Mechanism-Based Modeling of Nutritional and Leptin Influences on Growth in Normal and Type 2 Diabetic Rats

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Running title page
Running title: Modeling growth, food, fat, and leptin in diabetic rats

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List of nonstandard abbreviations used in the paper:
GK: Goto-Kakizaki rats, WKY: Wistar-Kyoto control rats, HF: high-fat diet, N: normal diet, T2DM: Type 2 diabetes mellitus, WT: total body weight, B, Bf c, cf: constants, LBM: lean body mass, AFat: abdominal fat mass, C_{Lept}: leptin concentration, k_{in_Food}: zero-order input constant for food.
All other abbreviations are explained in Tables 1 and 2.
Recommended section assignment: Endocrine and Diabetes
Abstract

Influences of genetic and nutritional factors on body weight, fat mass, and leptin production, and effects of leptin were assessed in normal (WKY) and diabetic (GK) rats by mechanism-based modeling. The study included 60 WKY and 60 GK rats: half received high-fat diet (HF), the others normal rat chow (N). Body weights and food consumption were measured twice weekly. Six rats per group were sacrificed at 4, 8, 12, 16, and 20 weeks. Abdominal fat was weighed and plasma leptin measured by ELISA. All data were co-modeled using NONMEM VI (FOCE-I). Weight gain was modeled as differences between energy intake and metabolic rate based on allometrically scaled lean body mass (LBM). The GK had higher metabolic rates (1.15 kcal/day/g LBM$^{0.75}$) than WKY-N (0.92) and WKY-HF (1.02) rats, and higher efficiency in transforming energy into body weight. Leptin effect was modeled as inhibition of food consumption. Total body fat was estimated from abdominal fat. Leptin production from fat was 4.7-fold higher for GK (3.03 ng/mL/day/g) than WKY (0.66 ng/mL/day/g). Leptin production rate from LBM was 0.53 ng/mL/day/g for all groups. The IC$_{50}$ for inhibition of food intake by leptin was about three-fold higher in GK vs. WKY, indicating leptin resistance for the effect on food consumption in GK. The GK had similar kcal intake, but lower body weights and fat mass than WKY, possibly due to higher metabolic rates. Our mechanism-based model explains intrinsic reasons for differences in growth, food intake and leptin concentrations among these two strains of rats.
Introduction

Type 2 diabetes (T2DM) is a major health risk in many countries and the number of diabetic patients and associated costs for health systems are increasing. More than 180 million people are diabetic worldwide and this number was estimated to more than double by 2030 (CDC, 2006). In 2005, an estimated 1.1 million people died from diabetes and such deaths were predicted to increase by more than 50% over the next 10 years (CDC, 2006).

T2DM is a chronic disease and characterization of the disease progression may provide useful insights for developing approaches to reduce such progression. Drugs may provide useful treatments. Many different animal models for diabetes are available. In contrast to human T2DM, a polygenic disease, many animal models involve single gene alterations such as the Zucker fatty rat, db/db mouse, and ob/ob mouse (Srinivasan and Ramarao, 2007). To test for disease-modifying drug effects, an animal model similar to human T2DM is preferable. Before studying drugs the natural disease progression needs to be investigated. The Goto Kakizaki (GK) rat is a polygenic model for spontaneous Type 2 diabetes, produced by repeated selective inbreeding of Wistar-Kyoto (WKY) rats with high glucose concentrations after oral glucose tolerance tests for more than 30 generations (Goto et al., 1988). Many of its characteristics are similar to human diabetes, although the GK is a lean rat model whereas most, but not all, T2DM patients are overweight. This study evaluates genetic and nutritional influences on disease progression in this animal model.
Adipose has long been viewed mainly as storage tissue. However, during the last decade the characteristic of adipose tissue as an endocrine tissue is becoming increasingly apparent. Fat cells secrete protein hormones called adipokines such as leptin and adiponectin. Leptin inhibits the appetite, stimulates metabolic rate, and increases insulin sensitivity. Leptin concentrations increase with increasing body weights, especially increasing fat mass. Leptin is produced mainly by adipose tissue, but also other tissues such as muscle, liver, and placenta. In addition to other actions, leptin decreases appetite and food intake by acting on the brain (Friedman and Halaas, 1998; Ahima and Flier, 2000). A direct inhibitory effect of leptin on lipogenesis in white adipose tissue has also been suggested (Swierczynski, 2006). Obesity can be viewed as a state of leptin resistance. Obese patients have high leptin concentrations but, despite this, an inadequate leptin effect similar to insulin resistance in T2DM patients. Possible mechanisms of the apparent leptin resistance in obesity that have been suggested are decreased activity of saturable leptin transport across the blood-brain-barrier leading to central leptin insufficiency and inhibition of the intracellular leptin receptor signaling cascade (Munzberg and Myers, 2005; Swierczynski, 2006; Kalra, 2008).

