Accepted Manuscript online: 2023-02-27 Article published online: 2023-04-05

Prolactin is a Key Factor for Nonalcoholic Fatty Liver Disease in Obese Children

Authors

Jianwei Zhang, Jieqiong Guan, Xiaoli Tang, Jinliang Xu

Affiliations

Paediatric Department, Shaoxing Women and Children's Hospital, Shaoxing, China

Key words

children, nonalcoholic fatty liver disease, prolactin, obesity, metabolic syndrome

received 04.11.2022
accepted after revision 23.02.2023
accepted manuscript online 23.02.2023

Bibliography

Horm Metab Res 2023; 55: 251–255
DOI 10.1055/a-2043-1044
ISSN 0018-5043

Georg Thieme Verlag KG, Rüdigerstraße 14, 70469 Stuttgart, Germany

Correspondence

Dr. Jianwei Zhang
Shaoxing Women and Children's Hospital
Paediatric Department, Zhejiang Shaoxing
Shaoxing 312000, China
sxfyzjw@alu.zcmu.edu.cn

ABSTRACT

This study investigates whether serum prolactin (PRL) is a key factor for nonalcoholic fatty liver disease (NAFLD) in children. A total of 691 obese childred participated in this study and were divided into a NAFLD group (n = 366) and simple obesity (SOB) group (n = 325) according to the hepatic ultrasound results. The two groups were matched for gender, age, pubertal development, and body mass index (BMI). All patients underwent an OGTT test, and fasting blood samples were collected to measure prolactin. Stepwise logistic regression was performed to identify significant predictors of NAFLD. Serum prolactin levels were significantly lower in NAFLD subjects than in the SOB subjects [82.4 (56.36, 118.70) vs. 99.78 (63.89, 153.82), $p < 0.001$] (mIU/l). NAFLD was strongly associated with insulin resistance (HOMA-IR) and prolactin, with lower levels of prolactin increasing the risk of NAFLD (adjusted ORs = 1.741; 95% CI: 1.059–2.860) across the prolactin concentration tertiles after adjustment for confounders. Low serum prolactin levels are associated with the presence of NAFLD; thus, increased circulating prolactin might be a compensatory response for obesity in children.

Introduction

Nonalcoholic fatty liver disease (NAFLD) is one of the most common chronic liver diseases in children [1], with a prevalence between 65% to 85% in obese patients [2]. Furthermore, it is expected to be the leading cause of liver transplantation in the next few decades. The mechanism by which obesity causes NAFLD is not fully understood, including increased hepatic de novo lipogenesis, genetic variability in pathways regulating hepatic lipid droplet formation and lipid secretion from the liver [3]. Fat accumulation in the liver promotes insulin resistance, which increases the sensitivity of the liver to subsequent damage. Other factors such as oxidative stress, lipid peroxidation, mitochondrial dysfunction, intestinal flora, adipose tissue dysfunction, and adipokines may also be involved [4].

Prolactin (PRL) is a polypeptide hormone secreted by the pituitary gland and is involved in reproduction, growth and development, metabolism, immune regulation, brain function, and behavior [5]. Recently, serum prolactin has been demonstrated to be associated with obesity and type 2 diabetes mellitus (T2DM). Serum prolactin levels were significantly lower in obese children compared to healthy-weight children, which suggests there is a negative correlation between prolactin levels and metabolic abnormalities such as obesity [6]. In addition, animal experiments depicted that prolactin intervention can alleviate serum free fatty acid levels in mice [7], and the fat cell volume of rats fed a high-fat diet was significantly reduced after prolactin treatment [8], indicating that prolactin has a protective effect on metabolism. Intervention in diabetic rats with high concentrations of prolactin exacerbates hepatic insulin resistance, while injection of low concentrations of prolactin enhanced insulin secretion under glucose stimulation in diabetic rats [9, 10]. However, patients with clinically pituitary prolactinoma also have disorders of glycolipid metabolism [11], which may be related to the different effects of elevated prolactin levels on metabolism in physiological and pathological conditions.

Therefore, whether prolactin under physiological conditions regulates insulin secretion to avoid insulin resistance and protect the liver is worthy of further investigation. The relationship between prolactin and NAFLD in obese youth with obesity has not yet been reported. In this present study, we sought to evaluate the association between serum prolactin and NAFLD in children with obesity. It will be very helpful to provide medical treatment at an appropriate time if serum prolactin could be as a simple effective and less-invasive biological marker, which reflect hepatic inflammatory change in obesity.

