

1 Developmental variations in plasma leptin, leptin soluble receptor and their molar ratio in
2 healthy infants

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10 WK participated in design and execution of the study, analysis and interpretation of the
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1 Abstract

2 Background: Leptin and its soluble receptor (sOB-R) are important to regulation of body
3 composition but there is no data on the developmental variations in these plasma
4 variables and their relationship with body composition measurements,

5 Methods: Weight, length, and body composition (bone, fat and lean mass) by dual energy
6 absorptiometry, and plasma variables were measured in healthy infants at 2, 4, 8 and 12
7 months.

8 Results: 15 whites and 29 African Americans (21 males and 23 females) with mean birth
9 weight 3357 +/- 45 (SEM) g and gestation of 39.3 +/- 0.17 weeks were studied. The
10 overall Z score for weight, length and weight for length during the study were 0.00 +/-
11 0.15, -0.08 +/- 0.11 and 0.12 +/- 0.14 respectively. With increasing age, plasma leptin
12 (1.0 to 18.2, median 5.5 ng/mL) and sOB-R:leptin molar ratio (10.1 to 247.4, median
13 59.9) were lowered ($r = -0.47, p < 0.01$; and $r = -0.37, p < 0.05$ respectively), best predicted
14 by weight Z scores, and higher in African American and female. Fat mass as a
15 percentage of total mass was predictive for plasma leptin and sOB-R:leptin ratio and the
16 presence of body composition measurements eliminated the race and gender effect on the
17 plasma variables. Plasma sOB-R (49.5 to 173.9, median 81.3 ng/mL) did not change
18 significantly with age and was correlated and predicted only by body composition
19 measurements.

20 Conclusions: In healthy growing infants, plasma leptin but not sOB-R decreases with age.
21 Gender, race and anthropometric measurements are additional physiological determinants
22 and predictive of plasma leptin and the receptor:ligand ratio. However, body
23 composition is the only variable that can predict plasma leptin and its soluble receptor

1 and the receptor:ligand ratio, and body composition measurements eliminate the race and
2 gender effect on these plasma variables. Awareness of these developmental variations
3 and measurement of body composition are critical to delineate the interplay of leptin and
4 its receptors in the regulation of growth and tissue accretion under normal and pathologic
5 states.
6

1 **Background**

2 The adipocyte hormone, leptin, is the protein product of the obesity (ob) gene.
3 Circulating leptin concentrations reflect the amount of energy stored in adipose tissue and
4 is considered a marker of nutritional status [1,2]. Leptin is bound to the soluble leptin
5 receptor (sOB-R) in the circulation, which modulates steady state leptin levels by
6 preventing the hormone from degradation and clearance [3], and sOB-R:leptin ratio may
7 be considered a marker of bioavailable leptin. However, dramatic changes in circulating
8 leptin and sOB-R occurs in the neonate with a consistent weight independent decrease in
9 plasma leptin concentration, by as much as 80% [4-6] and increase in sOB-R by >5 folds
10 [6] between delivery and first few days after birth. Further decrease in plasma leptin
11 concentration by almost 90% between birth and 1 year [7] has been reported, although
12 changes in plasma sOB-R in infants beyond the immediate newborn period are not well
13 defined.

14 At birth, weight and body mass index are positively correlated with plasma leptin
15 concentrations [4,5,8], and one report indicated that sOB-R is inversely related to birth
16 weight [6]. During infancy, data on the relationship of plasma leptin and sOB-R to
17 growth is limited with no information on the relation of these plasma variables on body
18 composition. The aim of this study is to define the developmental variations in circulating
19 leptin and sOB-R in healthy infants specifically the physiologic determinants including
20 age, gender, race, measures of growth or body composition on these plasma variables.

21

1 **Methods**

2 This is a cross sectional design with anthropometry, body composition and
3 circulating leptin and sOB-R measurements performed on the same day for each subject.
4 All subjects were singleton infants between 37 and 42 weeks gestation with appropriate
5 birth weight for gestation [9] and studied at approximately 2, 4, 8 and 12 months. None
6 of the infants had major malformation or medical or surgical conditions that may affect
7 long term growth.

