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The Effect of Age, Gender, and Variable Conditions on Plasma Adipokines, Other Parameters, and Glucose Regulating Hormones in Common Bottlenose Dolphins

Jinhai Yu[1], Guoliang Hu[1]*, and Zhaofei Xia[2]

[1]*Jiangxi Provincial Key Laboratory for Animal Health, Institute of Animal Population Health, College of Animal Science and Technology, Jiangxi Agricultural University, China*

[2]*Department of Veterinary Clinical Medicine, College of Veterinary Medicine, China Agricultural University, China*

***Corresponding author**

Guoliang Hu, Jiangxi Provincial Key Laboratory for Animal Health, Institute of Animal Population Health, College of Animal Science and Technology, Jiangxi Agricultural University, Nanchang, Jiangxi, China, Tel: 86-3807089905

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Keywords

- Common bottlenose dolphin
- Adiponectin, Leptin, Insulin, Glucagon, Sex hormones

Abstract

The reference data and associations of leptin, adiponectin, insulin, glucose, glucagon, HOMAIR, and HOMAβ with other parameters in healthy (adults, pubescents, and juveniles), inflammatory, pregnant, and lactated common bottlenose dolphins (*Tursiops truncatus*) were studied. Results showed that the levels of insulin, adiponectin, leptin, and glucagon varied with age and gender, presented variable values in gestation, lactation, and inflammation. The values of insulin in the adult females were higher than that of the adult males ($p < 0.01$). The value of insulin of in the pubescent males was higher than that of the adult males ($p < 0.05$). The value of insulin and HOMAIR of the lactated females were higher than that of the adult females ($p < 0.05$), while the value of adiponectin in the adult females were higher than that of the lactated females ($p < 0.05$). The value of adiponectin in healthy juvenile males was higher than that of the inflammatory males ($p < 0.05$). HOMAIR in the healthy adult females were higher than that of the adult males ($p < 0.05$). HOMAβ in healthy adult females were lower than that of the adult males ($p < 0.05$). HOMAβ in healthy pubescent males were lower than that of healthy adult males ($p < 0.05$). HOMAβ of inflammatory pubescent females reduced as compared with that of healthy pubescent females ($p < 0.01$), whereas HOMAβ of inflammatory juvenile females increased as compared with that of healthy juvenile females ($p < 0.01$). Taken together, leptin, adiponectin, insulin, and glucagon of common bottlenose dolphin are associated with glucose regulation, lipid metabolism, and sex hormone homeostasis.

ABBREVIATIONS

INS: Insulin; ADP: Adiponectin; LEP: Leptin; GLC: Glucagon; ADRL: Ratio of adiponectin to leptin values; HOMAIR: Homeostasis Model Assessment of Insulin Resistance; HOMAβ: Homeostasis Model Assessment of Beta Cell Function; WBC: Leukocyte or White Blood Cell; RBC: Erythrocyte or Red Blood Cell; PLT: Platelet; ESR: Erythrocyte Sedimentation Rate (mm/60min); TP: Total Protein; ALB: Albumin; GLB: Globulin; ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase; Fe^{++}: Iron Ion; BUN: Blood Urea Nitrogen; CRE: Creatinine; UA: Uric Acid; CHO: Total Cholesterol; TG: Triglyceride; HDL: High Density Lipoprotein Cholesterol; LDL: Low Density Lipoprotein Cholesterol; GLU: Glucose; CK: Creatine Kinase; CKMB: Creatine Kinase Isoenzyme Muscle Brain; LDH: Lactate Dehydrogenase; HBDH: **Hydroxybutyric Dehydrogenase;** COR: Cortisol; FSH: Follicle Stimulating Hormone; LH: Luteinizing Hormone; E: Estrogen; PRO: Progesterone; TES: Testosterone

INTRODUCTION

The blubber layer is the main site of metabolic energy accumulation and mobilization for cetaceans. Adipocytes derived from blubber can produce some adipokines such as leptin and adiponectin. Leptin controls body weight and regulates appetite and energy expenditure, while adiponectin modulates glucose and lipid metabolism, promotes fatty acid oxidation, and enhances insulin sensitivity in the liver and skeletal muscles [1]. Obese people have elevated leptin and reduced adiponectin which indicate that the expression and secretion of leptin and adiponectin are associated with lipid metabolism or fat accumulation and mobilization. Leptin and leptin receptors in mammals have a remarkably conserved tertiary structure evolutionarily [2]. There have been some reports on leptin and leptin receptors in marine mammals. Ball and colleagues indicated that leptin and leptin receptors of bowhead whales (*Balaena mysticetus*) [3] and belugas (*Delphinapterus leucas*) had high expressions in blubber, and demonstrated seasonal and age- related variations [4]. Russo found leptin distribution in the gastrointestinal tract of South American sea lions (*Otaria flavescens*) and common bottlenose dolphins (*Tursiops truncatus*) [5]. Leptin concentration was also high in the blubber of the grey seals *(Halichoerus grypus*) and the harbor seal (*Phoca vitulina*) [6], and another study indicated that

high amounts of adiponectin, adiponectin receptors, and leptin receptors in the blubber of the northern elephant seal (*Mirounga angustirostris*) during late- fasted lactation and molting [7]. In addition, Neely found that serum adiponectin was correlated with glucagon levels in bottlenose dolphins in managed care [8]. Plasma adiponectin decreased during suckling and early postweaning fasting in grey seal pups [9], but this study did not discuss the relationship between plasma leptin and adiponectin. In conclusion, leptin, adiponectin, and their receptors regulate the glucose and lipid metabolism in some marine mammals. However, there is no reported evidence of a correlation between leptin and adiponectin in the common bottlenose dolphins.

