Changes in plasma endocannabinoid levels in viscerally obese men following a 1 year lifestyle modification programme and waist circumference reduction: associations with changes in metabolic risk factors

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Abstract

Aims/hypothesis We previously reported that the plasma levels of the endocannabinoid, 2-arachidonoylglycerol (2-AG), in a cohort of viscerally obese men are directly correlated with visceral adipose tissue (VAT) accumulation and metabolic risk factors including low HDL-cholesterol and high triacylglycerol. It is not known, however, if such

correlations persist after vigorous lifestyle interventions that reduce metabolic risk factors. We analysed the changes in endocannabinoid levels in a subsample from the same cohort following a 1 year lifestyle modification programme, and correlated them with changes in VAT and metabolic risk factors.

Methods Forty-nine viscerally obese men (average age 49 years, BMI 30.9 kg/m², waist 107.3 cm) underwent a 1 year lifestyle modification programme including healthy eating and physical activity. Plasma levels of 2-AG and the other most studied endocannabinoid, anandamide, were measured by liquid chromatography–mass spectrometry. Anthropometric and metabolic risk factors, including VAT, insulin resistance and glucose intolerance, HDL-cholesterol and triacylglycerol, were measured.

Results Most risk factors were improved by the intervention, which led to a significant decrease in body weight (–6.4 kg, $p<0.0001$), waist circumference (–8.0 cm, $p<0.0001$) and VAT (–30%, $p<0.0001$), and in plasma 2-AG (–62.3%, $p<0.0001$) and anandamide (–7.1%, $p=0.005$) levels. The decrease in levels of 2-AG but not those of anandamide correlated with decreases in VAT and triacylglycerol levels, and with the increase in HDL₃-cholesterol levels. Multivariate analyses suggested that decreases in 2-AG and VAT were both independently associated with decreases in triacylglycerol.

Conclusions/interpretation This study shows that a strong correlation exists between 2-AG levels and high plasma triacylglycerol and low HDL₃-cholesterol in viscerally obese men.

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Abbreviations

2-AG	2-arachidonoylglycerol
CRP	C-reactive protein
EC	endocannabinoid
InsRes	variable of insulin resistance
RepIns30	variable of insulin resistance
SAT	subcutaneous adipose tissue
TG	triacylglycerol
VAT	visceral adipose tissue

Introduction

Elevated concentrations of endogenous agonists of cannabinoid CB₁ receptors (endocannabinoids [ECs]) in tissues are associated with obesity and with excess visceral adipose tissue (VAT) accumulation and related metabolic abnormalities (high plasma triacylglycerols [TGs], low HDL-cholesterol, high fasting glucose and insulin levels, insulin resistance; see [1] for a recent review). In obese patients, higher levels of the EC, 2-arachidonoylglycerol (2-AG), but not of another EC, anandamide, are found in VAT, but not subcutaneous adipose tissue (SAT), as compared with lean volunteers [2]. In two independent cross-sectional studies, increased plasma levels of 2-AG, but not anandamide, were shown to correlate with high levels of VAT, but not SAT, as well as with several of the aforementioned metabolic alterations [3, 4]. In view of the proposed pro-lipogenic and glucose intolerance-inducing actions of CB₁ receptor stimulation in rodents [1], an upregulated EC system in the VAT of obese patients might lead to an increased VAT accumulation and contribute to ectopic fat accumulation and insulin resistance. However, whether interventions aimed at specifically reducing VAT in abdominally obese patients also reduce circulating EC levels has not been investigated. Therefore, it is not known if intervention-induced changes in EC levels correlate with changes in metabolic dysfunctions associated with VAT. Such an observation would establish a relationship between the overactive endocannabinoid system and some metabolic risk factors that is stronger than that implied by cross-sectional studies [3, 4]. In the present study, we aimed to obtain this information by investigating, in a sample of viscerally obese patients, the effect of a 1 year lifestyle modification programme, consisting of healthy eating and regular physical activity/exercise, on EC levels, VAT accumulation and metabolic cardiovascular risk factors.

Methods

The study included 49 asymptomatic men aged 32–65 years without diabetes, characterised by visceral obesity and high

TG–low HDL-cholesterol. Men were assigned to a personalised nutritional and physical activity exercise management programme offered by a multidisciplinary team which included a monthly visit to a dietitian and an exercise physiologist. All participants gave their written consent to participate in the study, which was approved by the Medical Ethics Committee of Hôpital Laval.

