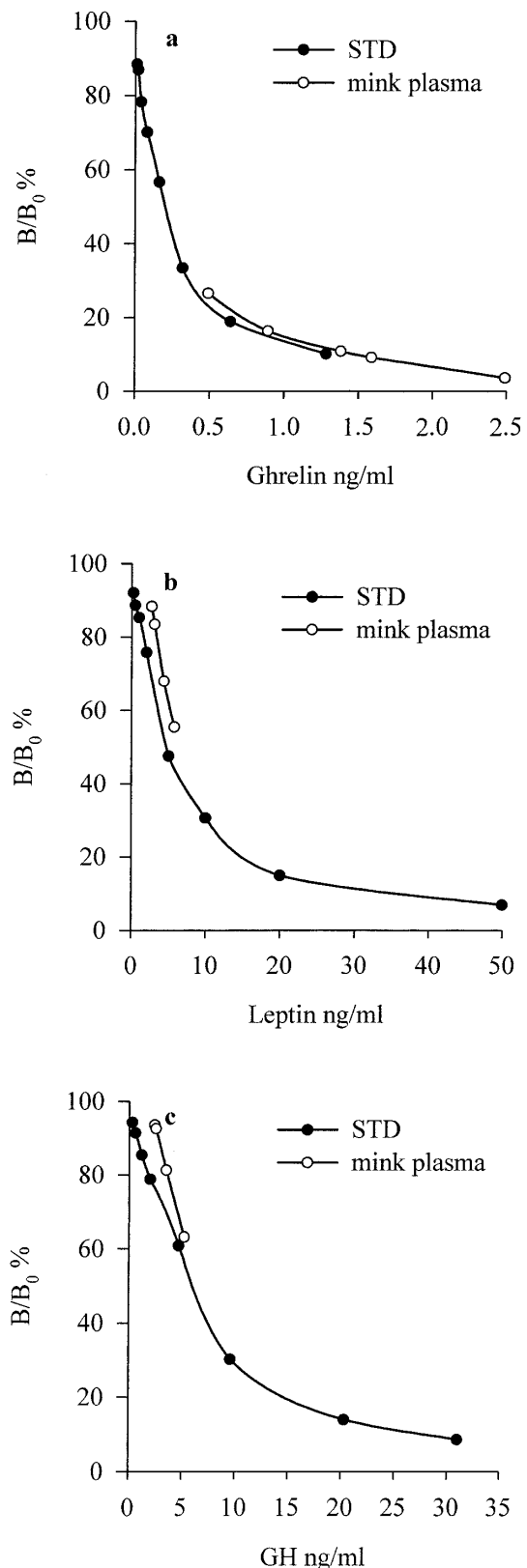


Fig. 1. Standard curves for ghrelin (a), leptin (b) and growth hormone (GH) (c) and the corresponding dose-response curves of serial dilutions of mink plasma (ghrelin=25, 50, 75, 100 and 200 μl , leptin=75, 100, 150 and 200 μl , and GH=100, 150, 200 and 300 μl ; B=sample or standard binding, B_0 =maximum binding).

Table 1. The body masses (BM) and the plasma ghrelin, leptin and growth hormone (GH) concentrations of the male and female minks (mean \pm SE).

| | BM* g | Ghrelin* ng/ml | Leptin ng/ml | GH* ng/ml |
|--------|---------------|-------------------|-----------------|-----------------|
| Female | 982 \pm 18 | 1.98 \pm 0.09 | 1.16 \pm 0.08 | 0.53 \pm 0.05 |
| Male | 1507 \pm 27 | 1.67 \pm 0.09 | 1.48 \pm 0.19 | 0.38 \pm 0.05 |

*Statistically significant difference between the sexes ($P < 0.05$).

females (1507 \pm 27 vs. 982 \pm 18 g, t -test, $P < 0.0004$, Table 1). On the contrary, the female minks had higher plasma ghrelin (1.98 \pm 0.09 vs. 1.67 \pm 0.09 ng/ml, t -test, $P = 0.013$) and GH levels (0.53 \pm 0.05 vs. 0.38 \pm 0.05 ng/ml, t -test, $P = 0.04$) than the males. There were no differences in the plasma leptin levels between the sexes (females 1.16 \pm 0.08 vs. males 1.48 \pm 0.19 ng/ml, t -test, $P = 0.134$).

