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ARTICLE

Clinical research

Adenovirus 36 seropositivity is related to obesity risk, glycemic control, and leptin levels in Chilean subjects

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Abstract

Background Adenovirus 36 (Ad-36) has been associated to adiposity in animal and in vitro studies. Ad-36 seropositivity has also been reported to contribute to obesity risk in children and adult populations. We investigated the relationship of Ad-36 serology with obesity and metabolic parameters in a Chilean population.

Subjects and methods Clinical and anthropometric data were obtained and blood samples were drawn from 99 lean (BMI: 18.5–24.9 kg/m^2) and 151 obese (BMI > 30 kg/m^2) subjects. Laboratory tests included lipid profile as well as glucose, insulin, leptin, and adiponectin levels. Ad-36 seropositivity was evaluated in serum samples by enzyme-linked immunosorbent assay.

Results Seroprevalence of Ad-36 was higher in the obese group (58%) than in lean controls (34%) demonstrating that individuals previously infected with Ad-36 have higher risk of obesity in the study population (OR: 2.67, 95%CI: 1.58–4.51, $p < 0.001$). Interestingly, Ad-36 was related to lower concentrations of triglycerides and VLDL cholesterol in lean subjects ($p = 0.049$) and lower leptin in obese individuals ($p = 0.014$). Previous Ad-36 infection was also related to lower glycemia, insulinemia, and HOMA-IR ($p < 0.05$) in obese subjects who were not under antidiabetic drugs.

Conclusions Our results provide evidence of the contribution of previous Ad-36 infection to an increased risk of obesity in adult Chilean population. Ad-36 seropositivity was also associated to lipid profile, glycemic control, and leptin levels in adult Chilean population.

Introduction

Obesity is a major public health problem considering that its prevalence has exponentially risen worldwide. A rapid increase in obesity and its associated health care costs prompt the search for better approaches to obesity prevention and management [1]. Chile has experienced increasing rates of obesity; data from the last National Health Survey (2016) showed that 39.8% of individuals over 15 years old were overweight, while 31.2% were obese and another 3.2% were considered morbidly obese.

The etiology of obesity is a complex process due to the participation of environmental, cultural, psychosocial, and genetic factors. In this way, the role of pathogens as a cause of obesity has gained attention in the last years. This includes the adipogenic effects of various pathogens including human and non-human viruses, bacteria, and gut microbiota [2]. Among them, adenovirus type 36 (Ad-36)

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has been the most widely studied infectious agent in animals and humans because of its association with obesity [3].

In vitro experiments using 3T3-L1 adipocytes as well as studies in animal models demonstrate an adipogenic effect of Ad-36 infection, showing an increased adipocyte differentiation and fat accumulation [2, 4]. Studies on the associations between seroconversion against Ad-36, fat accumulation, and lipid metabolism alterations in adults and children have been carried out by evaluating the presence of antibodies against Ad-36 in the blood of obese and normal subjects, and correlating them with anthropometric measures and biochemical parameters.

Studies in children have been more consistent in establishing an association between obesity and Ad-36. In South Korean children, Ad-36 infection was related to increased body mass index (BMI) and obesity [5, 6]. Other recent studies performed in children/adolescents have demonstrated an association between Ad-36 seropositivity and an increased risk of obesity in Turkish [7] and Midwestern US populations [8]. However, studies in adults have shown some controversial results. Higher seroprevalence has been reported in obese subjects from different origins, compared with lean controls [9–11]. Contrarily, other works have reported no association between previous Ad-36 infection and obesity [12–15]. Taken together, a meta-analysis study considering more than 10,000 children and adults reported that, despite discordance in some results, previous infection with Ad-36 increases the risk of obesity [16].

Our study aimed to evaluate the association of previous Ad-36 infection with the risk of obesity in an adult Chilean population, as well as its relationship with metabolic parameters in lean and obese subjects.