8 Weight, length, and head circumference were measured using standard methods
9 [10,11]. Infants were weighed in the nude to the nearest 5 g using an electronic scale
10 (Seca, Toledo, OH) that was calibrated daily. Length was measured in duplicate to the
11 nearest 0.1 cm with the infant in a recumbent position using O'Leary Lengthboard TM
12 (Ellard Instruments Ltd, Seattle, WA).

13 Body composition is indicated by total body bone mass as bone mineral content,
14 and fat and lean mass were determined by fan beam dual energy X-ray absorptiometry
15 (DXA) (QDR 4500A, Hologic Inc, Waltham, MA). Scan acquisition techniques have
16 been reported elsewhere [12]. Each infant was wrapped in cotton blanket for the scan.
17 The use of a diaper with or without light undergarment for the infant was allowed prior to
18 bundling the infant in the cotton blanket. However, all coverings were weighed with an
19 electronic scale and the weight recorded. Scan analysis used software vKHS11 validated
20 by carcass analysis [13,14]. Only scans with no significant movement artifacts [15] were
21 included in data analysis. In our laboratory, the precision error [16] from duplicate infant
22 whole body scans for bone, fat and lean mass were 2.6, 7.1 and 2.5% respectively. Bone,
23 fat and lean mass also were expressed as a percent of DXA measured total body mass.

1 Plasma samples for the measurement of leptin and sOB-R were kept at -70° C
2 until measurement. Plasma leptin and sOB-R were measured using the commercial
3 enzyme linked immunoassay kits from the same manufacturer (Diagnostic Systems
4 Laboratories, Inc., Webster, TX). Recombinant human leptin and human soluble leptin
5 receptor were used as standards and controls in the respective assays. In our laboratory,
6 the coefficient variation of the leptin assay was 9% and for the sOB-R assay was 5%.

7 Ethical approval for the study obtained from the Institutional Review Board for
8 Human Investigations at Wayne State University, Detroit, MI. Written informed consent
9 was obtained from the parent of each infant.

10 Statistical analysis

11 The absolute values of anthropometric measurements were normalized by
12 expression as Z scores using the age and gender matched normative data from the
13 National Center for Health Statistics [17]. The absolute values for bone, fat and lean
14 mass were transformed to a percentage of total weight. Plasma sOB-R:leptin molar ratio
15 was calculated according to the molecular mass of 130 kD for sOB-R and 16 kD for
16 leptin. All statistical analysis included the use of absolute and transformed
17 measurements.

18 Pearson's correlation was used to determine the relationship for each plasma
19 parameter (leptin, sOB-R and sOB-R:leptin ratio) with each anthropometric and each
20 DXA parameters. Analysis of covariance was used to determine the relation of gender,
21 race and age to plasma measurements. Stepwise regression analysis was used to
22 determine the relative contribution from each of the physiologic variables in the
23 prediction of plasma leptin, sOB-R, and sOB-R:leptin ratio. By design, the use of

1 absolute and percentage of body composition measurement was mutually exclusive in
2 regression analysis, as well as the use of anthropometric and DXA measurements, since
3 body weight is the sum of body composition measurements; and both weight and length
4 are predictive of various aspects of body composition [18-20]. Neither age nor gender
5 was entered as independent variables with any analysis using Z scores since the Z scores
6 were standardized to age and gender.

7 All values are mean +/- SEM. Statistical tests were performed with SPSS Version
8 13.5 for Windows (SPSS Inc., Chicago, IL) at an adopted significance level of 0.05 and
9 were two-tailed.

10

1 Results

2 There were 44 infants with mean birth weight 3357 +/- 45g and gestation of 39.3
3 +/- 0.17 weeks, with 15 whites and 29 African Americans, and 21 males and 23 females.
4 Anthropometric and DXA measurements and blood collection were performed at 56 +/-
5 0.8, 112 +/- 1.0, 240 +/- 1.5, and 366 +/- 2.7 days. Age was positively correlated with all
6 absolute values of anthropometric and DXA measurements ($p < 0.01$ for all comparisons).
7 Weight for age Z score (WAZ) decreased with age ($r = -0.36$, $p = 0.02$) but recumbent
8 length for age (HAZ) and weight for length (WHZ) Z scores were not significantly
9 different with age. The overall WAZ, HAZ and WHZ were 0.00 +/- 0.15 and -0.08 +/-
10 0.11, and 0.12 +/- 0.14 respectively. Bone mass as a percentage of total mass was
11 increased with age ($r = 0.58$, $p < 0.01$) but the percentages of fat and lean mass did not
12 change significantly with age. The overall percentages for bone, fat and lean mass were
13 2.5 +/- 0.04, 26.3 +/- 0.92, and 71.2 +/- 0.93 respectively.