Subjects and Methods

Study population

The study design is illustrated in ▶ **Fig. 1**. A total of 691 obese children admitted to our hospital from January 2017 to December 2020 were enrolled in this study. Obesity was defined as BMI for age and sex ≥ 95th percentile. The subjects were divided into two groups, a NAFLD and simple obesity (SOB) group, and then two groups were matched for gender, age, pubertal development, and BMI. NAFLD was defined based on hepatic ultrasound, which was conducted to assess the presence and extent of hepatic steatosis according to the following guidelines: (a) a diffuse hyperechoic texture (bright liver); (b) increased liver echo texture compared to the kidney; (c) deep beam attenuation; (d) vascular blurring (absence of normal echogenic walls of the portal veins and hepatic veins). Exclusion criteria were: drugs and genetic metabolic liver disease, history of severe heart, liver, and kidney disease, type 1 diabetes mellitus, malignancy, pituitary disease, hyperprolactinemia, viral hepatitis; previous drinking history, and other related endocrine and metabolic diseases [12].

Statement of ethics

This study was approved by the Ethics Committee of Shaoxing Maternity and Child Health Care Hospital, China (No. 2018035). Written informed consent was obtained from the guardians of all recruited children, and the study was performed in accordance with the principles of the Declaration of Helsinki.

Measurements

General measurements: body weight, height, waist circumference, and hip circumference were measured to the nearest 0.1 cm, body weight nearest 0.1 kg. BMI: weight (kg)/height^2 (m^2), waist to hip ratio: waist circumference/hip circumference. The blood pressure was measured twice at rest using a standard mercury sphygmomanometer, and the average value was calculated.

Laboratory tests: Blood samples were collected after fasting overnight for at least 8 hours, processed, refrigerated, and transported to the clinical laboratory for analysis within 8 hours. All clinical analyses were performed by the hospital laboratory, which is certified by the China National Accreditation Service for Conformity Assessment. The serum concentrations of glucose (hexose kinase method, TBA-200FR, Japan), insulin (chemiluminescence, Siemens, UK); glycated hemoglobin (HbA1c, HPLC, Tosoh, HLC-73G8, Japan), serum prolactin (PRL), (Chemiluminescence, Siemens, UK), alanine transaminase (ALT), aspartate transaminase (AST), triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) (automatic immunoassay analyzer, Abbott Laboratories) were measured. All children underwent a 2-hour OGTT (1.75 g/kg, maximum 75 g of glucose). Blood samples were obtained at 0, 30, 60, 90 and 120 minutes for the measurement of glucose, insulin. Insulin resistance (HOMA-IR) was determined by the homeostasis model and calculated using the following equation: HOMA-IR = [fasting insulin (μIU/ml) × fasting glucose (mmol/l)]/22.5.

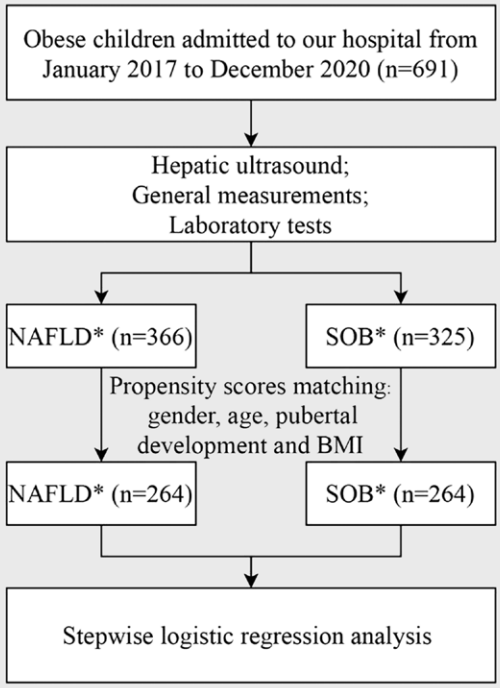
Statistical methods

All data were statistically analyzed using SPSS 20.0. Normally distributed variables are presented as mean ± standard deviation (SD), while non-normally distributed variables are presented as medians and interquartile range (25–75th percentiles). Normally distributed variables were analyzed by independent sample *t*-tests, while non-normally distributed variables were analyzed by the non-parametric Mann–Whitney U-test. Propensity analysis was conducted using logistic regression to generate a propensity score for NAFLD and SOB, and propensity scores matching analysis was performed using a 1:1 greedy method without replacement. The caliper used in this study was 0.01, and the variables entered into the propensity model were gender, age, and BMI. Stepwise logistic regression analysis was conducted to determine the significant predictors after controlling for all variables. A two-sided p-value < 0.05 was considered to be statistically significant.