Subjects and methods

Study population

The protocol was designed as a case and control study to evaluate the association of Ad-36 infection with the risk of obesity. Two-hundred and fifty individuals (Control group: 99 lean individuals with BMI between 18.5 and 24.9 kg/m^2; Case group: 151 obese individuals with BMI > 30 kg/m^2) were randomly selected in the Centro de Tratamiento de la Obesidad at the Clinica Alemana de Temuco and among workers from the Universidad de La Frontera in the city of Temuco, in southern Chile. All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of the Universidad de La Frontera (Protocol No. 159/15). Individuals of both sexes aged between 18 and 75 years, without liver or renal disease were selected. Individuals with secondary causes of obesity and pregnant women were not included in the study.

Clinical data and anthropometric measurements

All participants answered a questionnaire to collect personal information during an interview. Each individual provided information about cigarette smoking, alcohol consumption, physical activity, and medication use. Current tobacco smoking was considered as a daily intake of one or more cigarettes. Alcohol consumption was considered an intake of any dose of beer, wine, and/or distilled spirits according to World Health Organization recommendations. Physical exercise practice was considered as the practice of sports such as walking or running, for at least 2 h per week.

Anthropometric measurements were taken from each participant such as height, weight (to calculate BMI) as well as waist circumference. Weight in kilograms was measured in light indoor clothing without shoes to one decimal place. Height was measured without shoes in centimeters to one decimal place with a stadiometer. Waist circumference was determined at the narrowest point between the costal margin and iliac crest, at minimal respiration. Individuals were then included in the lean control group (BMI 18.5–24.9 kg/m^2) or in the obese case group (BMI ≥ 30 kg/m^2).

Systolic and diastolic blood pressure (SBP and DBP) were measured in supine position after resting for 30 min by a trained physician using a mercury column sphygmomanometer. Subjects with SBP/DBP over 140/90 mmHg or that were under anti-hypertensive therapy were considered hypertensive. Individuals with type 2 diabetes (T2D) were classified through prior clinical diagnosis, determined by reviewing their clinical records. Dyslipidemia was considered as any alteration in the lipid profile according to NCEP/ATPIII and/or use of hypolipidemics [17].

Laboratory tests

Blood samples were drawn for evaluation of Ad-36 serology and measurements of glycemic and lipid profiles after fasting for 12 h. Serum glucose, triglycerides, and total LDL and HDL cholesterol were measured by enzyme-colorimetric methods in a Cobas c311 analyzer (Roche Diagnostics, Risch-Rotkreuz, Switzerland). Briefly, Hexoquinasa method was used to determine glucose concentration, whereas CHOD/PAP and GPO/PAP methods were used for determination of cholesterol and triglycerides (Roche Diagnostics, Risch-Rotkreuz, Switzerland). Values of VLDL cholesterol were calculated as triglycerides divided into five (TG/5). Enzyme-linked immunosorbent assays (ELISA) purchased from Invitrogen (Carlsbad, CA, USA) were used to determine concentrations of insulin (Cat# KAQ1251), leptin (Cat# KAC2281), and adiponectin

(Cat# KHP0041). Concentrations of glucose and insulin were used to calculate the homeostasis model assessment for evaluation of insulin resistance (HOMA-IR). Individuals with HOMA-IR >2.6 were considered insulin resistant.

A qualitative determination for the presence of antibodies against Ad-36 was performed using the Adenovirus 36 Antibody (AdV36-Ab) ELISA Kit (MyBiosource *#MBS9310682*, San Diego, CA, USA) according to the manufacturer instructions. Serum samples were processed in duplicate and classified as seronegative or seropositive according to recommended cut-off absorbance.

Statistical analysis

The results were analyzed using SigmaStat 3.5 (Systat Software Inc., San Jose, CA, USA) and Minitab 17.10 statistical software (Minitab Inc., State College, PA, USA). Initially, descriptive analysis was performed, and then comparisons were done assuming a significance level of $p < 0.05$. Categorical variables are shown as counts and percentages and continuous variables are presented as mean ± standard deviation (SD). Chi-square test was used to compare categorical variables. Continuous variables were initially tested for normality using the K–S test. Then, comparisons between two groups were performed by *t*-test or Mann–Whitney *U* test for parametric and non-parametric data, respectively. A multiple logistic regression analysis was performed to assess the contribution of previous Ad-36 infection on the risk of obesity, using a step-wise method for variable selection among relevant clinical variables.