A causal relationship between consumption of energy-enriched diets, obesity, central leptin insufficiency and metabolic disorders such as T2DM has been proposed (Kalra, 2008). Wang et al. suggested that the degree of expression of genes regulating adipogenesis, such as leptin profoundly
influences onset and severity of diet-induced T2DM and that, contrary to general belief, obesity temporarily protects against T2DM (Wang et al., 2008).

Characterizing an animal model similar to human T2DM is valuable to better understand interactions between genetic and nutritional factors in diabetes and could support the development of new drugs that influence disease progression. For the development of T2DM both genetics and lifestyle factors, such as exercise and diet, are important. We aimed to explore both genetic and dietary influences and their interaction by comparing diabetic GK rats with WKY control rats and high-fat to normal diets. In the present report we explore growth and metabolic functions in diabetic and normal rats.

We sought to study the increase in body weight and fat mass dependent on food intake in rats, compare weight gains, fat mass and food consumption between normal (WKY) and diabetic rats (GK), build a population model, and explore reasons for possible intrinsic differences. A second aim was to assess the influence of high fat versus normal diet on weight and body composition of WKY and diabetic GK rats. Thirdly, we explored influences on leptin production and effects by population modeling, compared the progression of leptin concentrations in WKY and GK rats, and explored the influence of high fat diet. This report focuses on developing a model that can capture the relationships between the body weight, food consumption, abdominal fat, and leptin concentration. This model can be utilized to include other biomarkers and form the basis for modeling disease progression of T2DM in GK rats.
Methods

Animals: Sixty male Wistar-Kyoto rats (WKY) and 60 male Goto-Kakizaki rats (GK) were received from Taconic Farms (Germantown, NY) directly after weaning at 21 +/- 3 days of age. WKY rats were chosen for the control group as GK rats were originally produced by selective inbreeding of WKY with low glucose tolerance. During the study period, all rats were maintained on a strict 12 h light - 12 h dark cycle. All animal handling was performed within 1.5 to 3.5 h after the start of the light period. Body weights of all animals were measured twice weekly. The study was carried out in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the U.S. National Institutes of Health and approved by the University at Buffalo Institutional Animal Care and Use Committee.

Diets: Thirty WKY and 30 GK rats received normal rat chow (N, 2016 Harlan TekLad, Madison, WI) which included 4.2% soybean oil, 5% sugar, and had a digestible energy content of 3.3 kcal/g, with about 10% energy coming from fat. Thirty WKY and GK rats received a high fat diet (HF, TD.06415 Harlan TekLad, Madison, WI) which included 19.5% lard, 3% soybean oil, about 20% sucrose, and had an energy content of 4.6 kcal/g, with approximately 45% energy coming from fat. Animals were randomly assigned to the N or HF group and food was available ad libitum. The food was changed twice weekly and leftover food was weighed to assess food consumption.

Sacrifice: Six animals from each of the four groups (WKY-N, WKY-HF, GK-N, and GK-HF) were sacrificed at 4, 8, 12, 16, and 20 weeks of age. All
animals were anesthetized by intraperitoneal injection of 80 mg/kg ketamine and 5 mg/kg diazepam. Animals were sacrificed by aortic exsanguination using EDTA (4 mM final concentration) as anticoagulant. Blood was centrifuged at 2000 x g and 4 °C for 15 min. Abdominal fat and gastrocnemius muscle were harvested, weighed, and flash-frozen in liquid nitrogen. Plasma aliquots and tissue samples were stored at -80 °C until use. The data from one rat from the WKY-HF group were left out. This rat reached a 50 % higher body weight than the next largest rat and inclusion resulted in biased fittings. One of the rats delivered from the vendor was female and therefore left out of the analysis (WKY-HF group). Leptin concentration could not be obtained from one rat in the GK-HF group, which died before sacrifice.