14 Plasma leptin concentrations varied from 1.0 to 18.2 (median 5.5) ng/mL and
15 decreased with age ($r = -0.47$, $p < 0.01$) (**Fig 1**). The relationships between plasma leptin
16 concentrations and anthropometric measurements are shown in **Table 1**. Z scores were
17 better correlated with plasma leptin than absolute measurements. The relationships
18 between plasma leptin concentrations and body composition measurements are shown in
19 **Table 2**. Body compositions as percentage of total weight were better correlated with
20 plasma leptin than absolute measurements. Percent fat mass was positively correlated
21 with plasma leptin although both absolute and percent bone and lean masses were
22 negatively correlated with plasma leptin. African American infants had higher plasma
23 leptin concentrations ($p < 0.05$) after adjustment for age at study. There was no race and

1 gender interaction effect on plasma leptin. Neither race nor gender affected absolute or
2 percent fat mass.

3 Plasma sOB-R concentrations varied from 49.5 to 173.9 (median 81.3) ng/mL,
4 did not change significantly with age (**Fig 2**) and were not significantly correlated with
5 plasma leptin concentrations. There was no correlation between plasma sOB-R with any
6 anthropometric measurements (Table 1). Plasma sOB-R was positively correlated with
7 fat mass and negatively correlated with percent lean mass (Table 2). Plasma sOB-R was
8 not affected by race or gender.

9 Plasma sOB-R:leptin molar ratio varied from 10.1 to 247.4 (median 59.9) and
10 decreased with age ($r = -0.37$, $p = 0.05$). Weight and WAZ were the only anthropometric
11 variables significantly correlated with plasma sOB-R:leptin ratio. Whereas, plasma sOB-
12 R:leptin ratio was positively correlated with absolute and percent fat mass, but negatively
13 correlated with lean mass and percent lean and bone mass. The relationship was stronger
14 based on percent lean or fat mass than the absolute mass (Table 2). There was no race or
15 gender effect on plasma sOB-R:leptin ratio.

16 Weight or WAZ had positive and length or HAZ had negative predictive effect on
17 plasma leptin. Females have higher plasma leptin concentrations and sOB-R:leptin ratio
18 compared to males. None of the physiologic variables entered into analysis with
19 anthropometric measurements were predictive for plasma soluble receptor concentration
20 (**Table 3**).

21 The absolute or percent fat mass was consistently predictive of plasma leptin,
22 sOB-R and sOB-R:leptin molar ratio (**Table 4**). Percentage of body composition
23 component was slightly better than the absolute values in the prediction of the plasma

1 variables. Age was predictive of plasma leptin and sOB-R:leptin ratio. Presence of body
2 composition measurements eliminated any race or gender effect on plasma sOB-R and
3 sOB-R:leptin ratio.
4

1 **Discussion**

2 To our knowledge, this is the first report of the relationship of the circulating
3 leptin and sOB-R concentrations to various physiological variables of growth and body
4 composition specifically, bone, fat and lean masses, during infancy. In this study, the
5 measurement of circulating leptin and its soluble receptors after the neonatal period
6 probably better reflect the physiological status during infancy since it eliminates the
7 consideration of placental leptin contribution [21] to the dramatic weight independent
8 changes in these variables in the neonate.

9 After the initial dramatic decrease in plasma leptin concentrations after birth,
10 some investigators reported an increase during the first few months [22,23] but not by
11 others [7]. It is possible that the apparently transient increase during the early postnatal
12 period may not be detected depending on the blood sampling interval. One report of
13 plasma leptin concentrations in healthy infants show a progressive decrease from the cord
14 level until one year and the plasma leptin concentrations were found to discriminate both
15 long term and changes in energy status [7], although the infants were from Gambia and
16 had grown relatively poorly beyond the first two months when compared to Western
17 standards with average weight for age Z score of -1.9 at 52 weeks. Our data indicated
18 that similar decrease in plasma leptin concentrations occur in healthy infants with normal
19 age and gender specific weight and length Z scores.