Cetaceans evolved from cetartiodactyla such as hippopotami [10,11]. They have undergone some adaptive changes during evolution and are now fully aquatic and occupy the highest trophic level. Cetaceans are top predators in the marine food chain adapted to consuming high protein, high fat, and low carbohydrate food (fish or invertebrates). In addition, odontocetes (dolphins, whales, and porpoises) have a higher encephalization quotient than terrestrial mammals [12,13] and can dive for long periods of time to forage or escape. In this condition, their brains need a continuous supply of glucose to support neuronal activities and physiological functions [14]; therefore, they need to be able to sustain endogenous production of glucose via glycogenolysis or gluconeogenesis. Some studies with bottlenose dolphins have shown evolutionary changes to these vital pathways that signal cascades differed from terrestrial mammals. For example, bottlenose dolphins have presented hyperglycemia [15] and insulin resistance after a 10- 14 hour fasting time [16], and bottlenose dolphin is a good animal model for human diabetes mellitus to some degree [17]. Additionally, Venn- Watson [18] found managed bottlenose dolphins groups had higher insulin and glucose compared to free - ranging groups. However, it only contained data from two groups, and more data are required to confirm these results. The combination of long-term fasting in breeding [19] and lactation of Northern elephant seal depends on the efficient mobilization of lipid from blubber being directed into milk production [20]. The oxidation reaction of substrate glucose also requires an adequate oxygen supply, and the erythrocytes of cetaceans show adaptive changes with a higher concentration of hemoglobin than terrestrial mammals [21] to transport more oxygen to vital organs such as the brain and heart. The glucose transporter 1 (GLUT 1), a member of the GLUT family of mammalian glucose transport proteins, is a high - affinity transporter to the brain and erythrocytes that depends on glucose for energy metabolism [22]. Dolphin and human erythrocytes have superior transmembrane glucose transporter activities [23], which is attributed to GLUT 1- mediated glucose uptake [24]. Therefore, the objectives of this study were to compare the data of plasma parameters in different conditions in common bottlenose dolphin.

Insulin and glucagon are endocrine hormones synthesized and excreted by the pancreatic islets that are critical for regulating blood glucose in mammals. Pancreatic islet architecture varies among dolphins [25]. The pancreas of common bottlenose dolphins is divided into lobules separated by variably thick fibrous connective tissues, similar to humans and other mammals. Lobules contain many exocrine acini and variable numbers of randomly distributed islets of Langerhans cells containing α-cells (excrete glucagon), β- cells (produce insulin), and δ- cells (produce somatostatin). When the circulating glucose increases, β- cells of islets start to excrete insulin and blood glucose will decrease, which in turn promote the excretion of glucagon from α- cells. One study indicated that the serum adiponectin was correlated with glucagon in common bottlenose dolphins [8], and another study presented that the serum insulin level decreased and glucagon increased in 2h postprandial in healthy common bottlenose dolphins [26]. However, evidence about the association between glucose, insulin, glucagon, and other parameters in common bottlenose dolphins are scanty and not yet elucidated.

This manuscript will demonstrate the reference values of insulin, glucagon, leptin, and adiponectin, and other parameters in healthy common bottlenose dolphins in different groups.

MATERIALS AND METHODS

Animal information

Forty four common bottlenose dolphins (male: 24; female: 20) were selected to study the association of insulin, glucagon, leptin, adiponectin, and other plasma parameters from May 2019 to Dec 2019. Age were classified by a previous study [27]. Based on the results of Kasuya' report, ages were determined by birth dates or estimated from body length [28]. There were three age groups in this study: Juvenile: 0-5 y old; pubescent: 6-10 y old; adult: 10-30 y old. No geriatric dolphins (> 30 y old) were included in this study. All dolphins were born or raised in aquariums which met the requirements of the China National Industrial Standard (SC/T6073-2012). All data collection was done via voluntary behaviors without any restraint and drug use. Daily food intake was 2- 6% of body weight, and dolphin diets consisted of high- quality herring (*Clupea harengus*, 30- 40%), capelin (*Mallotus villosus*, 40- 60%), and squid (*Loligo* spp., 5-10%). Water temperature was maintained between 19 to 23°C for all dolphins. Natural photoperiod was main lighting in daily life, and the activities of dolphins were normal. Powerful life- support systems ensured that water quality at or above standards (China National Industrial Standard SC/T 9411 - 2012). Environment enrichments offered according to facility and exhibit schedules. The inflammatory juveniles and pubescents were suffered from pneumonia.

Blood samples collection, centrifugation, and storage- Blood samples were collected monthly at 8:30 - 10:00 in every fasted animal as schedules (i.e., no fish fed for 14h-16h, or before the first morning feed on sample collection days) from the caudal vascular bundle of the ventral or dorsal fluke using a disposable scalp vein set and a 10 mL syringe. Two to six blood samples were collected for each dolphin during research. Blood samples (2 mL) were placed in vacuum tubes (4- 5 mL) for plasma biochemistry and hormone tests, in anti- coagulant 3.8% sodium citrate (2 mL) for erythrocyte sedimentation rate (mm/60 min). Plasma samples were centrifuged at 1,000 g for 5 min by TRIAC Centrifuge (Becton Dickinson primary care diagnostics, Becton Dickinson and Company), and plasma was frozen and stored at -20º C until analysis. Laboratory tests were performed once or twice for each sample within 24- 48h after blood collection.