**Leptin analysis:** Leptin (Kline et al., 1997) concentrations in plasma were determined by use of a commercially available ELISA kit (Rat Leptin TiterZyme EIA, Assay Designs, Ann Arbor, MI). Two experimental samples were selected as “quality control standards” to assess experimental variations between different runs. Such inter-assay variation was less than 10%.

**Data analysis:**

**Application of published growth models:**

Two previously published growth models were first utilized to model the total body weights (WT, g). The Weibull function (Weibull, 1951) in the parameterization by Maruyama et al. (Maruyama et al., 2001) is

\[
WT = WT_{max} - (WT_{max} - B) \cdot e^{-\left(\frac{c-1}{c}\right)\left(\frac{t}{IP}\right)^c}
\]

Equation 1
where $WT_{\text{max}}$ is the maximum body weight (g), IP is the inflection point of the curve (day), and B and c are constants, which are “not intuitively biologically interpretable” (Maruyama et al., 2001).

The model for ontogenetic growth by West et al. (West et al., 2001) is

$$\frac{dWT}{dt} = a \cdot WT^{0.75} \cdot \left(1 - \left(\frac{WT}{WT_{\text{max}}}ight)^{0.25}\right)$$

Equation 2

where $WT_{\text{max}}$ is the maximum body weight (g). The constant $a$ is defined as $B_0 \cdot m_c / E_c$ where $B_0$ is a constant for a given taxon, $m_c$ the cell mass, and $E_c$ the metabolic energy required to create a cell.

**Mechanism-based growth model:**

Our mechanism-based model includes food consumption, abdominal fat weights and leptin concentrations in addition to total body weights (Figure 1). This model for total body weight (WT) is derived from the basic principles of energy homeostasis. Food accounts for input of energy and allometric scaling of metabolic rate describes the utilization of energy.

$$\frac{dWT}{dt} = EF \cdot (\text{Food} - A \cdot (WT - \text{Fat})^{0.75} \cdot 20.65)$$

Equation 3

where Food is the food consumption per day (kcal/day). The initial condition of the differential equation is $WT(0)$, i.e. the weight at study day 0, which was estimated. Fat is an estimate of total body fat (g), and $(WT - \text{Fat})$ is an estimate for lean body mass (LBM). Metabolic rate is related to allometrically scaled LBM by the factor $A$, as derived from the formula by West et al. (West et al., 1997; West et al., 2003): $B = a \cdot WT^b$, where $B$ is the whole-organism metabolic rate,
WT is body mass, $b$ is 0.75, and $a$ is a taxon-dependent normalization. The factor $EF$ describes the efficiency of conversion of unmetabolized energy to body weight (g/kcal). The factor 20.65 comes from conversion of units between watts as used by West et al. and kcal/day in our model.

Total body fat ($Fat$, g) was estimated from abdominal fat, which was weighed after sacrifice, using an equation from Newby et al. (Newby et al., 1990).

$$Fat = AFat \cdot 7.96 + 3.13$$

Equation 4

where $AFat$ is abdominal fat mass (g) which was modeled by the Weibull function.

$$AFat = AFat_{\text{max}} - (AFat_{\text{max}} - Bf) \cdot e^{-\frac{c_f}{c_f} \cdot \left(\frac{t}{IP_{Fat}}\right)^{c_f}}$$

Equation 5

where $Bf$ and $c_f$ are constants and $IP_{Fat}$ is inflection point of the curve (day).

Food intake was described by an indirect response model (Dayneka et al., 1993) and is inhibited by leptin.

$$\frac{dFood}{dt} = k_{in\_Food} \cdot \left(1 - \frac{I_{\text{max}} \cdot C_{Lept}}{IC_{50} + C_{Lept}}\right) - k_{out\_Food} \cdot Food$$

Equation 6

where $k_{in\_Food} = k_{out\_Food} \cdot Food_{\text{max}}$. The initial condition is $Food(0)$, which was estimated. Leptin concentration is $C_{Lept}$ (ng/mL), $I_{\text{max}}$ describes the maximal inhibition of food intake, $IC_{50}$ (ng/mL) is the leptin concentration for half-maximal inhibition of food intake, and $k_{in\_Food}$ (kcal/day/day) and $k_{out\_Food}$ (1/day) are parameters for input and loss. The $Food_{\text{max}}$ is hypothetical maximum food intake at steady-state which would be reached in absence of leptin. Indirect response
models describe biomarker turnover by input and loss functions. The term
\[
\left(1 - \frac{I_{\text{max}} \cdot C_{\text{Lept}}}{IC_{50} + C_{\text{Lept}}} \right)
\]
describes the inhibitory effect of leptin in plasma on food intake.

