Journal of Fluorine Chemistry 136 (2012) 3–7

Contents lists available at SciVerse ScienceDirect

Journal of Fluorine Chemistry

journal homepage: www.elsevier.com/locate/fluor

NaF treatment increases TNF-α and resistin concentrations and reduces insulin signal in rats

Fernando Yamamoto Chiba [a,b], Natália Helena Colombo [a], Daisy Jaqueline Shirakashi [a], Viviane Clície da Silva [a], Suzely Adas Saliba Moimaz [b], Cléa Adas Saliba Garbin [b], Cristina Antoniali [a], Doris Hissako Sumida [a,*]

[a] Department of Basic Sciences, Dental School of Araçatuba, Univ Estadual Paulista – UNESP, R. José Bonifácio, 1193, CEP 16015-050, Araçatuba, SP, Brazil
[b] Department of Child and Social Dentistry, Dental School of Araçatuba, Univ Estadual Paulista – UNESP, R. José Bonifácio, 1193, CEP 16015-050, Araçatuba, SP, Brazil

ARTICLE INFO

Article history:
Received 21 October 2011
Received in revised form 8 December 2011
Accepted 12 December 2011
Available online 20 December 2011

Keywords:
Fluorine
Insulin receptor substrate proteins
Diabetes mellitus
Insulin resistance
Fluorides

ABSTRACT

The use of fluoridated products has significantly contributed to the reduction in rates of dental caries. However, excessive sodium fluoride (NaF) intake promotes inhibition of glycolysis, decrease in insulin secretion, hyperglycemia, and insulin resistance. Seven-week-old castrated male Wistar rats were used to evaluate the chronic effect of NaF on insulin sensitivity, insulin signal transduction in white adipose tissue (WAT), and plasma TNF-α and resistin concentrations. The animals were randomly divided into two groups: (1) control group (CN); (2) fluoride (F) group, which was treated with NaF in the drinking water and F in the food pellets (estimated total F intake: 4.0 mg/kg bw/day). After 42 days, an intravenous insulin tolerance test (0.75 U/kg), plasma TNF-α and resistin quantification analysis, and insulin receptor substrate (pp185 – IRS-1/IRS-2) tyrosine phosphorylation and IRS-1 serine phosphorylation status tests in WAT were performed. The chronic treatment with F promoted: (1) decrease in pp185 (IRS-1/IRS-2) tyrosine phosphorylation status in the WAT; (2) increase in IRS-1 serine phosphorylation status in the WAT; (3) increase in plasma concentrations of TNF-α and resistin; and (4) decrease in insulin sensitivity.

1. Introduction

Over the last few years there has been a significant reduction in the incidence of dental caries in several regions of the world. This fact has been attributed to the exposure of the populace to fluoridated products [1]. However, excessive ingestion of fluoride (F) causes acute or chronic toxicity such as dental fluorosis [2] or alterations in the carbohydrate metabolism [3–6]. Furthermore, elevated serum F concentration is associated with the inhibition of glycolysis [7], hyperglycemia [5], and depletion of hepatic and muscular glycogen [7,8].

Rigalli et al. [3] found diminished insulin secretion both in human patients and in rats that ingested high doses of NaF. Trivedi et al. [9] showed that 40% of patients with endemic fluorosis had impaired glucose tolerance, but this anomaly was reversed by removing excess F from the drinking water. In 1995, Rigalli et al. [4] showed that when plasma NaF exceeds 5 μmol/l, it affects glucose homeostasis. In 1997, De La Sota et al. [10] showed that glucose tolerance tests on residents in an endemic fluorosis area indicated that plasma insulin levels as a function of time were inversely correlated with fluoremia. Xie et al. [11] reported that higher plasma glucose levels and a delay in the peak plasma insulin were observed after glucose tolerance test in people with high F intake. Our previous studies [6], using the same experimental model as in the present study showed that chronic fluoride intake (4.0 mg F/kg bw/day) decreases both insulin signal in the rat muscle tissue and insulin sensitivity measured by the HOMA-IR index.