20 In this study, the relationship between anthropometric measurements and plasma
21 leptin and sOB-R was better with Z scores compared to the absolute values. Our data is
22 also consistent with previous reports in infants showing the correlation between plasma
23 leptin with actual [23] or gain [22] in body weight; and actual [7,8] and changes [7] in

1 body mass index. The better correlation between fat percent rather than absolute fat mass
2 to plasma variables parallels our findings with anthropometric measurements. These data
3 would support that standardized measurements of anthropometric and body composition
4 would be preferable than absolute values in determining the relationship and physiologic
5 actions of leptin and its receptor.

6 Our data is consistent with the report that subcutaneous skinfold thicknesses are
7 correlated with plasma leptin [6,22,23] and that adipose tissue is the major source of
8 circulating leptin. The negative correlation of percent bone mass and percent lean mass
9 with plasma leptin is not surprising since an increase in the proportion of fat mass is
10 generally correlated with decreased proportion of lean and bone mass. However, a direct
11 relation between plasma leptin and other tissue mass may be possible since increasing
12 numbers of non-adipose tissues including skeletal muscle [24], chondrocyte [25] and
13 human osteoblast [26] are reported to synthesize leptin and may have cellular leptin
14 receptor forms with physiological activity in experimental models. However, the exact
15 role of leptin in the changes in skeletal muscle and bone in humans remain to be defined.

16 Our findings of higher leptin concentration in African American infants
17 independent of fat mass is consistent with the report on adult males and females that non-
18 Hispanic blacks have slightly higher values compared to non-Hispanic whites or Mexican
19 Americans [27]. Our data also are consistent with the presence of sexual dimorphism
20 with higher plasma leptin concentration in female infants [7,28]. However, our data
21 show that race and gender effects were eliminated in the presence of body composition
22 measurements, presumably body composition measurements more specifically reflect the
23 source of leptin and its receptors.

1 The soluble leptin receptor (sOB-R) represents the main leptin-binding activity in
2 human blood [29] and this protein potentially modulates steady-state leptin levels by
3 complexing to free leptin in the circulation. Our data indicated that plasma sOB-R
4 remained high throughout most of the infancy and persists during earlier childhood [30].
5 Our data indicate that variations in plasma sOB-R are independent of race, gender or age
6 but are predicted by body composition measurements. The positive relation between
7 plasma sOB-R and fat mass may be indicative of the increased membrane-bound leptin
8 receptor forms, the source of sOB-R. The negative correlation of percent lean mass with
9 plasma sOB-R is consistent with the generally inverse relation between percent lean and
10 fat mass.

11 Complexes of leptin with sOB-R reflect a molecular ratio of 1:1. There is
12 progressive increase in plasma sOB-R:leptin ratio as a result of a significant decrease in
13 leptin and simultaneous increase in sOB-R during the first 5 days after birth. The median
14 value for plasma sOB-R:leptin ratio is >10 by day 5 [6]. Our data indicated that plasma
15 sOB-R:leptin ratio remained relatively high throughout infancy and extend into early
16 childhood [30]. It is possible that high circulating concentrations of sOB-R may block
17 leptin function by its competition with the membrane receptor for the ligand, which in
18 turn may be an important stimulus for energy uptake in the rapidly growing infant or in
19 other conditions with a high energy demand. However, the decreasing sOB-R:leptin ratio
20 during later infancy is presumably associated with increasing bioavailable leptin, and is
21 consistent with slowing of growth [17] and tissue accretion [18-20].

22

1 **Conclusions**

2 We conclude that in healthy growing infants, plasma leptin but not sOB-R
3 decreases with age. Gender, race and anthropometric measurements are additional
4 physiological determinants and predictive of plasma leptin and the receptor:ligand ratio.
5 However, body composition is the only variable that can predict plasma leptin and its
6 soluble receptor and the receptor:ligand ratio, and body composition measurements
7 eliminate the race and gender effect on these plasma variables. Awareness of these
8 developmental variations and measurement of body composition are critical to delineate
9 the interplay of leptin and its receptors in the regulation of growth and tissue accretion
10 under normal and pathologic states.