Leptin concentrations are described by a turnover model where leptin production depends on both fat mass and lean body mass (LBM = WT - Fat).

\[
\frac{dC_{\text{Lept}}}{dt} = k_{\text{in1}_{\text{Lept}}} \cdot \text{Fat} + k_{\text{in2}_{\text{Lept}}} \cdot (\text{WT} - \text{Fat}) - k_{\text{out}_{\text{Lept}}} \cdot C_{\text{Lept}} \quad \text{Equation 7}
\]

where \(k_{\text{in1}_{\text{Lept}}}\) (ng/mL/day/g Fat) is the leptin production from fat, \(k_{\text{in2}_{\text{Lept}}}\) (ng/mL/day/g LBM) is leptin production from lean body mass, and \(k_{\text{out}_{\text{Lept}}}\) (1/day) is a parameter for loss of leptin. The initial condition is \(C_{\text{Lept}}(0)\), which was fixed to 16.1 ng/mL for WKY-N, 23.5 for WKY-HF, 18.4 for GK-N, and 28.0 for GK-HF.

**Parameter variability and observation model**

The between subject variability was described by an exponential parameter variability model. The unidentified residual variability was described by a combined additive and proportional error model for all independent variables.

**Model discrimination**

Competing models were distinguished by their predictive performance assessed via visual predictive checks, the NONMEM objective function, and residual plots. For the visual predictive check, we simulated profiles of 10,000 subjects for each of the four groups for competing models. From these data we calculated the median, the nonparametric 80% prediction interval (10% to 90% percentile), and the nonparametric 50% prediction interval (25% to 75% percentile) for the predicted body weights, food intake, abdominal fat, and leptin.
concentrations. These prediction interval lines were then overlaid on the original experimental measurements. If the model described the data adequately, then 20% of the observed data points should fall outside the 80% prediction interval and 50% of the data should fall outside the interquartile range. We compared the median predicted concentrations and the prediction intervals with the experimental measurements and tested whether such predictions mirrored the central tendency and the variability of the data for each model.

**Computation**

The first-order conditional estimation (FOCE) method with the interaction estimation option in NONMEM® version VI level 1.1 (NONMEM Project Group (Beal et al., 2007)) was used for all modeling and simulation. All data were modeled simultaneously. WinNonlin Professional Version 5.0.1 was used for ANOVA statistics.
Results:

Figures 2 to 5 present the individual observed data and the predictive performance of the model as assessed by visual predictive checks.

Body weight: Individual body weights over time for all groups are shown in Figure 2. Body weights increased steeply up to about 8 weeks of age and then started to approach a plateau. WKY rats reached higher body weights than GK rats and the high fat diet resulted in higher body weights for both strains. Median body weights at all time points after 8 weeks of age increased in the order of GK-N < GK-HF < WKY-N < WKY-HF.

Food consumption: Individual food consumptions over time are shown in Figure 3. Food consumption increased up to approximately 8 weeks in all groups and then approached a plateau. Until about 14 weeks of age, food consumption was similar among all groups. Subsequently, food consumption slightly decreased in WKY, whereas it stayed constant or slightly increased in GK.

Abdominal fat weight: Individual measurements of abdominal fat weights at sacrifice are shown in Figure 4. Abdominal fat increased until approximately 12 weeks of age in all groups. Thereafter it reached a plateau or increased slightly in GK, whereas it kept increasing in WKY. Abdominal fat was higher in WKY than GK and higher in HF than N.

Leptin concentration: Individual measurements of leptin concentrations at sacrifice are shown in Figure 5. Like abdominal fat, leptin concentrations increased in WKY throughout the course of the study, whereas in GK they
seemed to reach a plateau or only slightly increased after 12 weeks of age. For both strains, leptin concentrations were higher in HF than N.

**Population model**

**Growth models from literature**

Population parameter estimates and between subject variability for the Weibull and West models are listed in Table 1. For both models, estimated maximum body weights increased in the order GK-N < GK-HF < WKY-N < WKY-HF. In the Weibull model, the inflection point was similar for WKY and GK, and with the West model all groups could be described using the same constant a, which indicates a similar shape of the growth curves. Both models had good predictive performance as evaluated by visual predictive checks.