Anthropometric measurements were performed following standardised techniques [5]. Measurement of L4–L5 abdominal adipose tissue areas was performed by computed tomography [6]. Complete fasting plasma lipoprotein–lipid profile was obtained as described [4] and LDL particle size was measured by non-denaturing polyacrylamide gradient gel electrophoresis [7]. A 75 g OGTT was performed in the morning after an overnight fast. Plasma glucose and insulin were measured as described [8, 9]. The total glucose and insulin AUCs during the OGTT were determined with the trapezoid method. Variables of insulin resistance were calculated using the equations: $\text{InsRes} = \{[\text{fasting insulin (pmol/l)}/7.2] \times \text{fasting glucose (mmol/l)}\}/22.5$ and $\text{RepIns30} = \{[\text{fasting insulin (pmol/l)} + \text{insulin at 15 min (pmol/l)}]/2 \times 15\} + \{[\text{insulin at 15 min (pmol/l)} + \text{insulin at 30 min (pmol/l)}]/2 \times 15\} - [\text{fasting insulin (pmol/l)} \times 30]$, both derived from HOMA model at baseline.

EC levels were measured by isotope-dilution liquid chromatography–atmospheric pressure chemical ionisation–mass spectrometry as described [4]. Fasting plasma adiponectin, leptin (B-Bridge International, Mountain View, CA, USA) and IL-6 (R&D Systems, Minneapolis, MN, USA) concentrations were determined by ELISA. Concentrations of high-sensitivity C-reactive protein (CRP) were measured with a highly sensitive immunoassay and performed with a Behring BN-100 nephelometer (Dade Behring, Marburg, Germany) as described [10]. Data as means $\pm$ SD were analysed by the non-parametric Sign test for paired data. When variables were not normally distributed, a logarithmic transformation was used instead. Relationships between variables were assessed by Pearson's correlation, except for abnormally distributed variables which were assessed with Spearman's correlation and/or Pearson's correlation on log-transformed data. A *p* value <0.05 indicated statistical significance.

Results

The 1 year intervention reduced body weight (−6.4 kg), BMI (−2.1 kg/m²), waist circumference (−8.0 cm), VAT (−79.3 cm²) (Table 1) and supine diastolic and systolic BP (−5.9 $\pm$ 6.0 and −5.6 $\pm$ 9.6 mmHg from baselines of 82.3 $\pm$ 6.6 and 122.8 $\pm$ 10.6 mmHg, *p* $\leq$ 0.0002). It also significantly ameliorated nearly all conventional metabolic risk factors, including TGs (−0.74 mmol/l), HDL-cholesterol (+0.13 mmol/l),

Table 1 Anthropometric and biochemical variables at baseline and following a 1 year lifestyle modification programme

Variable	<i>n</i>	Baseline	Post 1 year intervention	Absolute change	Change (%)	<i>p</i> value
Body weight (kg)	49	93.9±12.5	87.5±13.4	−6.4±4.5	−6.9±4.9	<0.0001
BMI (kg/m ²)	49	30.9±3.3	28.8±3.6	−2.1±1.5	−6.8±4.8	<0.0001
Waist circumference (cm)	49	107.3±9.8	99.3±11.6	−8.0±4.9	−7.6±4.6	<0.0001
L4–L5 visceral AT (cm ²)	49	270.9±82.4	192.7±93.9	−79.3±56.2	−30.3±18.6	<0.0001
L4–L5 subcutaneous AT (cm ²)	49	295.8±93.3	242.0±97.6	−52.5±43.3	−18.5±13.0	<0.0001
Total cholesterol (mmol/l)	47	5.12±0.70	4.99±0.75	−0.12±0.57	−1.94±11.3	NS
LDL-cholesterol (mmol/l)	47	3.01±0.55	3.11±0.70	0.11±0.53	3.98±18.1	NS
VLDL-cholesterol (mmol/l)	47	1.16±0.55	0.80±0.41	−0.36±0.43	−27.8±29.3	<0.0001
HDL-cholesterol (mmol/l)	47	0.95±0.16	1.08±0.20	0.13±0.14	15.0±15.4	<0.0001
HDL ₂ -cholesterol (mmol/l)	47	0.33±0.10	0.39±0.14	0.07±0.11	25.9±36.5	0.0019
HDL ₃ -cholesterol (mmol/l)	47	0.62±0.10	0.69±0.09	0.06±0.08	11.8±14.5	0.0011
Non-esterified triacylglycerol (mmol/l)	47	2.72±1.08	2.00±0.77	−0.74±1.05	−22.2±29.4	<0.0001
LDL-triacylglycerol (mmol/l)	47	0.31±0.14	0.23±0.09	−0.08±0.07	−23.5±17.7	<0.0001
VLDL-triacylglycerol (mmol/l)	47	2.15±0.94	1.56±0.71	−0.61±0.96	−21.5±36.3	0.0002
HDL-triacylglycerol (mmol/l)	47	0.26±0.07	0.21±0.05	−0.05±0.06	−17.6±18.0	<0.0001
Apolipoprotein B (g/l)	47	1.08±0.16	1.01±0.19	−0.07±0.12	−6.88±11.6	0.003
Apolipoprotein A-I (g/l)	47	1.11±0.15	1.27±0.15	0.15±0.11	14.6±10.9	<0.0001
LDL particle size (Å)	49	251.4±4.1	252.9±3.9	1.6±3.8	0.65±1.49	0.066
Fasting glucose (mmol/l)	49	6.0±0.5	6.0±0.5	0.01±0.4	0.39±6.03	NS
Fasting insulin (pmol/l)	49	169.8±82.8	117.9±48.2	−51.9±71.4	−17.1±44.9	<0.0001
AUC glucose (mmol/l × min)	49	1,381±316	1,291±355	−90.2±318.2	1.63±70.9	0.09
AUC insulin (pmol/l × min ×10 ^{−4})	49	16.5±7.8	10.9±6.1	−5.6±6.6	−18.6±103.4	<0.0001
InsRes	49	6.3±3.1	4.4±1.9	−1.9±2.8	−15.9±47.5	0.005
RepIns30 (×10 ^{−3})	49	10.8±7.5	7.9±6.1	−2.9±7.3	−28.6±100.3	0.01
Leptin (ng/ml)	49	12.1±9.4	9.3±10.1	−2.8±3.9	−23.8±32.9	<0.0001
CRP (mg/l)	48	1.8±1.2	1.3±1.9	−0.6±1.5	−31.6±47.0	<0.0001
IL-6 (pg/ml)	49	1.3±1.0	1.2±0.8	−0.1±0.8	3.58±58.4	NS
Adiponectin (µg/ml)	46	3.4±1.4	4.1±1.3	0.6±1.0	27.7±47.0	0.02