Results

Comparison of general data of both groups

Among study children, 366 (53.0 %) met the criteria for NAFLD (mean age: 10.83 ± 2.46 years), while 325 (47.0 %) were classified as SOB (mean age: 10.87 ± 2.53 years) based on liver ultrasound findings. A total of 486 boys were enrolled, including 288 (59.3 %) with NAFLD, 198 (40.7 %) classified as SOB, and a total of 205 girls were enrolled, including 78 (38.0 %) with NAFLD, 127 (62.0 %) classified as an SOB. There was a significant difference in prevalence between boys and girls ($X^2 = 22.04$, $p < 0.001$), and 264 patients were matched for gender, age, pubertal development, and BMI. The baseline clinical characteristics and laboratory data of the study population are described in ▶ **Table 1**, and as expected, there was a significant difference between the NAFLD and SOB groups in HOMA-IR and prolactin ($p < 0.05$).



▶ **Fig. 1** Flow chart of the study design. *NAFLD was defined based on hepatic ultrasound, which was conducted to assess the presence and extent of hepatic steatosis according to the following guidelines: (**a**) a diffuse hyperechoic texture (bright liver); (**b**) increased liver echo texture compared to the kidney; (**c**) deep beam attenuation; and (**d**) vascular blurring (absence of normal echogenic walls of the portal veins and hepatic veins). SOB: Simple obesity.

Logistic regression analysis for predictors of NAFLD

According to ▶ **Table 1**, seven variables were identified as significant with a p-value < 0.1 (these are: TG, TC, LDL, fasting insulin, HOMA-IR, prolactin, ISI). We used stepwise logistic regression (method: forward IR) to eliminate unnecessary variables and generate a parsimonious model. Finally, only TG (OR: 1.366, 95 % CI: 1.043–1.788, $p < 0.05$), prolactin (OR: 0.996, 95 % CI: 0.994–0.999, $p < 0.005$) and HOMA-IR (OR: 1.239, 95 % CI: 1.148–1.338, $p < 0.001$) found to be independent markers for the prediction of NAFLD (▶ **Table 2**).

Associations between serum prolactin and NAFLD

All subjects were divided into three gradients based on prolactin tertiles (1st Q: PRL ≥ 113.64 mIU/l; 2nd Q: PRL was 68.68–113.63 mIU/l; and 3rd Q: PRL ≤ 68.67 mIU/l). Two models were obtained after controlling for gender, age, BMI, SBP, DBP, and waist circumference (Model 1), and Model 1 plus ALT, uric acid, creatinine, triglyceride, HDL-cholesterol, HbA1c, HOM-IR, WBC counts, HB (Model 2) using 1stQ (PRL ≥ 113.64 mIU/l). Low serum prolactin levels remained significantly independently associated with NAFLD in all models (▶ **Table 3**).

The ROC analysis revealed the relationship between serum prolactin and NAFLD, and the AUC was 0.547 (95 % CI: 0.497–0.596; $p = 0.064$). Using the best cut-off value of prolactin, the occurrence of NAFLD was ≤ 87.84 mIU/l with a sensitivity of 54.2 % and specificity of 56.8 %.

Discussion

Previous studies have revealed that the prevalence of NAFLD in obese patients is significantly higher, and in the present study of 691 obese children, 53.0 % had NAFLD, with a significantly higher prevalence of NAFLD in boys than in girls (59.3 % vs. 38.0 %). The specific mechanism is still unclear, but gender, insulin resistance, and hyperuricemia are risk factors for NAFLD in obese children. Clinical evidence indicates that children with NAFLD experience an increased incidence and mortality of cardiovascular disease in adulthood [13]; therefore, early identification of the clinical characteristics is particularly important for Chinese children with NAFLD in light of the current obesity epidemic.