Several studies have demonstrated that the adipose tissue has an important role in the energetic metabolism, since this tissue produces adipokines that alter insulin sensitivity, such as TNF-α and resistin.The first cytokine to be described as being increased in fat cells of obese animals and humans was tumor necrosis factor-α (TNF-α) [12]. *In vitro* and *in vivo* studies have shown that TNF-α may be related to a deficiency in insulin-stimulated glucose uptake [13–16]. Hotamisligil et al. [12] showed that the neutralization of the effects of TNF-α in obese rats significantly increased the peripheral uptake of glucose in response to insulin, indicating that TNF-α can play an important role in obesity and particularly in insulin resistance and diabetes that often accompany obesity. In another study, carried out on genetically obese mice, Uysal et al. [17] observed that mice lacking the TNF-α receptor showed an

* Corresponding author. Tel.: +55 18 3636 2819; fax: +55 18 3636 3332.
E-mail addresses: fernandoychiba@hotmail.com (F.Y. Chiba), nathycolombo@yahoo.com.br (N.H. Colombo), daisy_jaqueline@yahoo.com.br (D.J. Shirakashi), vivi_clicie@hotmail.com (V.C. da Silva), sasaliba@foa.unesp.br (S.A.S. Moimaz), cgarbin@foa.unesp.br (C.A.S. Garbin), crisant@foa.unesp.br (C. Antoniali), dorishs@foa.unesp.br (D.H. Sumida).

0022-1139/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.jfluchem.2011.12.006

increase in insulin sensitivity. Moreover, Lumeng et al. [18] verified that TNF-α can block insulin action in adipocytes *via* down-regulation of GLUT4 and IRS-1, leading to a decrease in Akt phosphorylation and impaired insulin-stimulated GLUT4 translocation to the plasma membrane.

Resistin has also been implicated in the pathogenesis of obesity-mediated insulin resistance and type II diabetes mellitus. Banerjee et al. [19] observed that mice deficient in resistin had low blood glucose levels after fasting and reduced hepatic glucose production due to a decrease in gluconeogenic enzymes in the liver, suggesting that resistin plays an important role in the maintenance of blood glucose during fasting. Hivert et al. [20] observed in a cross-sectional study of 2356 individuals with and without metabolic syndrome that adverse levels of the adipokines adiponectin, resistin, and TNF-α are associated with insulin resistance. It was observed that elevated levels of resistin and TNF-α had an additive effect in patients with metabolic syndrome, resulting in higher levels of insulin resistance.

A study using mice that produce resistin similar to that found in humans (humanized resistin mice), showed that a high-fat diet resulted in a significant decrease in insulin-stimulated glucose uptake in the skeletal muscle and WAT. In addition, WAT developed inflammation, inducing lipolysis and an increase in serum free fatty acids, contributing to an increase in insulin resistance [21]. Moreover, Steppan et al. [22] reported that obese mice had high resistin levels and these levels were reduced by anti-diabetic drugs of the thiazolidinediones class, suggesting that resistin may cause insulin resistance. Furthermore, it was observed that administration of anti-resistin antibody improves blood glucose and insulin action in mice with diet-induced obesity [22]. The literature indicates that resistin and TNF-α may have an important role in insulin resistance. Given that chronic treatment with F can interfere with carbohydrate metabolism, the aim of this study was to examine the chronic effect of elevated NaF exposure in a rat model on insulin sensitivity, insulin signal transduction in the WAT, and resistin and TNF-α concentrations in the plasma.

2. Material and methods

2.1. Animals

All experimental procedures were approved by the Institutional Committee on Animal Research and Ethics (Protocol No. 2006-08802). Four-week-old male Wistar rats were kept on a 12/12 h light/dark cycle (lights on at 0700) and room temperature of 23 ± 2 °C, with free access to a regular laboratory rat diet (LABINA Indústria de rações do Brasil LTDA, Paulínia, Brasil) and water (containing NaF or NaCl). The rats were castrated to avoid any influence of testosterone. After 21 days, 52 7-week-old castrated rats were divided into two groups: the control group (n = 26) received an average of 76.4 mg/l NaCl in their drinking water throughout the experiment; the F group (n = 26) received an average of 54.9 mg/l NaF in their drinking water throughout the experiment plus F present in the food pellets (total estimated F intake = 4.0 mg F/kg bw/day, resulting from 3.1 mg F/kg bw/day from drinking water and 0.9 mg/kg per day from diet). During the next 42 days, body weight and daily volume of water consumed per rat in the F group were measured every 2 or 3 days. At the end of this period, the control and F group rats were fasted for 14 h from food and deprived for 4 h from the NaF solution before the day of the experimental measurements to avoid an acute F effect. The F and control groups were then anesthetized with sodium thiopental (Thiopental® 3%, 5 mg/100 g bw, i.p.). Each group was divided into three subgroups for the intravenous insulin tolerance test, the determination of the phosphorylation status of the insulin receptor substrate and the quantification of TNF-α and resistin plasma concentrations. Ten control and 10 F-treated rats were submitted to the intravenous insulin tolerance test (0.75 U insulin/kg bw). Six control and six F-treated rats were used to quantify the pp185 (IRS-1/IRS-2) tyrosine phosphorylation status and IRS-1 serine phosphorylation status in the WAT after regular human insulin (1.5 U, i.v.) administration. Median laparotomy was performed in 10 control and 10 F-treated rats, and blood was collected from the inferior vena cava. The plasma was stored at −70 °C until quantification of plasma concentrations of TNF-α and resistin.