Results

Main characteristics of study groups

Table 1 shows clinical, demographic, anthropometric, and laboratory data of lean controls and obese individuals enrolled in this study. As expected, obese individuals had higher weight, BMI, and waist circumference ($p < 0.001$). Control and study groups did not differ according to age, current tobacco smoking, and alcohol consumption ($p > 0.05$); women and the practice of physical activity were less frequent in the obese group ($p < 0.05$). Moreover, mean values of SBP and DBP, as well as the frequencies of hypertension, dyslipidemia, T2D, insulin resistance, and the use of antihypertensive, hypolipemiant, and antidiabetic drugs were higher in obese as compared to lean control group ($p < 0.05$).

Laboratory analysis demonstrated a more atherogenic lipid profile in obese individuals, showing higher values of triglycerides, LDL and VLDL cholesterol, and lower concentrations of HDL cholesterol than lean individuals ($p < 0.05$; Table 1). Obese subjects also had higher glucose and insulin levels, with higher indices of insulin resistance demonstrated by elevated HOMA-IR values ($p < 0.001$; Table 1). As expected for obese individuals, this group had lower adiponectin and higher leptin concentrations compared to lean controls (Table 1).

Table 1 Demographic and clinical characteristics of lean controls and obese group

Variable	Lean controls ($n = 99$)	Obese ($n = 151$)	*p*-Value
Clinical and demographic data			
Age, years	45.5 ± 8.5	43.9 ± 10.7	0.216
Sex [women], %	76 (75)	64 (96)	**0.047**
Tobacco smoking, %	26 (26)	28 (42)	0.747
Alcohol consumption, %	65 (64)	55 (83)	0.130
Physical activity, %	72 (71)	23 (35)	**<0.001**
SBP, mmHg	115 ± 13	124 ± 15	**<0.001**
DBP, mmHg	68 ± 10	73 ± 13	**<0.001**
Hypertension, %	9 (9)	32 (48)	**<0.001**
Dyslipidemia, %	28 (28)	57 (86)	**<0.001**
Type 2 diabetes, %	1 (1)	12 (18)	**0.015**
Insulin resistance, %	15 (15)	70 (106)	**<0.001**
Medication, %			
Antihypertensive	6 (6)	25 (37)	**0.014**
Hypolipemiant	5 (5)	14 (21)	**0.024**
Antidiabetic	2 (2)	22 (34)	**<0.001**
Anthropometric measurements			
Weight, kg	61.2 ± 8.5	94.9 ± 17.4	**<0.001**
Body mass index, kg/m^2	23.1 ± 3.1	35.9 ± 5.2	**<0.001**
Waist circumference, cm	81.7 ± 6.8	106.7 ± 11.7	**<0.001**
Biochemical parameters			
Total cholesterol, mg/dL	199 ± 78	196 ± 37	0.717
LDL cholesterol, mg/dL	107 ± 25	118 ± 31	**0.005**
HDL cholesterol, mg/dL	63 ± 15	51 ± 12	**<0.001**
VLDL cholesterol, mg/dL	22 ± 11	32 ± 18	**<0.001**
Triglycerides, mg/dL	108 ± 57	160 ± 91	**<0.001**
Glucose, mg/dL	87 ± 23	101 ± 32	**<0.001**
Insulin, mUI/L	10.8 ± 9.7	22.4 ± 24.3	**<0.001**
HOMA-IR, a.u.	2.08 ± 1.05	5.75 ± 7.3	**<0.001**
Adiponectin, μg/mL	10.14 ± 6.19	8.17 ± 6.01	**0.003**
Leptin, ng/mL	14.09 ± 10.42	29.77 ± 23.58	**<0.001**