**Newly developed growth model**

The structure of our newly developed mechanism-based model is shown in Figure 1. Population parameter estimates and between subject variability are reported in Table 2. Visual predictive checks to assess predictive performance of the model are depicted in Figures 2 to 5.

**Body weight:** Body weights at the start of the study were similar for all groups. The efficiency of converting energy which is not needed for metabolism to body weight was slightly higher in GK-N than in GK-HF and WKY. Metabolic rate per g LBM$^{0.75}$ was higher in GK than in WKY. As LBM changed during the study, total metabolic rate also changed. The estimated LBM slightly decreased towards the end of the study period, i.e. the total body weight stayed about the same and fat mass still increased (Figures 2 and 4), therefore total metabolic rate
also decreased. The estimated LBM (WT - Fat difference) correlated well with gastrocnemius muscle weight measured in all rats at sacrifice ($r^2 = 0.83$ to 0.97 for the four groups). As the gastrocnemius is a large muscle in the rat and muscle tissue is a major part of LBM, we considered our estimate of LBM to be adequate. The metabolic rate per g LBM$^{0.75}$ was higher in GK than WKY. Bootstrapping the model without leptin, but with virtually identical parameter estimates as the full model yielded 90% confidence intervals for metabolic rate per g LBM$^{0.75}$: 0.85-0.96 for WKY-N, 0.91-1.06 for WKY-HF and 1.10-1.19 for GK. This suggests significantly higher metabolic rates in GK rats.

Body weights were higher in WKY than GK and higher in HF than N, despite similar calorie intake. We simulated from a previous model which did not include the leptin data but resulted in virtually identical parameter estimates for body weight as the full model. When assuming all groups had exactly the same calorie intake, the predicted body weights still increased in the order of GK-N < GK-HF < WKY-N < WKY-HF. Therefore different food intake was not the only reason for the different growth among the groups.

The model had highly sufficient predictive performance for the body weight growth curves for all groups. For WKY-HF and GK-HF, the variability was slightly overpredicted at the end of the study (Figure 2).

**Food consumption:** Energy intake at 3 weeks of age (start of the study) was higher in HF than N for both strains and was higher in WKY-N than GK-N. Maximum food intake without considering any leptin effect was estimated higher in WKY than GK. As shown in Figure 3, the model had good predictive
performance for food intake for all groups. The model predicts a slight decrease in food intake during the second half of the study for WKY rats, and a slight increase in GK-N rats, as seen in the experimental measurements. Food intake at the end of the study is slightly overpredicted in WKY rats and slightly underpredicted in GK-HF rats. The variability between rats was adequately predicted for all groups. With increasing leptin concentrations (Figure 5) in WKY after 12 weeks, food intake decreased. In contrast, leptin concentrations reached a plateau after 12 weeks in GK rats with ongoing slight increases in food consumption.

**Abdominal fat weight:** In agreement with the observed data shown in Figure 4, the estimated maximum abdominal fat was higher in WKY than GK and higher in HF than N. The inflection point of the curve was much later in WKY than GK, indicating that abdominal fat reached a plateau earlier in GK than in WKY, and the shapes of the curves were not influenced by the different diets within a strain. The model had good predictive performance for the central tendency and variability of the observed data.

**Leptin concentration:** Leptin production rate from adipose tissue was 4.7-fold (95% confidence interval from ANOVA: 4.1 - 5.4, p < 0.001) higher for GK compared to WKY. Leptin production from lean body mass was lower than from fat for both strains. The IC₅₀ for leptin action on food intake was higher in GK than WKY and higher in HF than N. This indicates that GK were leptin-resistant compared to WKY with regard to leptin action on food intake and HF
induced or increased leptin resistance in both strains. The observed leptin data were predicted well for all four groups (Figure 5).
Discussion

Disease progression in an animal model of polygenic diabetes and influences of HF versus N diet were assessed in GK and WKY rats.

The Weibull and West models could adequately fit the body weights, but underlying reasons for differences among groups were unclear. Our estimate for a in the West model was about 3-times higher than reported for rats, but closer to that reported for shrews (0.83) (West et al., 2001). Their rats likely were a different strain maintained under uncertain conditions and with a WT_max of 280 g considerably lighter than ours (WT_max 400-600 g). Also we did not have data before 3 weeks of age and predicted birth weight from this model were unrealistically low.