2.2. Short intravenous insulin tolerance test (ITT)

The tests were performed in the morning after a 12 h fast. The animals were anesthetized and the right jugular vein was cannulated under anesthesia by introducing a Silastic cannula close to the atrium (superior vena cava). This cannula was used to inject the 0.75 U/kg dose of insulin. Blood samples (about 50 μl) were collected from the tail at 0 (before insulin injection), 4, 8, 12, and 16 min (after insulin injection) and glycemia was measured using a glycemia monitor (One Touch Ultra; Johnson &Johnson, USA). The results were analyzed by comparison of the rate constant for glucose disappearance (K_{itt}) from 0 to 16 min of the test. The K_{itt} was calculated using the formula $0.693/t_{1/2}$. The glucose $t_{1/2}$ was calculated from the slope of the least square analysis of the plasma glucose concentration during the linear decay phase [23].

2.3. Assessment of the insulin receptor substrate (pp185 – IRS-1/IRS-2) tyrosine phosphorylation status and IRS-1 serine phosphorylation status

These tests were performed according to the method described by Carvalho et al. [24]. Two minutes after i.v. injection with human insulin a sample of WAT was extracted. This tissue sample was then treated by the western blotting method to quantify the tyrosine phosphorylation status of pp185 using an anti-phosphotyrosine antibody (Santa Cruz Technology, Santa Cruz, CA) and the IRS-1 serine phosphorylation status using an anti-IRS-1 phosphoserine antibody (Upstate, NY, USA).

2.4. Determination of TNF-α and resistin by enzyme-linked immunosorbent assay (ELISA)

Blood samples were collected from the inferior vena cava to measure plasma TNF-α and resistin concentrations. After centrifugation, supernatant was collected and maintained at −70 °C until use. The 96-well plate was coated with monoclonal antibody to TNF-α and resistin. Samples and recombinant TNF-α and resistin were added to the wells and after 2 h, unbound proteins were washed away, and an enzyme-linked polyclonal antibody was added to the wells; this antibody acted as a link between TNF-α and resistin and the dye, whereby a color change proportional to the amount of TNF-α and resistin was observed. This color change was quantified by comparing the optical densities of the samples to those of known dilutions using a plate reader at 450 nm. The concentrations of TNF-α and resistin were calculated from standard curves in pg/ml and ng/ml, respectively.

2.5. Statistical analysis

All numerical values are presented as means ± SEM. Statistical analysis was done by the Student's t-test for non-paired samples, and differences between two groups were considered significant when $p < 0.05$.