In this study, serum prolactin levels were significantly lower in obese patients with NAFLD, and a decreased serum prolactin level was associated with a significantly increased risk of NAFLD. Yan et al. reported that serum prolactin was a protective factor for NAFLD [14], and several studies have demonstrated that prolactin is involved in regulating whole-body insulin sensitivity and glucose metabolism [15, 16]. Prolactin upregulats pancreatic β-cells may be associated with cell cycle gene expression and DNA synthesis, which is known to result in increased glucose uptake and glucose utilization [17]. Physiologically elevated prolactin levels also improve hepatic insulin sensitivity and further improve energy and glucose homeostasis by increasing indirect effects of dopamine synthesis in the hypothalamus [10, 18]. The lower prolactin production involved in insulin sensitivity results in insulin resistance, which plays a key role in NAFLD development. Recent studies revealed that human adipose tissue produces prolactin and expresses prolactin receptors [19, 20]. Moreover, prolactin directly regulates the function of adipose tissue by downregulating lipoprotein lipase and fatty acid synthase, which consequently suppresses lipogenesis. The differences in precisely measured body fat distribution may be observed in children, as well as in adults, with high and low circulating prolactin levels and because body fat distribution strongly impacts on insulin resistance and NAFLD [21]. Zhang et al. demonstrated that PRL/PRLR improved hepatic steatosis via suppression of CD36 [22].

The present study revealed that a low prolactin level was significantly associated with HOMA-IR, which was confirmed by a recent study by Wang et al. that found that serum prolactin was associated with higher levels of HOMA-β [23]. However, no significant linear relationship was found, and any association between prolactin and HOMA-IR was also reported. In contrast, insulin inhibits prolactin expression and release from differentiated adipocytes, so the overall effect of insulin on prolactin is likely inhibitory [24]. Collectively, these results reveal that prolactin affects energy homeostasis through its action as an adipokine and is involved in the manifestation of insulin resistance.

Nonetheless, the present study has several limitations. First, the diagnosis of NAFLD was based on a hepatic ultrasound, and liver biopsy is the gold standard method. Second, other factors such as race/ethnicity, lifestyle-related parameters, and dietary intake of nutrients need to be further addressed in future studies.

▶ **Table 1** Comparison of general data and serum prolactin in the NAFLD and SOB groups.

Parameters	Before matching			After matching		
	SOB	NAFLD	p- Value	SOB	NAFLD	p- Value
Gender (male/female)	198/127	288/78	**<0.001**	196/68	196/68	1
Age (years)	10.87±2.53	10.83±2.46	0.836	10.77±2.55	10.82±2.47	0.819
Pubertal development (no/yes)	207/118	187/179	0.001	147/117	147/117	1
Course (years)	5.00 (3.00–7.50)	5.00 (3.00–8.00)	0.813	5.00 (3.00–7.00)	5.00 (3.00–8.00)	0.507
Height (cm)	150.22±14.64	149.81±14.95	0.714	150.25±14.90	150.22±14.47	0.977
Weight (Kg)	65.68±18.09	65.32±18.45	0.797	65.31±18.11	65.99±18.14	0.668
BMI	28.51±4.02	28.48±3.91	0.899	28.30±3.71	28.66±3.98	0.292
WC (cm)	90.53±11.08	90.43±11.45	0.907	89.79±10.87	90.74±11.43	0.331
HC (cm)	96.46±10.97	96.14±10.83	0.701	96.06±10.66	96.49±10.68	0.646
WHR	0.94 (0.90–0.98)	0.94 (0.90–0.98)	0.822	0.94±0.07	0.93±0.11	0.801
ALT (mmol/l)	28.00 (18.00–48.75)	30.00 (20.00–51.50)	0.087	28.00 (18.00–49.00)	29.00 (20.00–51.00)	0.219
AST (mmol/l)	25.00 (21.00–35.00)	27.00 (22.00–35.00)	0.1	25.00 (21.00–35.00)	27.00 (22.00–35.00)	0.223
UA (mmol/l)	377.50 (330.50–449.00)	384.00 (329.50–457.50)	0.729	381.00 (329.00–457.00)	380.00 (332.00–447.00)	0.903
TG mg/dl	1.16 (0.89–1.62)	1.24 (0.95–1.69)	0.092	1.12 (0.87–1.49)	1.32 (1.00–1.75)	**<0.001**
TC mg/dl	4.31±0.90	4.41±0.85	0.139	4.27±0.84	4.41±0.82	0.05
HDL mg/dl	1.24 (1.10–1.39)	1.22 (1.07–1.39)	0.729	1.27±0.26	1.25±0.25	0.363
LDL mg/dl	2.65±0.63	2.71±0.59	0.241	2.62±0.62	2.71±0.57	0.093
Apo A	83.0 (41.00–173.25)	80.0 (40.00–157.00)	0.754	83.00 (40.50–179.00)	80.50 (39.75–164.00)	0.822
IMT	0.06 (0.05–0.06)	0.06 (0.05–0.06)	0.077	0.06 (0.05–0.06)	0.06 (0.05–0.06)	0.213
HbA1c (mmol/mol)	5.69±0.81	5.71±0.72	0.756	5.68±0.85	5.67±0.75	0.948
FBG	5.30±0.39	5.38±0.78	0.107	5.30±0.39	5.39±0.88	0.160
Fasting insulin	20.43±9.64	25.72±15.63	<**0.001**	19.87±9.04	26.42±16.24	<**0.001**
HOMA- IR	4.53 (3.13–6.48)	5.03 (3.70–7.43)	**<0.001**	4.43 (3.05–6.33)	5.14 (3.75–7.59)	**<0.001**
LH (IU/l)	0.74 (0.07–3.21)	0.46 (0.07–2.15)	0.034	0.48 (0.07–2.65)	0.57 (0.07–2.17)	0.467
FSH (IU/l)	2.37 (0.91–4.24)	1.97 (0.69–3.46)	0.03	2.06 (0.77–4.03)	2.00 (0.69–3.57)	0.645
E2 (pmol/l)	158.15 (114.28–226.60)	149.34 (112.54–206.95)	0.087	156.90 (110.57–222.15)	146.94 (112.11–197.67)	0.135
Prolactin (mIU/l)	99.78 (63.89–153.82)	82.40 (56.36–118.70)	**<0.001**	93.87 (61.27–144.21)	82.48 (57.09–124.89)	0.063
T (nmol/l)	1.06 (0.68–2.33)	1.17 (0.64–2.91)	0.407	1.03 (0.65–2.56)	1.20 (0.66–2.77)	0.49

