

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

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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 are 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 predictive of plasma leptin and the receptor:ligand ratio. However, body composition is
23 the only variable that can predict plasma leptin and its soluble receptor and the receptor:

- 1 ligand ratio; and body composition measurements eliminated the race and gender effect
- 2 on these plasma variables.

1 **Background**

2 Hormonal responses from the gastrointestinal tract, brain and body tissues
3 associated with dietary intake and regulation of nutritional status and body weight are
4 well described in adults [1-3]. There are increasing data indicating that similar hormonal
5 changes can occur in children and infants [4-9]. Understanding their relationship with
6 body composition measurements as indicators of tissue accretion might shed light on the
7 physiological basis to integrate nutrition support, growth and tissue accretion.

8 Concentration of circulating leptin, an adipocyte hormone, reflects the amount of
9 energy stored in adipose tissue and is considered a marker of nutritional status [1-3].
10 Leptin is bound to the soluble leptin receptor (sOB-R) in the circulation, which modulates
11 steady state leptin levels by preventing the hormone from degradation and clearance [10],
12 and sOB-R:leptin ratio may be considered a marker of bioavailable leptin.

13 In the neonate, dramatic changes in circulating leptin and sOB-R with a decrease
14 in leptin by as much as 80% [5-7] and increase in sOB-R by >5 folds [7] are reported. At
15 birth, weight and body mass index are positively correlated with plasma leptin
16 concentrations [5,6,8], and one report indicated that sOB-R is inversely related to birth
17 weight [7]. This pattern of changes in leptin and its sOB-R and their relationship with
18 body weight and body mass index presumably reflect the loss of placental contribution
19 for leptin and the physiologic adaptation to lower availability of free leptin, thereby
20 allowing an increase in energy intake to initiate the phase of rapid postnatal growth.

21 Infancy is the period of most rapid postnatal growth and one report in healthy but
22 relatively malnourished infants with average weight for age Z score of -1.9 at 52 weeks
23 shows a decrease in plasma leptin from birth to 1 year. Changes in plasma sOB-R in

1 infants beyond the immediate newborn period are not well defined. Furthermore, the
2 relationship between body composition measurements as reflection of tissue accretion,
3 with these plasma variables is not known. The aim of this study is to define the
4 developmental variations in circulating leptin and sOB-R in healthy infants specifically
5 the physiologic determinants including age, gender, race, and measures of growth or
6 body composition on these plasma variables.

7

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 [11] 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 [12,13]. 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 [14]. 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 [15,16]. Only scans with no significant movement artifacts [17] were
21 included in data analysis. In our laboratory, the precision error [18] 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 was obtained from the Institutional Review Board
8 for Human Investigations at Wayne State University, Detroit, MI. Written informed
9 consent 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 [19]. 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 parameter. 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 [20-22]. 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 (7 white) males and 23
4 (8 white) females. Anthropometric and DXA measurements and blood collection were
5 performed at 56 +/- 0.8, 112 +/- 1.0, 240 +/- 1.5, and 366 +/- 2.7 days. Age was
6 positively correlated with all absolute values of anthropometric and DXA measurements
7 ($p < 0.01$ for all comparisons). Weight for age Z score (WAZ) decreased ($r = -0.36$, $p =$
8 0.02) but recumbent length for age (HAZ) and weight for length (WHZ) Z scores were
9 not significantly different with age. The overall WAZ, HAZ and WHZ were $0.00 +/-$
10 0.15 and $-0.08 +/- 0.11$, and $0.12 +/- 0.14$ respectively. Bone mass as a percentage of
11 total mass was increased ($r = 0.58$, $p < 0.01$) but the percentages of fat and lean mass did
12 not change significantly with age. The overall percentages for bone, fat and lean mass
13 were $2.5 +/- 0.04$, $26.3 +/- 0.92$, 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 Study of leptin and its regulation has demonstrated its importance as an integral
3 part of homeostatic mechanism in the regulation of body weight [1-3]. However, it is not
4 known whether this is applicable to all life stages and what changes it may have during
5 growth in which weight gain and tissue accretion rather than maintenance of body weight
6 is the physiologic norm.