Laboratory tests- Plasma biochemistry parameters including ALT, AST, ALP, GGT, BUN, CR, UA, GLU, CK, CKMB, Ca, Pi, Mg, CHO, TG, HDL, LDL, HBDH, and LDH were determined using an Olympus biochemical analyzer (*Olympus* AU400, Tokyo, Japan). The levels of TP, ALB, GLB, TBA, CHE, Fe^{++}, K^{+}, Na^{+}, and Cl^{-} were determined by biochemical analyzer (Roche Cobas c501, Roche diagnostics, IN, USA).COR, LH, FSH, E, PRO, and TES were determined by biochemical analyzer (Roche Cobas e411, Roche diagnostics, IN, USA). Insulin was examined with an Abbott automatic immunoassay analyzer (model: ARCHITECT i2000, Abbott Laboratories, Abbott Park, Illinois, USA). Glucagon was assayed with a radioimmunoassay kit (Beijing North Biotechnology Research Institute, Beijing, China) and automatic gamma immune counter XH6080 (Xi'an Zhonghe Nuclear Instrument Limited Company, Xi'an, Shanxi, China). Leptin and adiponectin were examined using ELISA kits (PL700, PA008, beyotime biotechnology, Beijing, China) with Multiskan Ascent (model: MK-III, Thermo Fisher Scientific, Waltham, MA, USA).

Homeostasis model assessment of insulin resistance (HOMAIR) and beta cell function (HOMAβ)- The HOMAIR and HOMAβ of dolphins were used formulas as follows:

HOMAIR = [fasting plasma glucose level (mmol/L)* fasting plasma insulin(μIU/mL)]/22.5 [29];

HOMAβ = 40*[fasting plasma glucose level (mmol/L) / [fasting plasma insulin(μIU/mL)*2-7] [30].

Statistical analysis- All data were analyzed using *SPSS* software (version 16.0, SPSS Inc., Chicago, Illinois, USA). The data were manifested as mean ± standard deviation. The data were analyzed with by nonparametric tests for independent samples. Differences that were significant at $p < 0.01$ and $p < 0.05$ are presented here.

RESULTS

The values of insulin in adult, pubescent, and juvenile males were 0.82 ± 0.30, 2.24 ± 1.10, and 2.60 ± 3.16 μ IU/mL, respectively. The values of insulin in adult, pubescent, and juvenile females were 2.20 ± 0.64, 1.92 ± 1.12, and 4.88 ± 4.42 μ IU/mL, respectively. The values of insulin in gestation or lactation were 2.68 ± 1.40 or 2.40 ± 0.19 μ IU/mL, respectively. The values of glucagon in adult, pubescent, and juvenile males were 110.01 ± 47.94, 114.40 ± 31.84, and 167.81 ± 70.52 pg/mL, respectively. The values of glucagon in adult, pubescent, and juvenile females were 104.37 ± 34.1, 129.64 ± 48.79, and 96.23 ± 25.58 pg/mL, respectively. The values of glucagon in gestation or lactation were 121.05 ± 51.20 or 78.00 ± 28.63 pg/mL, respectively. The values of leptin in adult, pubescent, and juvenile males were 2.33 ± 1.57, 3.27 ± 2.27, and 3.02 ± 0.17 ng/mL, respectively. The values of leptin in adult, pubescent, and juvenile females were 2.82 ± 1.94, 2.59± 2.83, and 2.66 ± 1.16 ng/mL, respectively. The values of leptin in gestation or lactation were 4.10 ± 1.91 or 3.38 ± 0.68 ng/mL, respectively. The values of adiponectin in adult, pubescent, and juvenile males were 3.34 ± 2.31, 4.45 ± 2.35, and 5.13 ± 0.65 mg/L, respectively. The values of adiponectin in adult, pubescent, and juvenile females were 4.10 ± 2.54, 5.40 ± 2.04, and 3.55 ± 1.23 mg/L, respectively. The values of adiponectin in gestation or lactation were 4.35 ± 2.46 or 2.81 ± 1.18 mg/L, respectively (see Table 1). The rate of adiponectin to leptin (ADRL) varied with age and gender, and the ADRL in inflammatory juvenile females decreased as compared with healthy juvenile females (Table 1).

Values of insulin in inflammatory female pubescents, inflammatory male and female juveniles were 5.65±8.81, 9.13±8.06, and 0.53±0.15 μIU/mL, respectively. Values of glucagon in inflammatory female pubescents or inflammatory male juveniles were 142.95±31.79 or 163.1±28.26 pg/mL, respectively. Values of leptin in inflammatory female pubescents, inflammatory male, and female juveniles were 2.79±0.76, 4.02±1.85, and 5.18±1.32 ng/mL, respectively. Values of adiponectin in inflammatory female pubescents, inflammatory male, and female juveniles were 3.62±1.72, 2.68±1.30, and 3.29±2.80 mg/L, respectively.

The value of insulin in the adult females was higher than that of the adult males ($p < 0.01$). The values of insulin of in the pubescent males were higher than that of the adult males ($p < 0.05$). The value of insulin and HOMAIR of the lactated females were higher than that of the adult females ($p < 0.05$), while the value of adiponectin in the adult females were higher than that of the lactated females ($p < 0.05$). The values of adiponectin in healthy juvenile males were higher than that of inflammatory males ($p < 0.05$). HOMAIR in healthy adult females were higher than that of the adult males ($p < 0.05$). HOMAβ in healthy adult females were lower than that of the adult males ($p < 0.05$). HOMAβ in healthy pubescent males were lower than that of healthy adult males ($p < 0.05$). HOMAβ of inflammatory pubescent females reduced as compared with that of healthy pubescent females ($p < 0.01$), whereas HOMAβ of inflammatory juvenile females increased as compared with that of healthy juvenile females ($p < 0.01$).