▶ **Table 2** Risk factors of NAFLD based on multivariate stepwise logistic regression.

Parameters	Beta	Standard error	Odds ratio	95 %CI	p-Value
TG	0.312	0.137	1.366	1.043–1.788	0.023
Prolactin	−0.004	0.001	0.996	0.994–0.999	0.002
HOMA-IR	0.214	0.039	1.239	1.148–1.338	<0.001

In conclusion, decreased serum prolactin may be a risk factor for NAFLD in obese children.

Author Contributions

Jian-Wei Zhang conceived and designed the study and wrote the manuscript. Jie-Qiong Guan, Xiao-Li Tang, and Jin-Liang Xu provided patient care and were responsible for communication with par-

▶ **Table 3** Associations between serum prolactin and NAFLD.

Parameters Prolactin	Case (n)	Unadjusted		Model 1		Model 2	
		Odds ratio (95 % CI)	p 1 Value	Odds ratio (95 % CI)	p 1 Value	Odds ratio (95 % CI)	p 1 Value
≤68.67	176	1.616 (1.061–2.461)	**0.026**	1.919 (1.198–3.076)	**0.007**	1.741 (1.059–2.860)	**0.029**
68.68–113.63	176	1.409 (0.926–2.144)	0.11	1.552 (0.998–2.416)	0.051	1.435 (0.905–2.274)	0.125
≥113.64	176	1		1		1	

ents. Jie-Qiong Guan performed data analysis. All authors have reviewed and approved the final version of the manuscript.

Acknowledgements

We are grateful to our participants and colleagues who provide help to our study.

Funding

This study is funded by the Health Commission of Zhejiang Province (2021KY1155) and the Science Technology Department of Shaoxing, China (Grant No. 2020A13033).

Conflict of Interest

The authors declare that they have no conflict of interest.