Number of individuals is in parenthesis. Continuous variables are presented as mean and standard deviation and compared by *t* test or Mann–Whitney *U* test. Categorical variables are presented as percentage and compared by Chi-square test

SBP systolic blood pressure, *DBP* diastolic blood pressure, *LDL* low density lipoprotein, *HDL* high density lipoprotein, *VLDL* very low density lipoprotein, *HOMA-IR* homeostasis model assessment of insulin resistance, *a.u.* arbitrary units

Association of adenovirus 36 infection with risk of obesity and clinical and laboratory data

Evaluation of antibodies against Ad-36 showed a seroprevalence of 34% in the control group and 58% among obese individuals (Fig. 1). This demonstrates that individuals previously infected with Ad-36 have higher risk of obesity in our study population (OR: 2.67, 95%CI: 1.58–4.51, $p < 0.001$). A logistic regression analysis using relevant clinical covariates (age, sex, physical activity, hypertension, dyslipidemia, and insulin resistance) confirmed that Ad-36 infection independently contributed to the risk of obesity (OR: 2.66, 95%CI: 1.06–6.65, $p = 0.036$; Table 2).

Table 3 shows clinical, anthropometric, and laboratory data from lean controls and obese individuals according to Ad-36 serology. Clinical and anthropometric data did not show any statistical difference between individuals seropositive and seronegative against Ad-36 in both groups ($p > 0.05$).

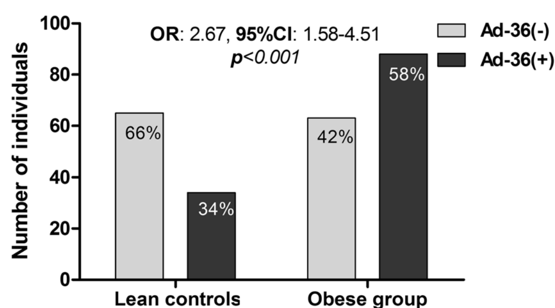

Fig. 1 Association of adenovirus 36 seropositivity with obesity in the study population. Adenovirus 36 (Ad-36) seroconversion was evaluated in serum samples of lean controls and obese individuals. Association between Ad-36 serology status and obesity was evaluated by Chi-square test and then the odds ratio (OR) and its 95% confidence interval (95%CI) were also calculated

Table 2 Contribution of previous Ad-36 infection to the risk of obesity: multiple logistic regression analysis

Variable	Odds ratio	95% Confidence interval	*p*-Value
Ad36 [ref: seronegative]	2.66	1.06–6.65	**0.036**
Age	0.93	0.88–0.98	**0.006**
Sex [ref: masculine]	0.37	0.13–1.03	0.058
Physical activity	0.10	0.04–0.27	**<0.001**
Hypertension	3.82	1.12–13.03	**0.033**
Dyslipidemia	3.30	1.21–8.98	**0.019**
Insulin resistance	13.09	4.69–36.50	**<0.001**

Results from a multiple logistic regression analysis introducing obesity as the dependent variable into the model. A stepwise method was used for the selection of relevant variables among clinical and demographic variables

Lean control subjects seropositive against Ad-36 had lower concentrations of triglycerides and VLDL cholesterol ($p = 0.049$). Moreover, a trend of lower glycemia was observed in this group; however, no statistical significance was reached ($p = 0.097$; Table 3). No other associations of Ad-36 infection and biochemical parameters were observed in the control group.

Parameters of lipid and glycemic profiles were not related to Ad-36 infection in obese subjects (Table 3). However, due to the number of obese subjects under anti-diabetic treatment, we also evaluated the association of Ad-36 with glycemic homeostasis parameters in users and non-users of anti-diabetic drugs. As observed in Fig. 2, values of glucose, insulin, and HOMA-IR were significantly lower in Ad-36 seropositive obese individuals who did not receive anti-diabetic treatment ($p < 0.05$). Individuals under anti-diabetic treatment also had lower concentration of glucose ($p < 0.05$).