The value of LDL, LDH, and HBDH of the adult males were higher than that of the adult females ($p < 0.05$), respectively. The value of BUN, TG, LDL, CK, CKMB, and HBDH of healthy pubescent females were higher than that of the males ($p < 0.05$), respectively. The value of LDH and HBDH of healthy juvenile females were higher than that of the males ($p < 0.05$), respectively. The value of TP, ALB, GLB, TG, and LDH of pregnant dolphins were lower than that of the adult females ($p < 0.05$), respectively; while the values of BUN of pregnant dolphins were higher than that of the adult females ($p < 0.05$). The values of LDL ($p < 0.01$) and GLU ($p < 0.05$) of the pregnant dolphins were higher than that of the adult females. The values of TP, GLB, CRE, CHO, TG, HDL, and CK of the lactated dolphins were higher than that of the pregnant dolphins ($p < 0.05$). The value of ESR, ALB, and ALT of the lactated dolphins were lower than that of the adult females. The value of PLT, BUN, CHO, CK, CKMB, LDH, and HBDH of the lactated dolphins were higher than that of the adult females ($p < 0.05$). The values of LDL in the lactated dolphins were significantly higher than that of the adult females ($p < 0.001$). The value of PRO in the pregnant dolphins was higher than that of the adult females ($p < 0.01$) or the lactated dolphins ($p < 0.05$). The values of PLT, ESR, ALT, AST, and BUN of healthy pubescent males were higher than that of healthy adult males ($p < 0.05$). The values of ALB, CRE, TG, and TES of healthy pubescent males were lower than that of healthy adult males ($p < 0.05$). The values of BUN, TG, LDL, CK, CKMB, and HBDH of healthy pubescent females were higher than that of healthy pubescent males ($p < 0.05$), while the values of AST

Table 1: Mean values of plasma insulin, adiponectin, leptin, glucagon, glucose and other parameters in common bottlenose dolphins (*Tursiops truncatus*).