11

1 **Competing interests:** None of the authors had any conflict of interest

2 **Authors' contributions:**

3 WK participated in design and execution of the study, analysis and interpretation of the
4 data, and completion of the manuscript. MH participated in execution of the study,
5 interpretation of the data, and manuscript writing. EH participated in statistical analysis,
6 interpretation of the data and manuscript writing.

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23

1 Figure 1. Variation in plasma leptin concentrations with age in healthy infants

2

- 1 Figure 2. Variation in plasma leptin soluble receptor (sOB-R) concentrations with age in
- 2 healthy infants
- 3

- 1 Table 1. Correlation of plasma leptin, leptin soluble receptor (sOB-R), and sOB-
 2 R:Leptin ratio with anthropometric measurements and their Z scores in term infants

	Leptin ng/mL	Leptin soluble receptor ng/mL	sOB-R:Leptin molar ratio
Absolute measurements			
Weight	-0.29	0.29	-0.14
Length	-0.44*	0.23	-0.30
Weight:length ratio	0.45*	-0.25	0.30
Z scores			
Weight	0.58*	0.22	0.66*
Length	0.05	0.29	0.21
Weight for length	0.57*	0.17	0.62*

- 3 Pearson correlation with 2 tailed significance: * $p \leq 0.01$, † $p \leq 0.05$

4

- 1 Table 2. Correlation of plasma leptin, leptin soluble receptor (sOB-R), and sOB-
 2 R:Leptin ratio with body composition measurements as absolute values or as percentage
 3 of total weight in term infants

	Leptin ng/mL	Leptin soluble receptor ng/mL	sOB-R:Leptin molar ratio
Absolute measurements			
Bone	-0.32†	0.20	-0.21
Lean	-0.43*	0.17	-0.34†
Fat	0.16	0.47*	0.38†
As percentage of total weight			
Bone	-0.33†	-0.16	-0.33†
Lean	-0.50*	-0.33†	-0.66*
Fat	0.52*	0.31	0.66*

- 4 Pearson correlation with 2 tailed significance: * $p \leq 0.01$, † $p \leq 0.05$

Table 3. Prediction values of physiologic variables including age, race, gender, and absolute values (left 2 columns) or age and gender specific Z scores (right 2 columns) of anthropometric measurements for plasma concentrations of leptin and leptin soluble receptor*, and leptin soluble receptor:leptin molar ratio.

Dependent variable – plasma leptin (ng/mL)			
Independent variables: R ² 0.49	Beta (p)	Independent variables: R ² 0.46	Beta (p)
Weight (g)	1.87 (0.001)	Weight Z score	0.83 (0.001)
Length (cm)	-2.17 (0.001)	Length Z score	-0.44 (0.01)
Gender (Female = 1, male = 0)	0.27 (0.03)		

Dependent variable - plasma soluble leptin receptor : leptin molar ratio			
Independent variables: R ² 0.50		Independent variables: R ² 0.44	
Age (d)	-0.75 (0.001)	Weight Z score	0.66 (0.001)
Weight (g)	1.51 (0.001)		
Gender (Female = 1, male = 0)	0.39 (0.01)		

* None of the physiologic variables was predictive for plasma soluble receptor concentration.

Table 4. Prediction values of physiologic variables including age, race, gender, and absolute values (left 2 columns) or percent bone, lean and fat mass (right 2 columns) for plasma leptin and leptin soluble receptor concentrations, and leptin soluble receptor:leptin molar ratio

Dependent variable – plasma leptin (ng/mL)			
Independent variables: R ² 0.37	Beta (p)	Independent variables: R ² 0.37	Beta (p)
Age (d)	-0.65 (0.001)	Age (d)	-0.34 (0.01)
Fat (g)	0.44 (0.003)	Fat%	0.42 (0.003)
Dependent variable – plasma soluble leptin receptor (ng/mL)			
Independent variables: R ² 0.22		Independent variables: R ² 0.43	
Fat (g)	0.47 (0.004)	BMC%	-0.63 (0.004)
		Lean%	-14.1 (0.001)
		Fat%	-13.8 (0.001)
Dependent variable – plasma soluble leptin receptor : leptin ratio			
Independent variables: R ² 0.54		Independent variables: R ² 0.59	
Age (d)	-0.71 (0.001)	BMC%	-0.59 (0.002)
Fat (g)	0.72 (0.001)	Lean%	-7.04 (0.02)
		Fat%	-6.42 (0.03)