7 To our knowledge, this is the first report of the relationship of the circulating
8 leptin and sOB-R concentrations to various physiological variables of growth and body
9 composition specifically, bone, fat and lean masses, during infancy. Age appears to be a
10 major physiologic determinant of plasma leptin concentrations. It is decreased during
11 infancy in those with normal age and gender specific weight and length Z scores as
12 indicated by our data, and is also decreased in infants with poor postnatal growth during
13 longitudinal measurement of plasma leptin from cord blood and at 8, 16 and 52 weeks
14 [9]. In this study, the initial measurement of circulating leptin and its soluble receptor at
15 2 months likely eliminated the confounding factors of placental leptin [23], the initial
16 physiologic adaptation that occurs commonly with other endocrine systems [24], and the
17 apparent transient increase in plasma leptin during the first weeks after birth observed by
18 some [25,26] but not by other [9] investigators. In the two reports on postnatal increase
19 in plasma leptin concentration, one reported an increase in plasma leptin at 30 days which
20 was significantly correlated with interval weight gain [25] but no data was available
21 beyond 30 days. In the other report, plasma leptin concentration was higher in term
22 versus preterm infants up to 30 days but there was no significant difference between
23 groups at 90 days. The increase in plasma leptin was correlated with weight gain and

1 increase in subcutaneous tissue [26]. Whether this transient increase in plasma leptin is
2 related to changes in leptin transport, metabolism or clearance is not known.

3 Plasma sampling in our study tend to correspond to ages when milk intake is the
4 exclusive or dominant source of nutrient, namely at 2 and 4 months, and when mixed diet
5 becomes increasingly established at 8 and 12 months respectively. Our preliminary data
6 suggest that usual dietary intake in healthy and normally grown infants probably does not
7 affect plasma leptin or its soluble receptor concentrations, although determination of the
8 relationship between leptin and its receptor with details of nutritional intake was not the
9 primary goal of this study and further studies are needed.

10 Anthropometric and body composition measurements are related to and predictive
11 of plasma leptin. This is consistent with other reports that plasma leptin is correlated
12 with actual [26] or gain [25,26] in body weight, and actual [8,9] and changes [9] in body
13 mass index, and with indirect indicators of body fat such as subcutaneous skinfold
14 thicknesses [7,25,26]. Plasma leptin was also found to discriminate both the long term
15 and changes in energy status based on skinfold thickness [9].

16 The consistent relation between the plasma leptin particularly with fat mass is
17 supportive of adipose tissue being the major source of circulating leptin. The negative
18 correlation of percent bone mass and percent lean mass with plasma leptin is not
19 surprising since an increase in the proportion of fat mass is generally correlated with
20 decreased proportion of lean and bone mass. However, a direct relation between plasma
21 leptin and other tissue mass may be possible since increasing numbers of non-adipose
22 tissues including skeletal muscle [27], chondrocyte [28] and human osteoblast [29] are
23 reported to synthesize leptin and may have cellular leptin receptor forms with

1 physiological activity in experimental models. In any case, the exact role of leptin in the
2 changes in skeletal muscle and bone in humans remain to be defined.

3 Our data show elevated sOB-R concentrations throughout infancy. Other
4 investigators have reported persistently elevated sOB-R concentrations during early
5 childhood [30]. The positive relation between plasma sOB-R and fat mass may be
6 indicative of the increased membrane-bound leptin receptor forms, the source of sOB-R.
7 The negative correlation of percent lean mass with plasma sOB-R is consistent with the
8 generally inverse relation between percent lean and fat mass.

9 The correlation between these plasma variables with anthropometric or body
10 composition measurements were better with the use of Z scores rather than absolute
11 measurements, with fat percent rather than absolute fat mass, and generally better with
12 body composition, specifically fat mass, rather than anthropometric measurements. Thus
13 the use of standardized rather than absolute measurements of anthropometry and body
14 composition is indicated in future studies on the interplay of leptin and its receptor with
15 different nutrition support in growing subjects. Furthermore, body composition
16 measurements are probably more sensitive indicators of leptin production and bioactivity.

17 It is interesting that even with the limited sample size, our findings of higher
18 leptin concentration in African American infants independent of fat mass is consistent
19 with the report on adult males and females that non-Hispanic blacks have slightly higher
20 values compared to non-Hispanic whites or Mexican Americans [31]. Our data of higher
21 plasma leptin concentration in female infants also are consistent with the presence of
22 sexual dimorphism [9,32]. Furthermore, our data show that race and gender effects were
23 eliminated in the presence of body composition measurements, presumably body

1 composition measurements more specifically reflect the source of leptin and its receptors.
2 Our data indicate that variations in plasma sOB-R are independent of race, gender or age
3 but are predicted by body composition measurements.