Parameters	Adults		Gestation	Lactation	Healthy Pubescents		Inflammatory pubescents	Healthy Juveniles		Inflammatory juveniles	
	Male (n=6)	Female (n=8)	(n=5)	(n=5)	Male (n=13)	Female (n=6)	female (n=6)	Male (n=5)	Female (n=6)	Inflammatory male (n=5)	Inflammatory female (n=5)
INS (μIU/mL)	0.82 ± 0.30	2.20 ± 0.64••	2.68 ± 1.40	2.40 ± 0.19*	2.24 ± 1.10▲	1.92 ± 1.12	5.65 ± 8.81	2.60 ± 3.16	4.88 ± 4.42	9.13 ± 8.06	0.53 ± 0.15
ADP (mg/L)	3.34 ± 2.31	4.10 ± 2.54	4.35 ± 2.46	2.81 ± 1.18*	4.45 ± 2.35	5.40 ± 2.04	3.62 ± 1.72	5.13 ± 0.65	3.55 ± 1.23	2.68 ± 1.30▼	3.29 ± 2.80
LEP (ng/mL)	2.33 ± 1.57	2.82 ± 1.94	4.10 ± 1.91	3.38 ± 0.68	3.27 ± 2.27	2.59 ± 2.83	2.79 ± 0.76	3.02 ± 0.17	2.66 ± 1.16	4.02 ± 1.85	5.18 ± 1.32
GLC (pg/mL)	110.01 ± 47.94	104.37 ± 34.10	121.05 ± 51.20	78.00 ± 26.83	114.40 ± 31.84	129.64 ± 48.79	142.95 ± 31.79	167.81 ± 70.52	96.23 ± 25.58	163.1 ± 28.26	85.12
ADRL	1778.5 ± 1134.34	1827 ± 1021.36	562.71 ± 477.55	985.53 ± 448.30	1810 ± 1476.82	3362.6 ± 2010.3	1410.5 ± 874.27	1693.9 ± 138.57	1481.4 ± 563.6	895.61 ± 720.98	574.20 ± 371.53
HOMAIR	0.16 ± 0.06	0.47 ± 0.16•	0.58 ± 0.52	0.62 ± 0.08*	0.48 ± 0.32	0.43 ± 0.39	2.22 ± 4.19	0.77 ± 1.07	1.43 ± 1.35	3.27 ± 2.96	0.14 ± 0.07
HOMAβ	207.45 ± 198.36	-94.07 ± 51.18•	-76.20 ± 74.64	-86.79 ± 18.77	-63.29 ± 106.72▲	0.58 ± 91.14	-83.95 ± 104.15••	284.44 ± 510.14	-69.33 ± 154.73	-199.6 ± 76.53	569.20 ± 126.32••
WBC (10^9/L)	5.82 ± 0.97	7.96 ± 2.22	10.88 ± 3.00	7.54 ± 1.39	8.64 ± 3.65	7.78 ± 1.97	13.95 ± 2.35••	11.63 ± 5.50	10.22 ± 2.26▽◇	17.28 ± 5.10	16.07 ± 3.19
RBC (10^{12}/L)	4.14 ± 0.20	3.81 ± 0.27	3.85 ± 0.24	3.71 ± 0.06	4.37 ± 0.36	4.18 ± 0.25	4.44 ± 0.33	4.42 ± 0.43	4.13 ± 0.14▽	4.08 ± 0.06	4.55 ± 0.08
PLT (10^9/L)	125.00 ± 12.83	118.50 ± 18.38	131.25 ± 7.89	220.00 ± 11.47*	131.78 ± 37.86▲	135.83 ± 27.73△	150.50 ± 75.56	130.50 ± 38.06	162.20 ± 57.94	207.60 ± 59.95*	110.67 ± 19.66
ESR (mm/h)	1.17 ± 0.41	2.38 ± 1.06•	2.33 ± 0.58	1.40 ± 0.55*	3.33 ± 4.03▲	1.60 ± 0.54	12.67 ± 23.38	5.40 ± 3.05▼	1.40 ± 0.55	9.20 ± 10.23	10.67 ± 15.89
TP (g/L)	79.43 ± 3.21	80.89 ± 6.19	64.84 ± 1.94★	79.92 ± 4.46☆	83.27 ± 10.31	78.15 ± 10.30	88.22 ± 14.41	76.22 ± 2.63	76.80 ± 3.54	79.08 ± 13.52	84.67 ± 3.93
ALB (g/L)	44.77 ± 1.58	41.59 ± 3.14	38.02 ± 1.07★	36.86 ± 1.79*	40.88 ± 2.14▲	39.12 ± 2.91	39.55 ± 2.35	41.88 ± 1.12	43.05 ± 1.93◇	39.46 ± 1.24	42.83 ± 2.63
GLB (g/L)	34.67 ± 2.58	39.30 ± 5.47	26.82 ± 2.51★	43.06 ± 3.75☆	42.39 ± 10.95	39.03 ± 11.46	48.67 ± 14.07	34.34 ± 2.23	33.75 ± 4.03	39.62 ± 12.88	41.83 ± 5.09
ALT(U/L)	24.83 ± 2.56	25.25 ± 11.90	23.60 ± 5.90	18.20 ± 2.28*	42.54 ± 13.93▲	22.50 ± 4.97•	26.50 ± 10.60	27.75 ± 7.89	31.83 ± 8.45▽◇	26.40 ± 3.13	28.33 ± 4.93
AST (U/L)	181.50 ± 15.24	162.75 ± 47.81	154.60 ± 32.45	140.40 ± 7.54	231.54 ± 71.48▲	181.00 ± 50.06	203.50 ± 28.85	158.00 ± 12.41	163.17 ± 31.56	165.20 ± 22.52	131.67 ± 7.57
Fe^{++} (μmol/L)	33.94 ± 2.56	30.39 ± 3.79	25.01 ± 2.12	27.61 ± 5.30	38.71 ± 12.25	28.96 ± 5.31	25.28 ± 4.25	29.37 ± 7.35	35.71 ± 12.45	28.17 ± 5.91	24.64 ± 20.37
BUN (mmol/L)	11.05 ± 1.76	12.34 ± 2.94	14.48 ± 0.61★	15.85 ± 0.89*	17.05 ± 1.50▲	20.45 ± 1.64•△	20.28 ± 2.68	15.71 ± 1.88▼	14.09 ± 2.42▽◇	15.82 ± 2.50	15.75 ± 1.01
CRE (μmol/L)	169.14 ± 18.38	140.62 ± 30.27	119.71 ± 8.09	141.45 ± 13.90☆	120.62 ± 26.30▲	104.06 ± 19.63	87.05 ± 38.33	129.00 ± 28.62	122.77 ± 27.51	128.87 ± 34.12	116.71 ± 26.03
UA (μmol/L)	8.29 ± 1.56	6.81 ± 1.97	7.44 ± 3.00	5.80 ± 2.30	7.92 ± 1.72	8.31 ± 3.01	5.01 ± 1.75	8.53 ± 5.14	7.66 ± 1.57	8.42 ± 5.51	9.20 ± 0.69
CHO (mmol/L)	6.04 ± 1.05	4.74 ± 0.78	5.45 ± 0.16	6.95 ± 0.29*☆	4.76 ± 1.12	7.26 ± 1.55△	8.96 ± 2.63	5.56 ± 0.37	5.76 ± 1.54	4.25 ± 1.05	5.45 ± 0.65
TG (mmol/L)	0.65 ± 0.08	0.62 ± 0.24	0.42 ± 0.02★	0.62 ± 0.10☆	0.45 ± 0.10▲	1.07 ± 0.46•	0.84 ± 0.83	0.55 ± 0.20	0.73 ± 0.39	0.58 ± 0.19	0.57 ± 0.14
HDL (mmol/L)	4.24 ± 0.39	3.47 ± 0.63	3.45 ± 0.29	3.93 ± 0.09☆	3.47 ± 0.67	4.75 ± 0.99	4.97 ± 0.70	3.57 ± 0.28	3.67 ± 0.58◇	3.06 ± 0.53▼	4.25 ± 0.43
LDL (mmol/L)	0.61 ± 0.14	0.42 ± 0.09•	0.84 ± 0.06★★	1.33 ± 0.03***	0.43 ± 0.13	1.11 ± 0.33•	1.35 ± 0.68	0.57 ± 0.17	0.63 ± 0.47◇	0.58 ± 0.25	0.60 ± 0.24
GLU (mmol/L)	4.39 ± 0.72	4.79 ± 0.64	6.00 ± 0.53★	5.77 ± 0.46	4.93 ± 0.74	5.61 ± 0.88△	5.98 ± 2.34	5.49 ± 1.07	6.49 ± 0.87▽	5.97 ± 0.91	5.55 ± 1.20
CK (U/L)	123.75 ± 40.19	85.08 ± 36.10	127.94 ± 19.07	151.78 ± 9.45*☆	88.12 ± 19.91	163.23 ± 51.15•△	202.35 ± 145.49	211.80 ± 66.88▼◆	210.30 ± 24.58▽	210.54 ± 93.41	122.50 ± 52.31
CKMB (U/L)	238.03 ± 102.10	152.62 ± 62.51	225.90 ± 31.70	277.82 ± 27.50*	166.82 ± 51.99	304.45 ± 107.91•△	380.17 ± 276.90	359.98 ± 95.86◆	363.65 ± 46.03▽	327.56 ± 148.55	215.20 ± 94.28
LDH (U/L)	370.84 ± 27.84	334.32 ± 31.41•	293.89 ± 17.36★	533.65 ± 67.66*☆	389.73 ± 48.50	430.34 ± 98.95△	479.19 ± 126.16	367.03 ± 29.21	466.39 ± 65.93•▽	338.56 ± 35.63	385.81 ± 9.41
HBDH (U/L)	363.28 ± 27.12	330.85 ± 39.15•	286.14 ± 22.17	527.14 ± 54.29*☆	391.58 ± 60.71	447.58 ± 119.93•△	518.27 ± 58.27	361.20 ± 22.90	464.15 ± 78.93•▽	337.56 ± 45.74	393.33 ± 9.16
COR (μg/dL)	0.46 ± 0.46	0.27 ± 0.26	0.69 ± 1.05	0.24 ± 0.18	0.50 ± 0.43	1.12 ± 1.23	0.67 ± 0.67	0.35 ± 0.13	0.37 ± 0.08	0.62 ± 0.47	0.37 ± 0.25