The plasma ghrelin levels of the female minks correlated with their BMs ($r_s = 0.411$, $P = 0.024$, Fig. 2a). The BMs of the male minks correlated positively with their plasma leptin ($r_s = 0.424$, $P = 0.019$) and GH levels ($r_s = 0.493$, $P = 0.006$),

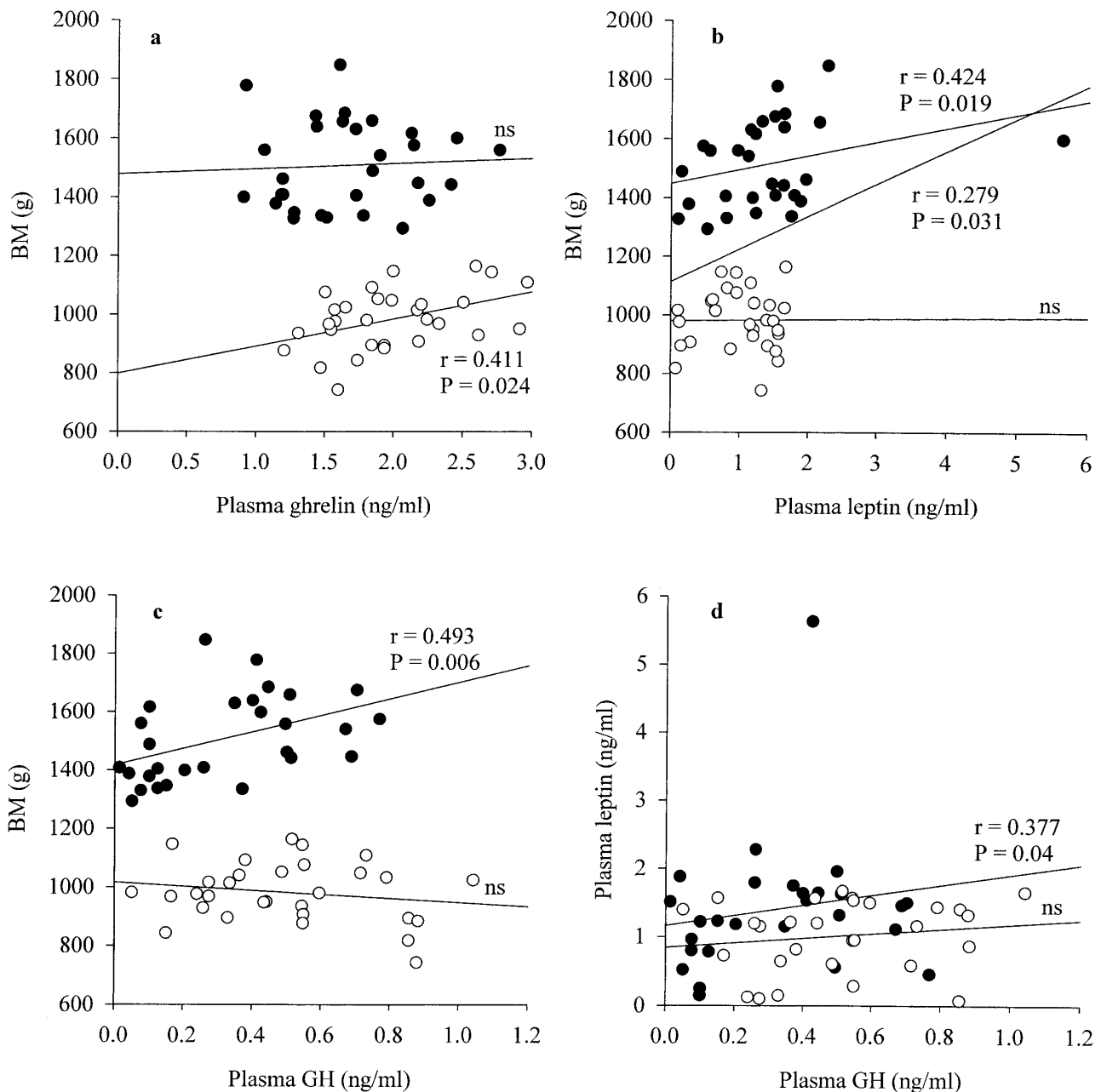


Fig. 2. Correlations between the body masses (BM) and the plasma ghrelin (a), leptin (b), growth hormone (GH) concentrations (c) or between the plasma leptin and GH levels (d) in the female (○) and the male (●) minks (ns=not significant).

but this was not observed in the females (Fig. 2b, c). The plasma leptin levels of all minks (females plus males) correlated positively with their BMs ($r_s=0.279$, $P=0.031$, Fig. 2b). There was also a positive correlation between the plasma leptin and GH levels in the male minks ($r_s=0.377$, $P=0.04$, Fig. 2d), but not in the females. The plasma ghrelin levels did not correlate with the GH or leptin concentrations (data not shown).

Ghrelin immunoreactivity could be detected and measured from mink plasma using a routine RIA method. The plasma ghrelin concentrations were quite similar to the levels measured from the raccoon dog (2.2–2.7 ng/ml in August, Nieminen *et al.*, 2002a) and the European polecat (*M. putorius*, 3–6 ng/ml, Nieminen *et al.*, 2002b) with the same ghrelin RIA kit. Similar values have been reported for rodents (mouse 2–5 ng/ml and rat 1.7–5.5 ng/ml, Tschöp *et al.*, 2000; rat 1.6 ng/ml, Mustonen *et al.*, 2001). However, the plasma ghrelin levels are lower in humans (0.2–0.8 ng/ml, Tschöp *et al.