4 Complexes of leptin with sOB-R reflect a molecular ratio of 1:1 [33] and reached
5 a median value of >10 as early as 3 days after birth because of a decrease in circulating
6 leptin with an accompanied increase in sOB-R [7]. Our data indicated that plasma sOB-
7 R:leptin ratios remained >10 throughout infancy. Limited data indicate the high plasma
8 sOB-R:leptin ratios may persist until 2 to 3 years [30]. It is possible that high circulating
9 concentrations of sOB-R may block leptin function by its competition with the membrane
10 receptor for the ligand, which in turn may be an important stimulus for energy uptake in
11 the rapidly growing infant or in other conditions with a high energy demand. However,
12 the decreasing sOB-R:leptin ratio during later infancy is presumably associated with
13 increasing bioavailable leptin, and is consistent with slowing of growth [19] and tissue
14 accretion [20-22].

15 Our report represents an exploratory step to determine the developmental
16 variations of plasma leptin and its soluble receptor during the period of most rapid
17 postnatal growth when the body weight and tissue accretion triples over a one year
18 period. These data when coupled with the body composition measurements are critical to
19 the design of future studies to determine the interplay of leptin and its receptors with
20 nutrition support and the regulation of growth and tissue accretion.

1 **Conclusions**

2 We conclude that in healthy growing infants, plasma leptin and sOB-R:leptin ratio
3 but not sOB-R decreases with age. Body composition is the only variable that can predict
4 plasma leptin and its soluble receptor and the receptor:ligand ratio, and body composition
5 measurements eliminate the race and gender effect on these plasma variables. Based on
6 limited size of subgroups in this study, the race and gender effect on these plasma
7 variables appears to be consistent with that for adults.

8

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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- 7

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)