The West model describes ontogenetic growth over a large range of species and takes solely WT into account. In contrast, our model includes effects of food intake, fat mass and leptin and aims to explore the underlying relationships. Our model for body weight is based on fundamental principles of mass balance and energy conservation (Figure 1). We aimed to explore differences in weight, food consumption, and leptin production and effects among groups and study potential underlying reasons.

In equation 3 (weight) we first used WT^{0.75} as proposed by West et al. however this yielded inadequate fittings. Fat is less metabolically active than LBM and fat mass was considerably different among the groups. Therefore including an estimate of LBM markedly improved the fitting. The estimated % fat mass in WKY-N and GK-N was within the range of literature reports in Wistars
≤12 weeks (Iossa et al., 1999; Larsen et al., 2001; Venu et al., 2004; Buckley et al., 2005; Morel et al., 2005; Escriva et al., 2007; Movassat et al., 2008).

Our model assumes constant metabolic rate per g LBM\(^{0.75}\). Towards the end of the study, LBM in WKY slightly decreased and therefore absolute metabolic rate also decreased. This agrees with reports indicating decreasing metabolic rate with aging in humans can be explained almost completely by changes in body composition (Roberts and Rosenberg, 2006). GK rats gained less weight than WKY despite similar energy intake. Our model suggests this was due to a higher metabolic rate per g LBM\(^{0.75}\).

For the different groups EF was 0.30-0.51 g/kcal. If no energy was needed for transformation of ingested kcal to body weight, this would be equivalent to 2.0-3.3 kcal/g rat tissue. Total carcass energy was reported at 1,500 kcal for Wistar rats weighing 600 g (Newby et al., 1990), corresponding to an energy content of approximately 2.5 kcal/g, similar to our EF. This interpretation of EF is simplified, but suggests our EF estimates are reasonable.

Our model assumes that all energy from food is absorbed. Wistar-CFT rats on a diet including 20% oil, absorbed 90-95% of dietary fat (Purushothama et al., 2003). GK rats on a 5% fat diet, had 86-89% fat absorption (Rubino et al., 2006). These data suggest that differences in fat absorption between GK and WKY might be small, and assuming the same extent of absorption among all groups seems reasonable.

When exploring differences in leptin production and effects among the groups, leptin production per g fat was estimated to be higher than per g LBM.
This agrees with literature reports stating that leptin is mainly produced in adipose tissue (Zhang et al., 1994; Ahima and Flier, 2000), but also in muscle (Wang et al., 1998), liver, and stomach (Bado et al., 1998; Cinti et al., 2000). . .

Our model suggests considerably higher leptin production per g fat in GK compared to WKY, potentially due to leptin resistance in GK as indicated by the higher IC₅₀. Rats on HF diet had higher absolute leptin concentrations than N due to more body fat. The IC₅₀ describes the potency of leptin to inhibit food intake, or the concentration required to reach half-maximal effect. The higher IC₅₀ in GK compared to WKY (Table 2) means therefore that a higher concentration of leptin is required in GK compared to WKY to have the same magnitude of inhibitory effect on food consumption. This is expressed by the term leptin resistance. Therefore leptin resistance in GK rats relative to WKY and in HF rats relative to N may be concluded. Leptin resistance was reported in obese humans (Gale et al., 2004) and rats (El-Haschimi et al., 2000; Dyck et al., 2006). Our model suggests increasing leptin resistance in the order of WKY-N < WKY-HF < GK-N < GK-HF, where the strain had more influence than diet.

Apparent leptin resistance might be due to impaired leptin transport across the blood-brain-barrier which decreases with age and results in central leptin insufficiency. Leptin insufficiency or resistance can cause increased fat deposits and T2DM by increasing food intake, decreasing energy expenditure, decreasing peripheral glucose metabolism, and causing hyperinsulinemia (Kalra, 2008). The latter two factors might influence the metabolic state of our GK rats which displayed apparent leptin resistance, increased leptin, glucose, and insulin.
concentrations (data not shown) compared to the control group, despite being non-obese and non-overeating. By including equation (7) in the model, differences in leptin production among the groups could be described by differences in fat mass and LBM. Differences in food intake could be explained by the different leptin concentrations and potencies of leptin to inhibit food intake (IC50).