FSH (mIU/mL)	ND	0.04 ± 0.04	0.02 ± 0.02	0.03 ± 0.02	ND	0.03 ± 0.06	ND	ND	0.04 ± 0.04	ND	ND
LH (mIU/mL)	ND	0.11 ± 0.14	0.13 ± 0.12	0.03 ± 0.02	ND	0.05 ± 0.02	ND	ND	0.08 ± 0.08	ND	ND
E (pg/mL)	ND	20.22 ± 9.77	17.75 ± 10.23	21.59 ± 13.42	ND	20.13 ± 9.49	ND	ND	24.11 ± 10.66	ND	ND
PRO (ng/mL)	ND	0.08 ± 0.06	13.09 ± 6.14★★	0.06 ± 0.05☆	ND	0.11 ± 0.10	ND	ND	0.05 ± 0.03	ND	ND
TES (ng/mL)	1.14 ± 0.88	ND	ND	ND	0.06 ± 0.06▲	ND	ND	0.03 ± 0.05	ND	ND	ND

※INS: insulin; ADP: adiponectin; LEP: leptin; GLC: glucagon; ADRL: ratio of adiponectin to leptin values; HOMAIR: homeostasis model assessment of insulin resistance; HOMAβ: homeostasis model assessment of beta cell function;WBC: leukocyte or white blood cell; RBC: erythrocyte or red blood cell; PLT: platelet; ESR: erythrocyte sedimentation rate (mm/60min); TP: total protein; ALB: albumin; GLB: globulin; ALT: alanine aminotransferase; AST: aspartate aminotransferase; Fe++: iron ion; BUN: blood urea nitrogen; CRE: creatinine; UA: uric acid; CHO: total cholesterol; TG: triglyceride; HDL: high density lipoprotein cholesterol; LDL: low density lipoprotein cholesterol; GLU: glucose; CK: creatine kinase; CKMB: creatine kinase isoenzyme muscle brain; LDH: lactate dehydrogenase; HBDH: hydroxybutyric dehydrogenase. COR: cortisol; FSH: follicle stimulating hormone; LH: luteinizing hormone; E: estrogen; PRO: progesterone; TES: testosterone; ND: not determined. *, compared the values of adult females with that of lactated ones. * <0.05, ** <0.01; *** <0.001;, Compared the values of adult females with that of pregnant ones. ★< 0.05, ★★< 0.01; ☆, Compared the values of lactated females with that of pregnant ones; ☆< 0.05;●,Compared the values of females with that of males in adults, pubescents, and juveniles, respectively; ●<0.05, ●●<0.01.▲, Compared the values of adult males with that of pubescent males;▲< 0.05.△, Compared the values of adult females with that of pubescent females; △< 0.05. ▼, Compared the values of adult males with that of juvenile males; ▼< 0.05; ▽, Compared the values of adult females with that of juvenile females; ▽< 0.05. ◆, Compared the values of pubescent males with that of juvenile males; ◆< 0.05. ◆◆< 0.01. ◆◆◆< 0.001. ◇, Compared the values of pubescent females with that of juvenile females; ◇< 0.05; ◇◇< 0.01. ♠♠, Compared the values of healthy pubescent females with that of inflammatory pubescent females; ♠♠< 0.01. ♥, Compared the values of healthy juvenile males with that of inflammatory juvenile males; ♥< 0.05. ♣, Compared the values of healthy juvenile females with that of inflammatory juvenile females; ♣ < 0.05. Juveniles: 0-5 years old; pubescents: 6-10 years old; adults: 10-30 years old

of healthy pubescent females were lower than that of healthy pubescent males ($p < 0.05$). The values of PLT, BUN, CHO, GLU, CK, CKMB, LDH, and HBDH of healthy pubescent females were higher than that of healthy adult females ($p < 0.05$). The values of WBC of inflammatory pubescent females were higher than that of healthy pubescent females ($p < 0.01$). The values of ESR, BUN, and CK of healthy juvenile females were higher than that of healthy adult females ($p < 0.05$). The values of CK and CKMB of healthy juvenile females were higher than that of healthy pubescent females ($p < 0.05$). The values of PLT and HDL of inflammatory juvenile males were higher than that of healthy juvenile males ($p < 0.05$).

DISCUSSION

Adipokines (leptin and adiponectin) were secreted by adipocytes and other cells [31]. They play an important role in regulating metabolism and homeostasis.There were no differences of leptin between the adults to the pubescents, the same as between the adults to the juveniles, as between the juveniles to the pubescents, respectively; it suggested the metabolism of leptin and adiponectin was consistent in healthy and fasting condition and had a similar action from the juvenile to the adult group *in vivo*. Adipokines dysregulation has emerged as a common characteristic of chronic inflammation and insulin resistance that are central components of vascular and metabolic diseases. Adiponectin in inflammatory juvenile males decreased when compared with healthy ones; and adiponectin in inflammatory pubescent females also reduced as compared with healthy ones (although statistical significance was not detected). It indicated that inflammation was accompanied by suppression of adiponectin levels, it might result from the blubber depletion. The present study found the adiponectin in the adult females were higher than that of the lactated ones. The mammary glands comprise most of adipose tissues which are composed of branched epithelial ducts infiltrating the blubber of dolphins. During lactation, the mammary gland changes with a progressive of reduction of blubber depth.