Adiponectin levels were not related to Ad-36 infection in lean controls and obese groups (Fig. 3). Regarding leptin levels, previous Ad-36 infection contributed to lower concentrations of leptin among obese individuals ($p = 0.014$), but no association was observed in lean subjects (Fig. 3).

Discussion

Our results from a sample of adults from southern Chile showed a higher prevalence of Ad-36 antibodies in obese subjects as compared to lean controls. This demonstrates that previous Ad-36 infection contributed to an increased risk of obesity in our population, which was confirmed in a logistic regression analysis adjusted for relevant clinical confounders. This is the first report evaluating the association of Ad-36 with obesity in South American populations.

Higher seroprevalence of Ad-36 in obese subjects has been described in early studies in adult populations [9–11]. The results of the first study to screen humans for the presence of Ad-36 neutralizing antibodies showed a significantly greater prevalence of the virus in obese people (30%) than in non-obese people (11%) in 502 participants from the USA [9]. The prevalence of Ad-36 infection was about 60% in severely obese participants ($BMI \geq 50$). Similar results were reported by Trovato et al. [10] in obese and normal subjects from Italy. Authors concluded that previous infection with Ad-36 was significantly more common in the obese than in the control group (64% vs. 32% for obese and lean subjects, respectively). Subsequently, other studies have shown higher seroprevalence in obese than in lean subjects in Chinese (47% vs. 32%) [18], Swedish women (28% vs. 15%) [19], and Turkish (18% vs. 4%) [11] populations. Conversely, other studies performed in Belgium [12], USA [13], Korea [14], and China [15]

Table 3 Clinical, anthropometric, and biochemical data in normoweight and obese individuals according to Ad-36 serology status

Variable	Lean controls			Obese		
	Ad-36(−)	Ad-36(+)	*p*-Value	Ad-36(−)	Ad-36(+)	*p*-Value
Ad-36 prevalence, %	66 (65)	34 (34)	–	42 (63)	58 (88)	–
Age, years	45.1 ± 8.5	46.1 ± 8.4	0.614	43.9 ± 11.7	43.8 ± 10.0	0.999
Sex [women], %	75	78	0.678	59	68	0.264
SBP, mmHg	116 ± 11	114 ± 16	0.426	124 ± 15	123 ± 15	0.693
DBP, mmHg	68 ± 10	66 ± 11	0.334	73 ± 14	73 ± 12	0.880
Hypertension, %	9 (6)	8 (3)	0.928	40 (25)	26 (23)	0.894
Dyslipidemia, %	31 (20)	24 (8)	0.774	54 (34)	59 (52)	0.631
Type 2 diabetes, %	2 (1)	0 (0)	–	13 (8)	11 (10)	0.901
Insulin resistance, %	14 (9)	17 (6)	0.737	71 (45)	69 (61)	0.878
Anthropometric measures						
Weight, kg	61.6 ± 8.7	60.5 ± 8.0	0.524	94.6 ± 14.8	95.1 ± 19.27	0.863
Body mass index, kg/m^2	23.4 ± 2.0	22.4 ± 4.5	0.225	35.9 ± 4.6	35.9 ± 5.6	0.928
Waist circumference, cm	82.0 ± 6.7	80.9 ± 6.9	0.453	107.5 ± 11.8	106.1 ± 11.7	0.517
Biochemical parameters						
Total cholesterol, mg/dL	202 ± 93	193 ± 38	0.475	193 ± 37	198 ± 38	0.373
LDL cholesterol, mg/dL	108 ± 25	106 ± 26	0.742	114 ± 32	121 ± 30	0.230
HDL cholesterol, mg/dL	64 ± 16	60 ± 12	0.130	51 ± 14	50 ± 11	0.662
VLDL cholesterol, mg/dL	22 ± 12	18 ± 11	**0.049**	32 ± 18	31 ± 18	0.920
Triglycerides, mg/dL	112 ± 58	91 ± 37	**0.049**	161 ± 3	159 ± 92	0.920
Glucose, mg/dL	87 ± 12	83 ± 10	0.097	104 ± 30	99 ± 24	0.406
Insulin, mUI/L	11.1 ± 11.3	10.1 ± 3.2	0.569	25.4 ± 34	20 ± 11	0.300
HOMA-IR, a.u.	2.10 ± 1.16	2.08 ± 0.71	0.447	6.56 ± 9.43	5.17 ± 5.12	0.362
Adiponectin, μg/mL	10.57 ± 6.43	9.26 ± 5.73	0.319	8.04 ± 4.75	8.27 ± 6.80	0.811
Leptin, ng/mL	14.46 ± 11.22	13.32 ± 8.60	0.596	36.2 ± 28.5	25 ± 18.3	**0.014**