References

[1] Petroni ML, Brodosi L, Bugianesi E et al. Management of non-alcoholic fatty liver disease. BMJ 2021; 372: m4747

[2] Zhang X, Wan Y, Zhang S et al. Nonalcoholic fatty liver disease prevalence in urban school-aged children and adolescents from the Yangtze River delta region: a cross-sectional study. Asia Pac J Clin Nutr 2015; 24: 281–288

[3] Stefan N, Cusi K. A global view of the interplay between non-alcoholic fatty liver disease and diabetes. Lancet Diabetes Endocrinol 2022; 10: 284–296

[4] Pappachan JM, Babu S, Krishnan B et al. Non-alcoholic fatty liver disease: a clinical update. J Clin Transl Hepatol 2017; 5: 384–393

[5] Ben-Jonathan N, Hugo ER, Brandebourg TD et al. Focus on prolactin as a metabolic hormone. Trends Endocrinol Metab 2006; 17: 110–116

[6] Chirico V, Cannavò S, Lacquaniti A et al. Prolactin in obese children: a bridge between inflammation and metabolic-endocrine dysfunction. Clin Endocrinol (Oxf) 2013; 79: 537–544

[7] Matsuda M, Mori T, Sassa S et al. Chronic effect of hyperprolactinemia on blood glucose and lipid levels in mice. Life Sci 1996; 58: 1171–1177

[8] Ruiz-Herrera X, de Los Ríos EA, Díaz JM et al. Prolactin promotes adipose tissue fitness and insulin sensitivity in obese males. Endocrinology 2017; 158: 56–68

[9] Park S, Kim DS, Daily JW et al. Serum prolactin concentrations determine whether they improve or impair β-cell function and insulin sensitivity in diabetic rats. Diabetes Metab Res Rev 2011; 27: 564–574

[10] Park S, Kang S, Lee H-W et al. Central prolactin modulates insulin sensitivity and insulin secretion in diabetic rats. Neuroendocrinology 2012; 95: 332–343

[11] dos Santos Silva CM, Barbosa FRP, Lima GAB et al. BMI and metabolic profile in patients with prolactinoma before and after treatment with dopamine agonists. Obesity (Silver Spring) 2011; 19: 800–805

[12] Zhang J, Cao J, Xu H et al. Ferritin as a key risk factor for nonalcoholic fatty liver disease in children with obesity. J Clin Lab Anal 2021; 35: e23602

[13] Feldstein AE, Charatcharoenwitthaya P, Treeprasertsuk S et al. The natural history of non-alcoholic fatty liver disease in children: a follow-up study for up to 20 years. Gut 2009; 58: 1538–1544

[14] Zhang P, Feng W, Chu X et al. A newly noninvasive model for prediction of non-alcoholic fatty liver disease: utility of serum prolactin levels. BMC Gastroenterol 2019; 19: 202

[15] Cejkova P, Fojtikova M, Cerna M. Immunomodulatory role of prolactin in diabetes development. Autoimmun Rev 2009; 9: 23–27

[16] Fleenor DE, Freemark M. Prolactin induction of insulin gene transcription: roles of glucose and signal transducer and activator of transcription 5. Endocrinology 2001; 142: 2805–2810

[17] Arumugam R, Fleenor D, Lu D et al. Differential and complementary effects of glucose and prolactin on islet DNA synthesis and gene expression. Endocrinology 2011; 152: 856–868

[18] Lyons DJ, Hellysaz A, Broberger C. Prolactin regulates tuberoinfundibular dopamine neuron discharge pattern: novel feedback control mechanisms in the lactotrophic axis. J Neurosci 2012; 32: 8074–8083

[19] Zinger M, McFarland M, Ben-Jonathan N. Prolactin expression and secretion by human breast glandular and adipose tissue explants. J Clin Endocrinol Metab 2003; 88: 689–696

[20] Ling C, Svensson L, Odén B et al. Identification of functional prolactin (PRL) receptor gene expression: PRL inhibits lipoprotein lipase activity in human white adipose tissue. J Clin Endocrinol Metab 2003; 88: 1804–1808

[21] Stefan N. Causes, consequences, and treatment of metabolically unhealthy fat distribution. Lancet Diabetes Endocrinol 2020; 8: 616–627

[22] Zhang P, Ge Z, Wang H et al. Prolactin improves hepatic steatosis via CD36 pathway. J Hepatol 2018; 68: 1247–1255

[23] Wang T, Lu J, Xu Y et al. Circulating prolactin associates with diabetes and impaired glucose regulation: a population-based study. Diabetes Care 2013; 36: 1974–1980

[24] Hugo ER, Borcherding DC, Gersin KS et al. Prolactin release by adipose explants, primary adipocytes, and LS14 adipocytes. J Clin Endocrinol Metab 2008; 93: 4006–4012