The difference in leptin concentrations between N and HF is more pronounced in GK than WKY, as the ratio of $\text{kin}_{1,\text{Lept}}$ to $\text{kin}_{2,\text{Lept}}$ is much larger in GK than WKY. Therefore the model suggests that leptin production from fat tissue relative to LBM is larger in GK compared to WKY. Due to greater abdominal fat mass in GK-HF than GK-N, leptin concentrations are higher in GK-HF than GK-N. The increase in leptin concentrations over time is more pronounced in GK-HF than GK-N, as abdominal fat increases over time in GK-HF, whereas it almost reaches a plateau in GK-N. As most leptin is produced in adipose tissue in the GK rats, the time course of leptin concentrations mirrors the time course of abdominal fat weight.

The turnover components in the leptin equation do not reflect production and loss of leptin, but physiological changes over a longer time period, similar to turnover models for describing disease progression (Post et al., 2005). Therefore leptin $k_{\text{out}}$ does not reflect its plasma half-life.

Plasma leptin in the present study was higher than in most previous literature reports. Results were confirmed by repeat-assay of several samples. However the relative changes over time and differences among the groups agree
well with expectations based on previous reports and could be described by modeling based on the underlying physiology. If the measurements were biased, e.g. being a factor 2 too high, the model could accommodate this. Initial conditions for leptin, $k_{in1\_Lept}$, $k_{in2\_Lept}$, and $IC_{50}$ would be half of current estimates and all other parameter estimates remain the same.

Differences in time course of food intake among the groups were explored and could be explained by the different leptin concentrations and potencies. The estimates for $Food_{max}$ suggest higher food intake in WKY than GK without any leptin effect. This is difficult to interpret as there was no group without leptin, an unphysiological situation. The turnover model described the increasing food intake over time due to increasing body weight and incorporated effects of leptin on food intake. This flexible model allowed leptin effects to be readily included. Other growth models were considered, but more parameters would be necessary to describe food intake for all groups.

Our model describes development of food intake, fat mass and leptin concentrations with age in relation to body weight in normal and diabetic rats. As the model is derived from basic principles and physiology it can be applied to other rat strains and, perhaps a modified version, also to humans and be used for translational research. Drug effects on leptin and food intake may be incorporated, such as down-regulation of the leptin gene by rosiglitazone (Kallen and Lazar, 1996; Seda et al., 2008). Increasing leptin availability in the hypothalamus by gene therapy enforced euglycemia in mice due to increased glucose metabolism throughout 7 weeks despite persisting severe insulinopenia.
(Ueno et al., 2006). The authors propose designing new leptin mimetics and central leptin delivery approaches for use in humans as a promising therapeutic approach for T2DM (Kalra, 2008). Such efforts might be supported by our model which can be expanded to include other leptin effects.

In summary, body weight, food intake, and leptin production and effects were integrated in a mechanism-based model. Lower body weights and fat mass in GK versus WKY despite similar food intake were ascribed to a higher metabolic rate in GK rats. Leptin is produced mainly by adipose tissue and higher leptin concentrations in rats on HF diet were explained by their higher fat mass. Continuously increasing leptin concentrations possibly caused decreasing food intake in WKY. Modeling suggested occurrence of leptin resistance in GK vs. WKY and HF vs. N. This model could be beneficial in designing pharmacological studies in GK rats.
References:


Footnotes

a) Unnumbered footnotes:

Footnote to the title:

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Legends for figures:

Figure 1: Model diagram for weight, food intake, abdominal fat, and leptin all modeled simultaneously. Symbols are also defined in the text and Table 2.

EF: Efficiency to convert energy which is not needed for metabolism to body weight (g/kcal), Food: Food intake (kcal/day), WT: total body weight (g), A · 20.65: Metabolic rate per LBM^{0.75} (kcal/d/gLBM^{0.75}), LBM: Lean body mass (g), \(k_{in2 \_Lept}\): Leptin production from lean body mass, \(k_{in1 \_Lept}\): Leptin production from adipose tissue (ng/mL/day/g), Fat: Total body fat (g), AFat: Abdominal fat mass (g), \(C_{Lept}\): Leptin concentration in plasma (ng/mL), \(k_{out \_Lept}\): Loss parameter for leptin (day^{-1}), \(I_{max}\): Maximum inhibition of food intake by leptin (-), \(IC_{50}\): Leptin concentration which results in half-maximal inhibition of food intake (ng/mL), \(k_{in \_Food}\) = \(k_{out \_Food}\) · \(Food_{max}\), input parameter for food (kcal/day/day), \(k_{out \_Food}\): loss parameter for food (day^{-1}), Weibull function: see Equation 5, closed bar: inhibition of food intake by leptin.