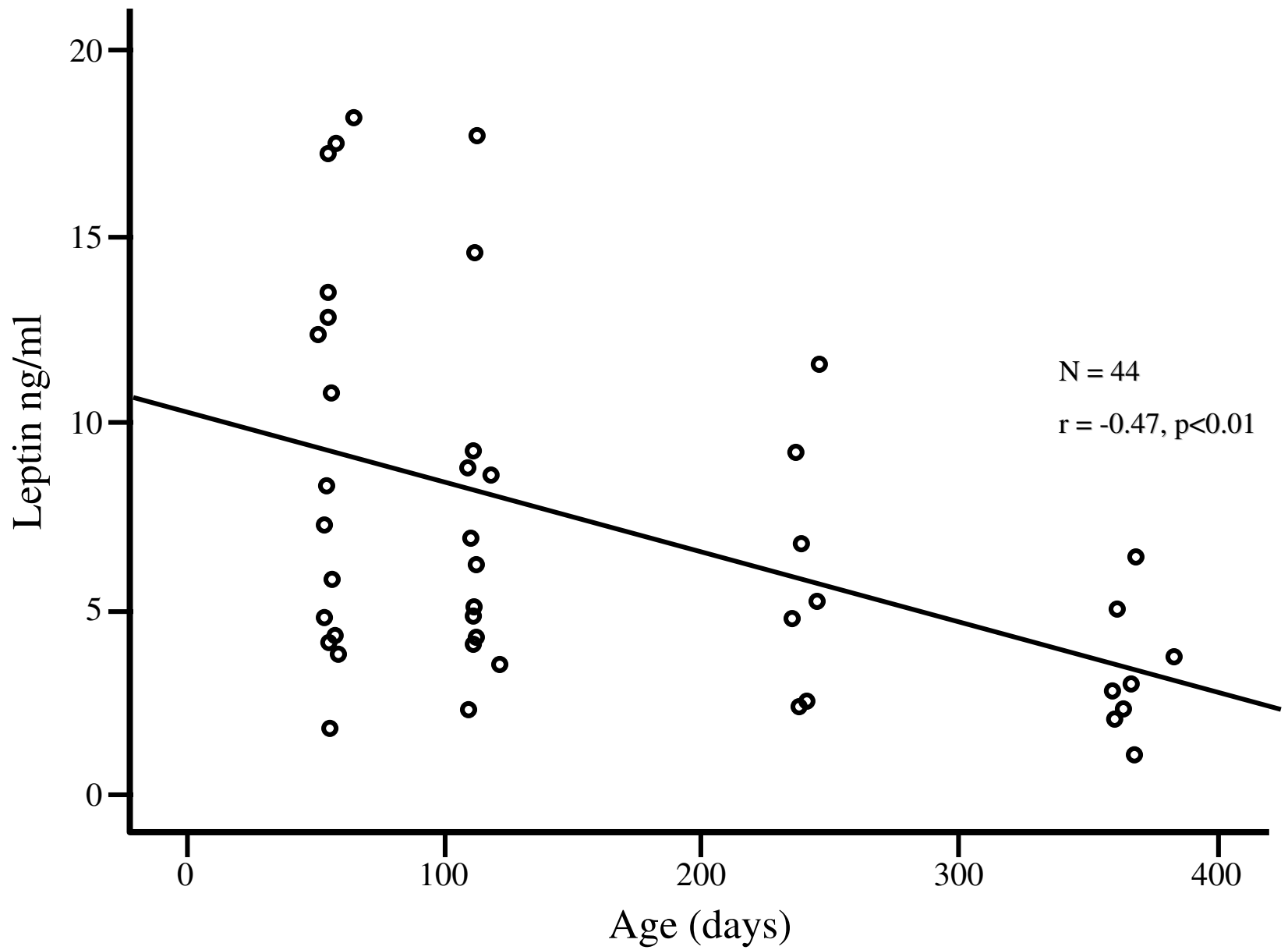


Figure 1

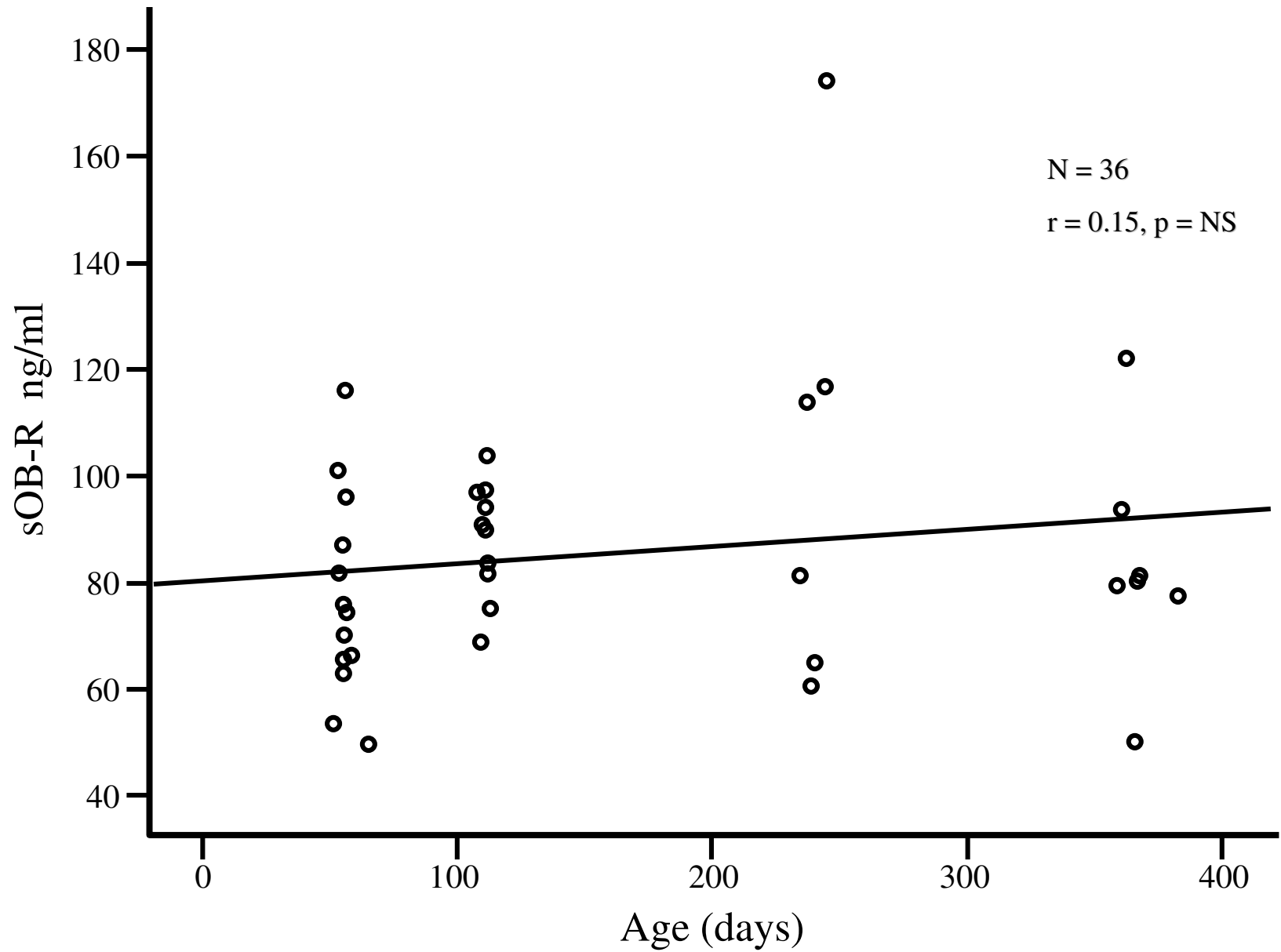


Figure 2