*, 2000; Barkan *et al.*, 2003). It has been previously observed that the plasma ghrelin levels show seasonal variations in the raccoon dog (Nieminen *et al.*, 2002a). Thus, seasonal changes in BM and appetite could partly explain the higher circulating ghrelin levels in carnivores such as the mink compared to humans.

Female minks had higher plasma ghrelin levels than the males. A similar difference between the sexes has been recently observed in the raccoon dog (Mustonen *et al.*, unpubl. data) and humans (Barkan *et al.*, 2003). In addition, the BMs of the female minks correlated positively with their plasma ghrelin concentrations. A similar positive correlation between body mass index and plasma ghrelin concentrations has been observed in the raccoon dog (Nieminen *et al.*, 2002a). These data are different from previous observations on humans and rats, in which the plasma ghrelin levels correlate negatively with the BMs (Tschöp *et al.*, 2001; Beck *et al.*, 2002). These opposite findings in different species require future study. The results of this study cannot totally explain the higher circulating ghrelin concentrations observed in the female minks. The clear sexual dimorphism in the BMs can, however, have an indirect influence on the plasma ghrelin levels. The lower BM of the females could be a cause for their higher circulating ghrelin concentrations and lead to their substantially higher relative food intake compared to the males (Mustonen, unpubl. data). This orexigenic effect of ghrelin has been described in rodents (Tschöp *et al.*, 2000).