Number of individuals is in parenthesis. Continuous variables are presented as mean and standard deviation and compared by *t* test or Mann–Whitney *U* test. Categorical variables are presented as percentage and compared by Chi-square test

SBP systolic blood pressure, *DBP* diastolic blood pressure, *LDL* low density lipoprotein, *HDL* high density lipoprotein, *VLDL* very low density lipoprotein, *HOMA-IR* homeostasis model assessment of insulin resistance, *a.u.* arbitrary units

have shown no association between Ad-36 seropositivity and overweight/obesity. Various factors could explain discordant results about the association of Ad-36 and obesity as well as differences in the seroprevalence reported here and in previous works. These confounding factors could include inadequate sample size, geographical and age group differences, viral load and persistence of the virus in the body as well as inappropriate methods of evaluating serum Ad-36 antibodies [3]. Furthermore, despite controversial results among different populations, evidence from two meta-analyses which evaluated cross-sectional and case–control studies including both children and adult populations suggests an overall increased risk of obesity in seropositive individuals, with a pooled odds ratio of 1.90 (95%CI: 1.01–3.56) [20] and 2.0 (95%CI: 1.46–2.74) [16].

In vitro and animal studies have proposed mechanisms that explain the adipogenic effect of Ad-36, supporting its role in promoting obesity in human beings. Dhurandhar et al. [21] found that chickens and mice infected with Ad-36 showed a sharp increase in body weight due to substantial fat accumulation; whereas there was no variation in the animals inoculated with an avian adenovirus. Subsequently, an adiposity-promoting effect was demonstrated in two species of nonhuman primates [4]. Further studies in humans also indicated that some individuals carry Ad-36 DNA in the visceral adipose tissue, suggesting that adipogenic effect from the virus is present in obese patients [22, 23]. In vitro experiments using 3T3-L1 preadipocytes, as well as human primary preadipocytes, demonstrated that Ad-36 increases differentiation and lipid accumulation [24]. An enhanced expression of fatty acid synthase (*FASN*) and acetyl-CoA carboxylase 1 (*ACC1*) was observed in Wistar rats infected with Ad-36. The latter suggests that it promotes the conversion of glucose to lipids in adipocytes via the up-regulated de novo lipogenic pathway [25]. Interestingly, further evidence has suggested that the viral gene