HOMAIR and HOMAβ are used as surrogate biomarker of insulin resistance and beta cell function in human medicine [32]. We found the insulin resistance increased in the lactated females and healthy adult females, similar to the previous study on the lactated cow [33]. HOMAβ increased with age from the pubescent to the adult in healthy males. HOMAβ of inflammatory pubescent females reduced as compared with that of healthy pubescent females, whereas HOMAβ of inflammatory juvenile females increased when compared with that of healthy juvenile females.

FSH is a glycoprotein hormone derived from the pituitary, which participates in reproductive events by coupling with FSH receptors. The target organs of FSH receptors are ovaries, testes, and adipose tissues [34]. Leptin modulates hypothalamic -pituitary- gonadal (HPG) axis in mammals, and in turn, both leptin and the leptin receptors are regulated by sex steroids. Leptin receptors are not only expressed in the pituitary gland and the gonads, but also found in human, mouse fat tissues, and adipocytes. The combination of leptin and leptin receptors transmits signal to the HPG axis to stimulate the sex hormones release. FSH also altered the secretion of leptin. Collectively, it suggested that leptin and FSH levels presented bidirectionally regulation. FSH is functionally expressed in human and mouse fat tissues and adipocytes and could promote lipid biosynthesis and lipid droplet formation [34]. However, there was only one report on leptin distribution in the gastrointestinal tract of common bottlenose dolphins [5]; it was hypothesized the existence of leptin and leptin receptors in the blubber or HPG axis in common bottlenose dolphins. Additional research is required to better assess these relationships.

Leptin played an important role in controlling reproductive function [35,36]. At physiological levels, leptin stimulates steroidogenesis and follicle maturation. Siawrys and Smolinska indicated that high dose PRO administration had higher level of leptin mRNA expressions in the porcine luteal cells [37]. One study showed high leptin treatment stimulated PRO increase in both prepubertal and cycling pigs [38]. Meanwhile, it suggested the sex hormone PRO can affect the leptin expression *in vivo*. Another study revealed high dose of PRO can cause circulating PRO concentration increase which was associated with higher

leptin mRNA expression of inguinal white adipose tissues in female rats [39].

Cetartiodactyla comprises artiodactyla (even- toed ungulates) and Cetacea (whales, dolphins, and porpoises). The artiodactyls are composed of over 190 living species including pigs, peccaries, hippocampi, camels, llamas, deer, pronghorns, giraffes, sheep, goats, cattle, and antelopes [40]. Cetaceans and bovids had divergent evolution of lipid metabolism since the divergence of these taxa from a common ancestor - Cetartiodactyla [41]. Domain and motif structures of the adiponectin protein among chicken and mammalian homologs are highly conserved [42]. Adiponectin influences glucose utilization, insulin sensitivity, and energy homeostasis by signaling pathways through two distinct receptors (adiponectin receptor 1, AdipoR1; and adiponectin receptor 2, AdipoR2). The chicken AdipoR1 and AdipoR2 complementary DNA sequences were identical to (76-83%) the respective mammalian [43]. Adiponectin improves insulin sensitivity in cows by activating adiponectin receptors (AdipoR1 and AdipoR2) [44]. Another study indicated that fatter cows had a higher abundance of adiponectin mRNA expression in discrete adipose tissues depots. Adiponectin and adiponectin receptors were also extensively expressed in various tissues and organs, such as the backfat of two breed pigs [45], adipose tissues, muscle tissues, visceral organs [46], ovaries, and testes of chicken [47,48]. Therefore, there may be adiponectin and adiponectin receptors in the blubber and other organs of common bottlenose dolphins, and it needs further study.

There were some differences for the level of adiponectin in variable physiologic and pathologic conditions. The level of adiponectin was significantly higher than leptin in short- term fasting condition (ADRL: from 1778.5 ± 1134.34 to 1827 ± 1021.36 folds) for the adult common bottlenose dolphins, similar to the results of cows [49] or human [50]. Our results indicated that the value of adiponectin in the adult females were higher than that of the lactated females, and similar results presented in wild grey seal pups in suckling and the early postweaning fasting [9]. In lactation, Dolphins consume the blubber store to meet the energy need in short- term fasting condition. It induced hyperlipidemia (the values of CHO, TG, and HDL of the lactated dolphins were higher than that of the pregnant dolphins. The value of LDL and CHO of the lactated dolphins were higher than that of the adult females), the adiponectin excretion will decrease. In addition, the parameters of heart and liver function increased. The values of TP, GLB, CRE, and CK of the lactated dolphins were higher than that of the pregnant dolphins; Meanwhile, the value of BUN, CK, CKMB, LDH, and HBDH of the lactated dolphins were higher than that of the adult female).

In addition, the level of leptin and adiponectin present opposite changes in sickness condition. Higher leptin and lower adiponectin (or adiponectin to leptin ratio) are biomarkers of adipose tissue dysfunction [51], and dairy cows with ketosis [49]. Our study found that adiponectin decreased and leptin increased in inflammatory dolphins (although the data had no statistical difference).