The plasma leptin levels of the minks were, on the average (1.2–1.5 ng/ml), similar to previous results on minks obtained with the same multi-species RIA kit (September–November below detection limit–6.8 ng/ml, Mustonen *et al.*, 2000; February–April 1.4–5.5 ng/ml, Tauson and Forsberg, 2002). The observed differences compared to previous studies are probably due to seasonal fluctuations in the plasma leptin levels (see also Mustonen *et al.*, 2000). The plasma leptin levels of the minks were also quite similar to rats (2.8–3.0 ng/ml, Mustonen *et al.*, 2001) and some other

species of the order Carnivora such as the European brown bear (1–4 ng/ml, Hissa *et al.*, 1998), the blue fox (*Alopex lagopus*, 0.5–4.3 ng/ml, Nieminen *et al.*, 2001), the raccoon dog (0.5–2.6 ng/ml, Nieminen *et al.*, 2001), and the Antarctic fur seal (1.4–4.8 ng/ml, Arnould *et al.*, 2001) measured with the same multispecies RIA kit. In most cases, the leptin levels were lower than recorded in humans (8–53 ng/ml, Friedman and Halaas, 1998; Tschöp *et al.*, 2001) and rats (2–20 ng/ml, Beck *et al.*, 2002). This can be due to the fact that the multi-species leptin RIA kit is not specific to the leptin of the mink. Therefore, the absolute concentrations could be higher than the values measured in this study.

Plasma leptin levels correlate positively with BMs of humans and rodents (Maffei *et al.*, 1995) and of the European brown bear (Hissa *et al.*, 1998). Tauson and Forsberg (2002) observed previously in female minks ($n=6$) that there was a positive correlation between their BMs and plasma leptin levels. This could not be confirmed by the results of this study, as there was a positive correlation between the BMs and the plasma leptin levels in the male minks but not in the females. Women and female rodents have higher leptin concentrations than males for any given body fat content (Frederich *et al.*, 1995; Ma *et al.*, 1996). On the contrary, there were no differences in the plasma leptin levels between the male and female minks of this study. This finding is in concert with the data of Mustonen *et al.* (2000), who found no sexual dimorphism in the leptin concentrations of minks. Unlike previously observed in rodents (Shintani *et al.*, 2001), no antagonism could be detected between the plasma leptin and ghrelin concentrations in the mink. The mink is, however, in this regard similar to the raccoon dog — another member of the order Carnivora — as a negative correlation between the plasma leptin and ghrelin levels is absent in the raccoon dog, too (Nieminen *et al.*, 2002a).

The plasma GH concentrations of the minks were relatively low but similar to the levels observed earlier in juvenile raccoon dogs in August (0.1–0.3 ng/ml, Nieminen *et al.*, 2002a). However, it has been observed that there are great seasonal changes in the plasma GH levels in the raccoon dog and the lowest levels are measured in the early autumn and from the middle of November to the middle of December. In this study, the plasma GH levels were lower than measured in rats (2.4–2.8 ng/ml, Mustonen *et al.*, 2001) with the same GH kit, and in humans (women 1.86 ng/ml and men 1.08 ng/ml, Barkan *et al.*, 2003). The plasma GH levels were higher in the female minks than in the males similar to previous observations in humans (Barkan *et al.*, 2003). The GH concentrations correlated positively with the plasma leptin concentration in the male minks. This observation is in concordance with previous studies showing that leptin stimulates the GH secretion of mammals (Barb *et al.*, 1998; Vuagnat *et al.*, 1998). GH secretion is also increased by ghrelin (Kojima *et al.*, 1999), which may explain the higher GH concentrations in the female minks, who had higher plasma ghrelin levels, too. This finding supports recent observations in humans with higher ghrelin and GH concen-

trations in women compared to men (Barkan *et al.*, 2003).

In conclusion, this study demonstrated that ghrelin, leptin and GH could be measured from mink plasma using routine methods. The female minks had higher plasma ghrelin and GH levels than the males, but the plasma leptin levels did not differ between sexes. The plasma ghrelin concentrations of the females correlated positively with their BMs. In the male minks, both the plasma leptin and GH concentration correlated positively with their BMs. However, there was no correlation between the plasma ghrelin and GH or leptin concentrations.

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