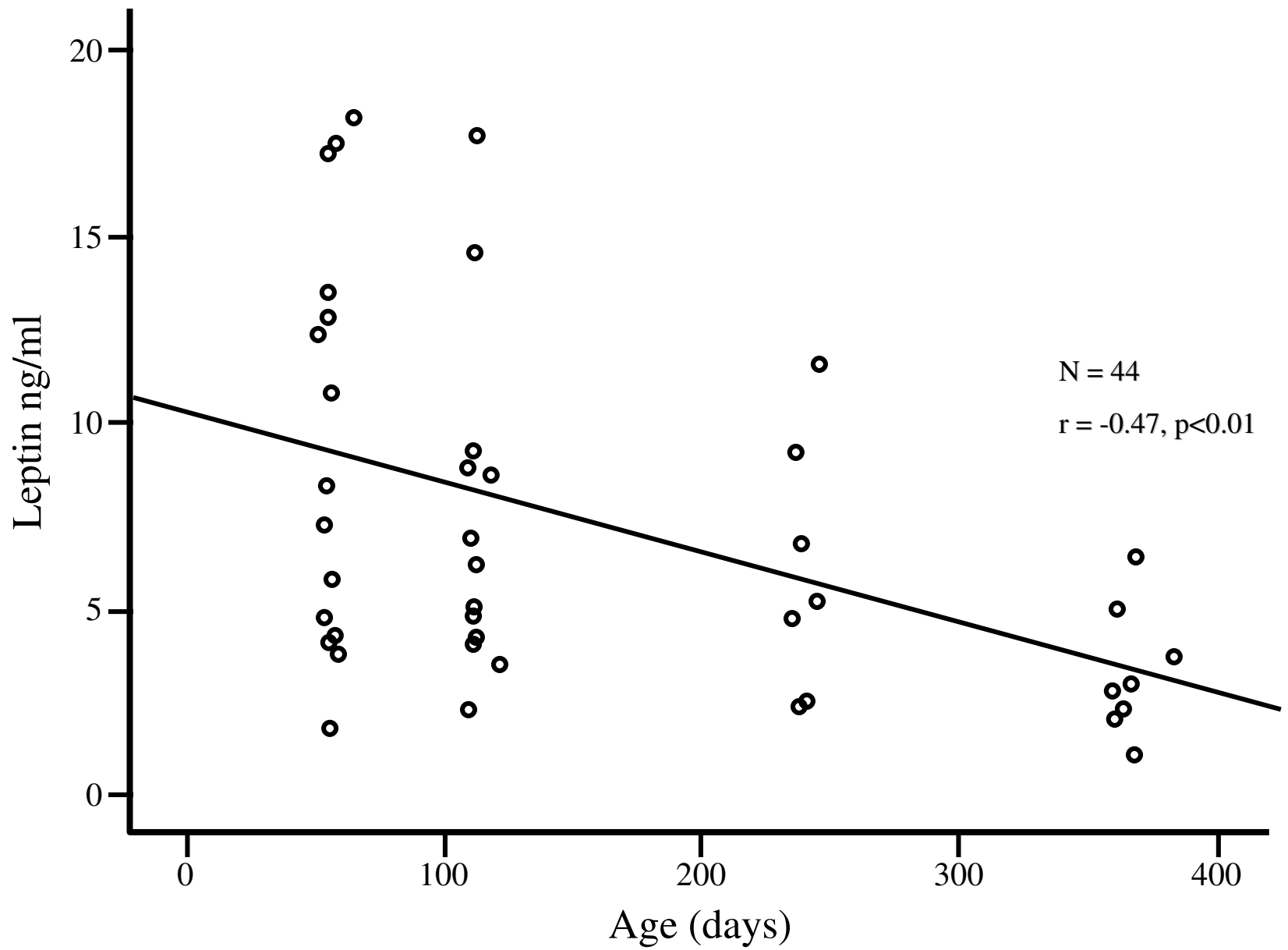


Figure 1