Data are presented as means±SD

VLDL-cholesterol (−0.36 mmol/l), fasting insulin levels (−51.9 pmol/l), AUCs for insulin and glucose levels (−5.6 mmol/l×min×10^{−4} and −90.2 mmol/l×min), as well as LDL particle size, high sensitivity-CRP and adiponectin levels, with no significant changes in LDL-cholesterol and fasting glucose levels (Table 1).

However, the 1 year intervention also caused a significant decrease in the plasma levels of anandamide (−7.1%) and, particularly, 2-AG (−62.3%; Fig. 1a,c).

Decreases in plasma 2-AG levels correlated positively with decreases in VAT ($r=0.30$, $p=0.04$), total ($r=0.54$, $p<0.0001$) and HDL- and VLDL-associated TGs ($r=0.48$, $p<0.0007$ and $r=0.50$, $p=0.0003$, respectively), and RepIns30, a measure of insulin resistance ($r=0.44$, $p=0.002$), and with increases in HDL₃-cholesterol ($r=−0.35$, $p<0.0001$). Decreases in anandamide levels correlated inversely with decreases in fasting C-peptide levels ($r=−0.29$, $p=0.05$), glucose and C-peptide AUCs ($r=−0.30$, $p=0.04$ and $r=−0.33$, $p=0.02$, respectively) and directly with decreases in IL-6 levels ($r=0.56$, $p<0.0001$) (Electronic supplementary material Table 1). A stratification of partic-

ipants according to tertiles of decreases in plasma 2-AG levels confirmed that these were associated with decreases in VAT and TG levels (Fig. 1b,d). Decreases in VAT correlated not only with decreases in TG ($r=0.62$, $p<0.0001$) and increases in HDL-cholesterol ($r=−0.39$, $p=0.008$), but also with decreases in leptin ($r=0.56$, $p<0.0001$) and increases in adiponectin ($r=−0.59$, $p<0.0001$).

Multivariate analyses using log-transformed changes in TG as dependent variable, and log-transformed changes in VAT and 2-AG as independent variables, showed that 13.7% ($p<0.001$) and 38.0% ($p=0.0001$) of the variation observed for TGs was explained by changes in 2-AG and VAT, respectively. When changes in log-fasting insulin and log-insulin AUC were the dependent variables, VAT was the only independent determinant to changes in insulin sensitivity variables, explaining 18.7% of the changes in fasting insulin ($p=0.002$) and 12.3% of changes in AUC insulin ($p=0.02$). When using changes in log-RepIns30 as dependent variable and changes in log-2-AG as independent variable, 20.3% ($p=0.002$) of the changes of the former were explained by the latter. However, changes in