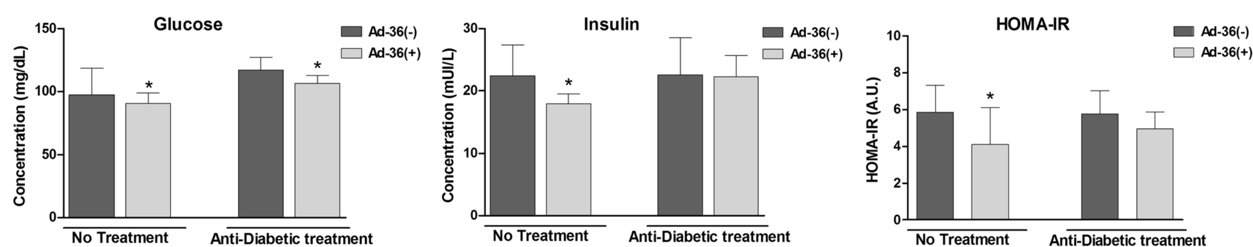

Fig. 2 Glycemic control parameters in users and non-users of anti-diabetic drugs according to Ad-36 serology in obese subjects. Columns and error bars represent mean and standard deviation. Glycemia and insulinemia were measured in obese individuals without treatment (no treatment) and users of anti-diabetic drugs (anti-diabetic treatment). The homeostasis model assessment of insulin resistance (HOMA-IR) was estimated. Variables were compared between individuals seronegative [Ad-36(−)] and seropositive [Ad-36(+)] against adenovirus 36 using *t* test. A.U. arbitrary units. $*p < 0.05$

E4orf1 (E4 open reading frame orf-1) is mainly responsible for fat cell stimulation [26]. In that work, authors also demonstrated that the Ad-36 *E4orf1* gene is necessary and sufficient for Ad-36-induced adipogenesis.

Ad-36 was slightly associated to reduced triglycerides ($p = 0.049$) in lean controls from our study, which was not observed in the obese group. A paradoxical reduction of serum lipids has been previously reported in animals infected with Ad-36 [4, 21], and in studies evaluating humans carrying antibodies against Ad-36 [9, 14]. Particularly, Ad-36 seropositivity was associated with lower serum total cholesterol and triglycerides concentrations in obese participants from the USA [9]. Meanwhile normo-weight, overweight, and obese participants who tested positive for Ad-36 antibody had lower levels of triglycerides in Korean population [14]. Nevertheless, the association of Ad-36 with serum lipids in human studies has shown contradictory results. Whereas no association with serum lipids has been reported by some authors [13, 27], other studies have shown increased concentrations of triglycerides in individuals previously infected with Ad-36 [6, 10]. In addition, regarding clinical outcomes from relationship of Ad-36 with serum lipids, Ad-36 seropositivity was associated with a lower occurrence of non-alcoholic fatty liver disease (NAFLD) and, moreover, seropositive patients with NAFLD submitted to a lifestyle-nutritional intervention have a more consistent decrease in insulin resistance, fatty liver severity, and body weight in comparison with Ad-36 seronegative patients [28, 29]. Current evidence has failed to explain both, the mechanisms involved in alterations of serum lipids and the contradictory results from human studies, creating the necessity to perform further investigations considering a large number of confounding variables, such as a more controlled report of diet and medication use.

Ad-36 can increase glucose uptake by murine fat cells [25] and human primary skeletal muscle [30]. Interestingly, our results showed that Ad-36 seropositive subjects had a trend of lower glycemia in the lean control group, as well as a significant reduction of glycemia, insulinemia, and HOMA-IR in obese individuals who were not under anti-diabetic treatment. Regarding studies evaluating the relationship between Ad-36 and glycemic control in humans, only a few studies have been published. In Swedish adults [19], it was reported that Ad-36 infection was associated with lower occurrence of T2D and better insulin sensitivity in adults, particularly among females. Authors described that Ad-36 seropositivity was more common in those with normal glucose tolerance (NGT) than in those with diabetes (females: OR 17.2, 95%CI 4.0–74.3; males: OR 3.5, 95%CI 1.8–6.7). Also, females with NGT had a higher frequency of Ad-36 seropositivity than females with prediabetes. Similarly, a study that compared longitudinal observations in indices of adiposity and glycemic control in Ad-36-infected vs. uninfected adults ($n = 1400$) informed that Ad-36 increased adiposity and attenuated deterioration of glycemic control [31]. The last study measured BMI and fat composition as well as fasting glucose and insulin at baseline and at ≈10 years past the baseline, and compared seropositive and seronegative subjects.