HDL produced in the liver and small intestine, and transported cholesteryl esters to the liver. Adiponectin accelerates reverse cholesterol transport via increasing HDL synthesis in the liver and cholesterol efflux from macrophages. It suggested that the changes of HDL and adiponectin were involved in the lipid metabolism for dolphins. Reverse cholesterol transport pathway is considered as HDL- mediated cellular cholesterol efflux and transport excess cholesterol from peripheral tissues through the action of APO- A1 and various transporters, such as adenosine triphosphate (ATP)- binding cassette transporter A1 (ABCAI) and ATP- binding cassette transporter G1 to the liver for bile excretion. Adiponectin plays a key role in promoting ABCA1-dependent cholesterol efflux and modulating HDL biogenesis [52]. A Japanese study indicated that adiponectin bound and inactivated oxidized LDL to inhibit its downstream effects [53]. The LDL receptor- related protein 1 is an essential regulator of beta cell function [54], and the LDL and LDL receptor play a critical role in beta cell compensation and lipid metabolism. Oxidized LDL produced a decrease in insulin - induced glucose uptake, increase adiponectin secretion [55]. Because LDL is the main transporters of cholesterol and cholesteryl esters to tissues, can scavenge cholesterol from cell membranes and other lipoprotein particles. And LDL is approximately 50 % phospholipid and 50 % cholesteryl esters, unesterified cholesterol, and triacylglycerol. In addition, platelets treated with ox-LDL exhibited a significant increase in the expression of CD147 (the extracellular matrix metalloproteinase inducer) whereas HDL decreased these effects. We found the values of PLT and HDL of inflammatory juvenile males were higher than that of healthy juvenile males.

Glucagon is an essential regulator of glucose homeostasis and energy metabolism. Glucagon receptors activation induces hepatic leptin receptors expression in liver- specific leptin receptors deficient mice [56]. The reduced glucose level will stimulate glucagon release in fasting condition, which promotes the excretion of insulin. It is a part of circadian clock in managed dolphins. Circadian clocks operative in pancreatic islets participate in the regulation of insulin and glucagon secretion in humans [57]. Shimizu indicated that leptin effects on insulin secretion by the existence of glucagons [58].

Some biochemical parameters varied with age or gender, including LDL, LDH, and HBDH. LDH is an enzyme that catalyzes the reversible conversion of pyruvate to lactate, and concentrated mainly in heart, muscle, and liver. HBDH was widely distributed in various tissue and organs, including brain, heart, kidney, and erythrocyte. We found the LDH and HBDH in the females decreased as age is growing. The age effect would be related to the sensitivity of the young animals to the muscular effort of continuous swimming in aquatic life. The value of LDH and HBDH of the adult males were higher than that of the adult females, respectively. It may be associated with rutting activity, over aggressive behaviors and social behaviors in adult male dolphins. CK catalyzes the creatine phosphate to creatine. This reaction is important for the production of energy in the muscle fibers, such as skeletal muscle and heart. The value of CK, CKMB, and HBDH of healthy pubescent females were higher than that of the males, respectively. It suggested that there was a higher energy need in heart for the pubescent females. In addition, some part of LDH in the plasma derived from erythrocytes and platelets[59]. The present study indicated that the value of PLT, BUN, CHO, CK, CKMB, LDH, and HBDH of the lactated dolphins were higher than that of the adult females, the same as healthy pubescent females

versus healthy adult females. The higher LDH and HBDH may be associated with higher PLT.

WBC and ESR are prognostic biomarkers of infection or inflammation in cetaceans [60] using in clinical practice of marine mammal medicine. We also found WBC and ESR of the juvenile bottlenose dolphins increased in the inflammatory condition (although without statistical differences). Some studies indicated that ESR had prognostic value for classification and evaluation infection in stranded or rehabilitated dolphins [61]. It is known that ovulation triggers an acute inflammatory cascade in human and animals [62,63]. Inflammation is involved in many reproductive processes such as ovulation, folliculogenesis, corpus luteum development, luteolysis, and uterine clearance after insemination and postpartum in cows [64]. The pre-ovulatory LH surge induces ovulation through disruption of the follicle structure that promotes pro- inflammatory (Th1) responses before fertilization [65]. Therefore, it may elucidate the association of LH and ESR in common bottlenose dolphins. Dolphins will present an acute inflammation reaction during estrous cycle and need to be pay attention to this natural phenomenon.

Our results indicated that the TES of adult males was higher than that of the pubescents and juveniles. TES is a major hormonal regulator of protein metabolism that affected plasma BUN level. Donahue indicated that exogenous TES could increase the protein content of kidney and spleen weight [66]. Another study showed that TES administration in rats with ovariectomy had higher level of BUN than the control [67]. It meant TES might regulate the proteins metabolism such as muscle. One study indicated that TES was positively associated with skeletal muscle mass in older women [68].Previous studies found that exogenous TES promoted expression of myoblast markers in cattle [69], improved skeletal muscle strength, endurance, and volume of septic rats [70]. Therefore, an elevation in TES could stimulate and increase muscle mass while reducing BUN in common bottlenose dolphins.

In gestation, dolphins had higher energy requirement for fetus development. Therefore, while the values of BUN, LDL, and GLU of the pregnant dolphins were higher than that of the adult females. Dolphin will motivate the blubber to meet. In addition, the higher PRO values also guarantee the growth and development of fetus. We found the value of PRO in the pregnant dolphins were higher than that of the adult females or the lactated dolphins.

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Authors' contribution

JHY conducted the data analysis and drafted the manuscript. JHY and GLH involved in the experimental design and data collection; discussed the interpretation of the data, discussed results, and commented on the final manuscript. All authors read and approved the final manuscript.

AVAILABILITY OF DATA AND MATERIALS

Common bottlenose dolphins in managed care were selected to study in this study. They were reared in aquariums that were met the requirements of aquatic mammal rearing facility of the China National Industrial Standard (SC/T6073-2012). All measures were collected using voluntary behaviors without any constraints or drugs.

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