Fig. 1 Changes in anandamide (a) and 2-AG (c) plasma levels following 1 year lifestyle modification programme, and their baseline levels at the beginning of the study. Anandamide, change -0.54 pmol/l, $p < 0.005$; 2-AG, change -0.98 pmol/l, $p < 0.005$. Variation in VAT (b) and TG (d) among tertiles of changes in 2-AG plasma levels (on the x axis) following 1 year lifestyle modification programme [tertile (1): 2-AG changed from -0.08 to -0.62 pmol/ml; tertile (2): 2-AG changed from -0.62 to -1.20 pmol/ml; tertile (3): 2-AG changed from -1.20 to -3.67 pmol/ml]. Data are presented as means with SE. Value significantly different from tertile (1): * $p < 0.05$; value significantly different from tertiles (1) and (2): † $p < 0.05$

2-AG did not contribute independently to changes of other insulin sensitivity indices (fasting insulin, AUC insulin, InsRes).

We then examined whether changes in other variables correlated with changes in metabolic cardiovascular risk factors. Changes in TG correlated with changes in fasting insulin ($r = 0.43$, $p < 0.002$), AUC insulin ($r = 0.35$, $p < 0.01$), AUC glucose ($r = 0.47$, $p = 0.0008$), InsRes ($r = 0.43$, $p < 0.0031$) and leptin ($r = 0.60$, $p < 0.0001$), but not CRP, IL-6 and adiponectin. Changes in HDL-cholesterol correlated with changes in adiponectin ($r = 0.39$, $p = 0.01$), and in glucose and insulin AUCs ($r = -0.41$, $p = 0.004$ and $r = -0.48$, $p = 0.0007$). Changes in InsRes, fasting insulin and insulin AUC also correlated with changes in leptin ($r = 0.34$, $p = 0.02$; $r = 0.33$, $p = 0.02$; and $r = 0.37$, $p = 0.008$, respectively) and, for InsRes and fasting insulin only, also with changes in adiponectin ($r = -0.38$, $p = 0.01$ and $r = -0.37$, $p = 0.01$, respectively). However, a multivariate analysis including log-transformed changes in VAT, SAT, 2-AG, leptin, adiponectin, CRP and IL-6 as independent variables and log-transformed changes in TG as dependent variable showed that changes in VAT, 2-AG and leptin formed the best predicting model, explaining 38.0% ($p < 0.0001$), 13.7% ($p = 0.001$) and 6.0% ($p = 0.02$) of the changes in TG levels independently of other metabolic variables.

Discussion

The present data indicate that a 1 year lifestyle modification programme leading to abdominal fat loss decreases, at the same time, several metabolic cardiovascular risk factors and plasma ECs. However, only the reduction in 2-AG correlated with intervention-induced beneficial changes in VAT, TG and HDL₃-cholesterol, thus resembling in this way other metabolic factors such as leptin. Although with the unavoidable limitations of all correlative studies, the present results suggest a relationship between peripheral EC and CB₁ receptor activity (in as much as these can be measured by plasma 2-AG levels) and VAT and dyslipidaemia (high TG and low HDL-cholesterol levels), a relationship which is possibly stronger than that suggested by previous cross-sectional studies [3, 4]. This finding is in agreement with the causative role, established in animal studies, of dysregulated EC levels in dyslipidaemia via direct actions on the adipose tissue and the liver [1]. In contrast, decreases in the levels of anandamide correlated with decreases in glucose tolerance and IL-6, reflecting, as in previous studies [2–4], different roles of plasma anandamide and 2-AG.

In view of the well-established role of VAT as a predictor of dyslipidaemia [1, 3, 4, 7], the above observations do not allow us to establish whether plasma 2-AG levels and VAT accumulation are independently associated with this risk factor. However, multivariate analyses allowed us to propose that elevated VAT accumulation and high peripheral 2-AG levels, although related to each other, act as two independent correlates of high TG levels. Clearly, 2-AG is only one of possibly several metabolic variables that correlate with this metabolic risk factor, since we have shown here that changes in plasma leptin levels also act as an independent predictor of changes in TG levels.

In conclusion, we have described here the first intervention study designed to assess whether changes in plasma EC levels correlate with changes in anthropometric and biochemical metabolic risk factors in visceroally obese men. Our data, together with previous findings in animals [1], might suggest that a peripherally overactive EC system in visceral obesity contributes to dyslipidaemia. However, since our data are still of a correlative nature, mechanistic studies will have to be performed to establish a cause–effect relationship between dysregulated EC levels and metabolic cardiovascular risk factors in humans.

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Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

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