E4orf1 is also involved in mechanisms that lead to hypoglycemic action of Ad-36 [32]. In fact, a comprehensive in vitro study demonstrated that the E4orf1 protein of Ad-36 is necessary and sufficient for enhancing glucose disposal [33]. This study showed that the glucose uptake enhanced by Ad-36 can be abrogated by knocking down *E4orf1* with siRNA. Furthermore, transfection with *E4orf1* significantly increases glucose uptake in preadipocytes, adipocytes, or myoblasts, and reduces glucose output by hepatocytes. Recently, in db/db or diet-induced obesity mice, hepatic expression of Ad-36 *E4orf1* robustly improved glycemic control and promoted glucose metabolism through AKT activation which lead to induction of GLUT4 translocation [34].

As expected, increased leptin and decreased adiponectin circulation levels were observed in obese individuals from this study. Ad-36 seropositive individuals from this group also had lower leptin concentration. Reduced leptin expression and secretion due to Ad-36 infection was

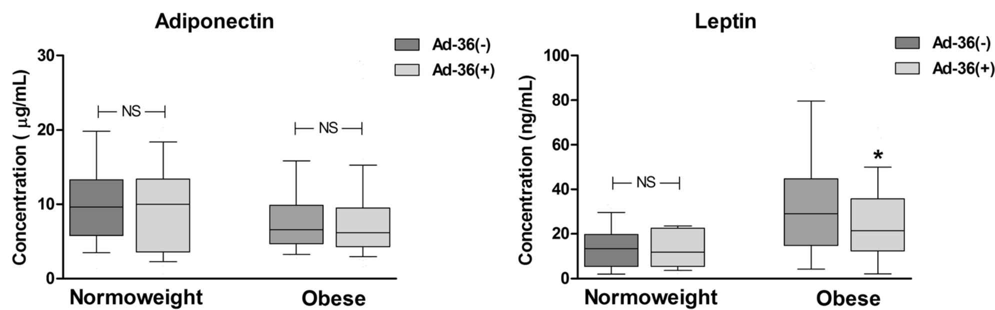

Fig. 3 Adenovirus 36 infection and adiponectin and leptin levels in lean controls and obese individuals. Box plots represent plasma concentrations of adiponectin and leptin in individuals seronegative [Ad-36(−)] and seropositive [Ad-36(+)] against adenovirus 36. Values were compared by Mann–Whitney *U* test. NS not significant; $^{*}p < 0.05$

reported early in fat cells [25]. Vangipuram et al. [25] also described a reduction in adipose tissue leptin expression in a group of male Wistar rats infected with Ad-36. Influence of Ad-36 on adipokine levels is less understood and there are only a few reports in humans. Different from basic in vitro research and the results presented here, Ergin et al. reported that subjects carrying Ad-36 antibodies had higher leptin and reduced adiponectin in 49 obese Turkish adults [35]. On the other hand, no differences in leptin and lower adiponectin levels in Ad-36 seropositive individuals were reported in 71 obese children from the same population [7]. Differences in sample size, geographical area, and age of the study population could explain the contradictory results with previous reports in humans.

This study has some limitations, such as the method used to screen Ad-36 antibodies. The serum neutralization assay (SNA) is the gold standard to specifically detect neutralizing antibodies to Ad-36. Although an enzyme immunoassay provides a quicker and more objective determination, it could be non-specific with some false-positive results. Nevertheless, reported cross-sectional and case–control studies have used both ELISA and SNA [16]. Also, it is important to consider that our results are in line with previous reports regarding the association of Ad-36 with obesity and biochemical metabolic parameters. Moreover, better-controlled data regarding diet as well as dose and time of medication would contribute to a more comprehensive evaluation of Ad-36 on anthropometric and biochemical variables. Also, an important difference was observed between lean controls and obese group regarding the prevalence of physical activity in our study population, however there was no association of Ad-36 seropositivity with physical activity, and moreover, the multiple logistic regression model also showed that Ad-36 contributed to obesity risk even considering physical activity as a covariate.

In conclusion, our results provide evidence about previous Ad-36 infection and its effect on the risk of obesity in an adult Chilean population, as well as its association with lipid profile and glycemic control.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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