Figure 2: Visual predictive check for total body weights (g) versus age for the four groups of rats

Diamonds are experimental measurements and lines are the 10% (broken line), 25% (dotted line), 50% (solid line), 75% (dotted line), and 90% (broken line) percentiles of the prediction interval. For the visual predictive check, we simulated profiles of 10,000 subjects for each of the four groups for competing models. From these data we calculated the median, the nonparametric 80% prediction interval (10% to 90% percentile), and the nonparametric 50%
prediction interval (25% to 75% percentile) for the predicted body weights, food intake, abdominal fat, and leptin concentrations. These prediction interval lines were then over-layed on the original experimental measurements. If the model described the data adequately, then 20% of the observed data points should fall outside the 80% prediction interval and 50% of the data should fall outside the interquartile range.

Figure 3: Visual predictive check for Food intake (kcal/day) versus age for the four groups of rats. Symbols and lines as defined in Figure 2

Figure 4: Visual predictive check for abdominal fat weight (g) versus age for the four groups of rats. Symbols and lines as defined in Figure 2

Figure 5: Visual predictive check for leptin plasma concentrations (ng/mL) versus age for the four groups of rats. Symbols and lines as defined in Figure 2
Table 1: Estimates from Weibull and West growth models

<table>
<thead>
<tr>
<th>Group</th>
<th>Parameter (unit)</th>
<th>Definition</th>
<th>Estimate</th>
<th>BSV (%)</th>
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<tbody>
<tr>
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<td>West model</td>
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<td>WKY-N</td>
<td>$WT_{\text{max}}$ (g)</td>
<td>Maximum body weight</td>
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<td>Maximum body weight</td>
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<td>Proportional error for body weight</td>
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<td>ALL</td>
<td>SD$_{WT}$ (g)</td>
<td>Additive error for body weight</td>
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<td>Weibull model</td>
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<td>WKY-N</td>
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<td>GK-N</td>
<td>IP$_{WT}$ (days)</td>
<td>Inflection point of body weight curve</td>
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<tr>
<td>ALL</td>
<td>CV$_{WT}$ (%)</td>
<td>Proportional error for body weight</td>
<td>2.3</td>
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<tr>
<td>ALL</td>
<td>SD$_{WT}$ (g)</td>
<td>Additive error for body weight</td>
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Table 2  Population parameter estimates for the mechanism-based growth model

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<th>Group</th>
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<th>Definition</th>
<th>Estimate</th>
<th>BSV (%)</th>
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<td>GK-HF</td>
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<td>WKY</td>
<td>kout_Food (day^-1) Loss parameter for food</td>
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<td>GK</td>
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<td><strong>Body weight</strong></td>
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<td>WT(0) (g)</td>
<td>Body weight at start of study</td>
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### Metabolic Rate per LBM^{0.75}

<table>
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### Abdominal Fat

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<tr>
<th>Group</th>
<th>AF_{\text{max}} (g)</th>
<th>Maximum abdominal fat mass</th>
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### Proportional and Additive Errors

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<th>SDWT (g)</th>
<th>Additive error for body weight</th>
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### Proportional and Additive Errors for Abdominal Fat

<table>
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<tr>
<th>Group</th>
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<th>SDAFAT (g)</th>
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Note: This table represents data from a scientific study, potentially involving metabolic rate and abdominal fat mass measurements in different groups. The values and formulas indicate specific calculations and error metrics associated with these measurements.
<table>
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<tr>
<th></th>
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<th>Leptin production from adipose tissue</th>
<th>Leptin production from lean body mass</th>
<th>Maximum inhibition of food intake by leptin</th>
<th>IC\textsubscript{50} (ng/mL)</th>
<th>half-maximal inhibition of food intake</th>
<th>Proportional error for leptin concentration</th>
<th>Additive error for leptin concentration</th>
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<td>GK</td>
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<td>ALL</td>
<td>( l_{\text{max}} (-) )</td>
<td>Maximum inhibition of food intake by leptin</td>
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<tr>
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<td>Maximum inhibition of food intake by leptin</td>
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<td>( CV_{\text{Lept}} ) (%)</td>
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Figure 2

Total body weight (g) vs Age (weeks) for WKY-N, WKY-HF, GK-N, and GK-HF.