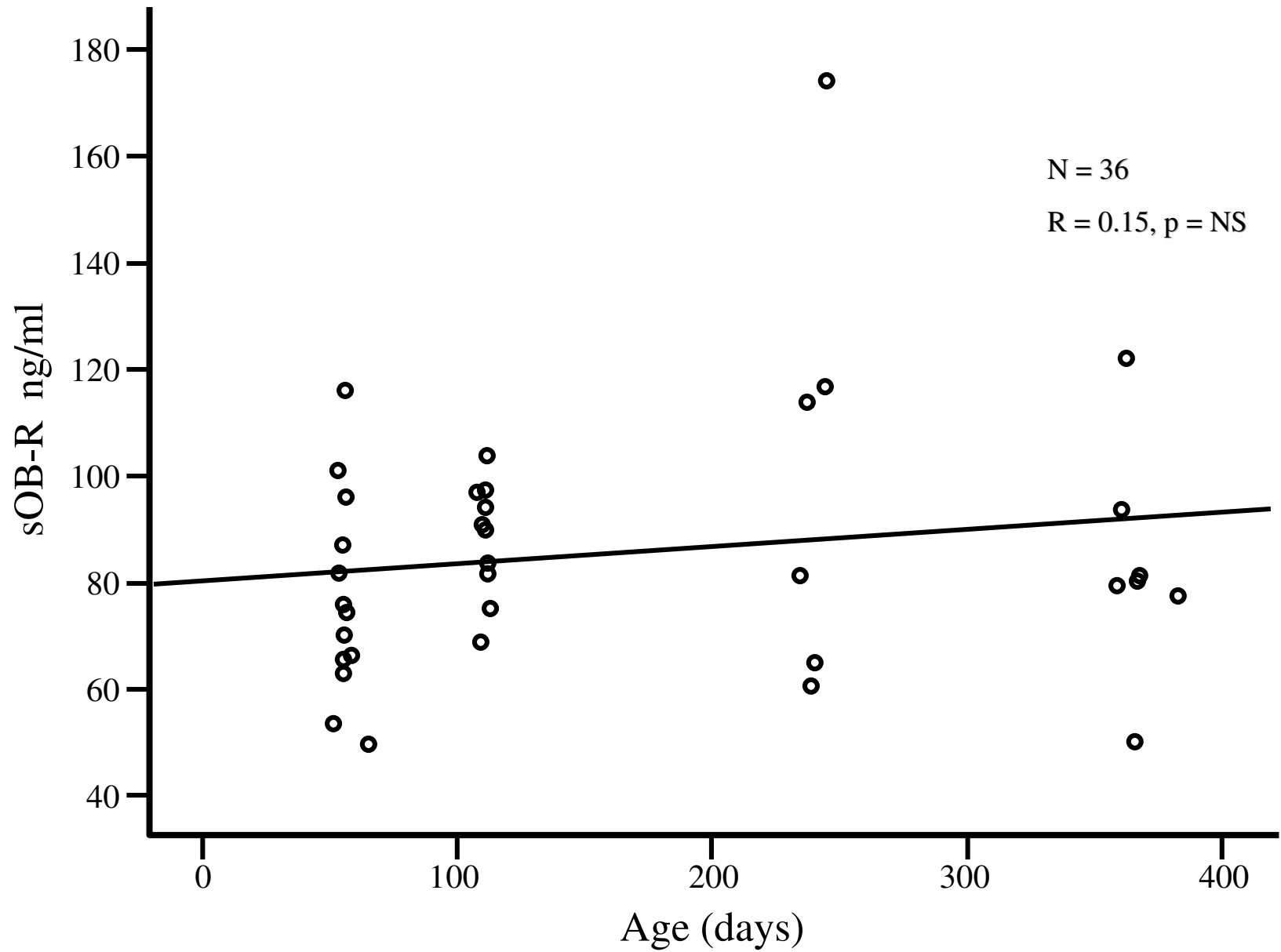


Figure 2