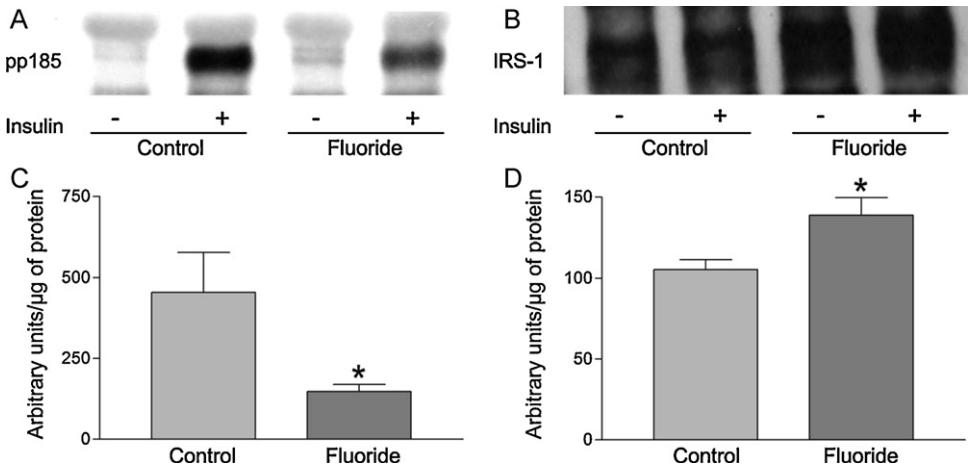

Fig. 1. Insulin-stimulated tyrosine phosphorylation status of pp185 (IRS-1/IRS-2) and insulin-stimulated serine phosphorylation status of IRS-1 in white adipose tissue (WAT) of control and F groups, before (−) and after (+) insulin infusion. In (A) and (B), typical autoradiogram of insulin-stimulated tyrosine phosphorylation status of pp185 (IRS-1/IRS-2) and serine phosphorylation status of IRS-1 in WAT, respectively. (C) and (D) Data after insulin infusion, expressed in arbitrary units per μg of protein (mean ± SEM, $n = 6$). $^{*}p < 0.05$ compared to control group.

3. Results

Fig. 1A and B shows a typical autoradiogram of the insulin-stimulated tyrosine phosphorylation status of pp185 (IRS-1/IRS-2) and serine phosphorylation status of IRS-1 in the WAT, respectively. Fig. 1C and D shows the data expressed in arbitrary units per μg of protein. After insulin infusion, the F group showed: (1) a decrease in pp185 tyrosine phosphorylation status compared to the control group, and (2) an increase in IRS-1 serine phosphorylation status compared to the control group.

Table 1 shows the glucose disappearance rate (K_{itt}), during the insulin tolerance test performed in the first 16 min after hormone infusion in the control and F groups. The group treated with sodium fluoride (F group) showed a glucose rate of disappearance (K_{itt}) significantly lower than the control group (CN group). As seen in Table 1, F-treated rats exhibited a significant increase in plasma concentration of resistin and TNF-α in comparison with the control group.

4. Discussion

The present study demonstrated that chronic treatment with NaF decreases the insulin signal in rats by reducing the pp185 tyrosine phosphorylation status (IRS-1/IRS-2) and increasing the IRS-1 serine phosphorylation status after insulin stimulus in the WAT. Previous *in vivo* studies of Chiba et al. [6] also demonstrated that chronic treatment with NaF induced a decrease in the pp185 tyrosine phosphorylation status in the muscle tissue of rats after insulin stimulation. These results are in agreement with an *in vitro* study using rat muscle tissue and human placenta, which showed that F decreased the insulin-induced tyrosine auto-phosphorylation of insulin receptors [25]. On the other hand, the study of Chehoud et al. [5], which utilized acute treatment with NaF, did not report any alterations in the pp185 tyrosine phosphorylation status after insulin stimulus in either gastrocnemius muscle or WAT in rats. This disagreement can be at least partly explained by the difference in methodology used between these studies and the present study. Our results showed an increase in the IRS-1 serine phosphorylation status in the WAT (Fig. 1), resulting in attenuation of insulin signal transmission by reducing the capacity of the insulin receptor to phosphorylate tyrosine residues after insulin stimulation. This inhibitory phosphorylation causes negative feedback in insulin signaling and can promote insulin resistance [26,27].

In view of the present results, it is possible to suggest that both the decrease in the pp185 tyrosine phosphorylation status and the increase in the IRS-1 serine phosphorylation status in the WAT may have favored the decrease in insulin sensitivity in the F group (Table 1).

The increase in plasma resistin and TNF-α may have caused, as mentioned above, the decrease in pp185 tyrosine (IRS-1/IRS-2) phosphorylation status and the increase in the phosphorylation status of IRS-1 serine in this tissue. These results are in agreement with studies suggesting that TNF-α can alter the insulin signal transmission through an increase in the IRS-1 serine phosphorylation status and possibly in other IRS proteins [26,28–30]. Similar results were also presented in *in vitro* studies by Lumeng et al. [18], which demonstrated that TNF-α can block insulin action in adipocytes by altering the expression of signaling and glucose transport proteins. By examining adipocyte insulin signaling under the influence of macrophage-derived factors, these researchers identified a decrease in IRS tyrosine phosphorylation and a mild reduction in Akt phosphorylation. These alterations were partially restored after treatment with anti-TNF-α antibodies.

Studies carried out on TNF-α-deficient mice demonstrated that these animals showed significantly improved insulin sensitivity, even when placed on a high fat diet. This indicated that in the absence of TNF-α, the signaling capacity of the insulin receptor is significantly protected from obesity-induced downregulation of this cytokine in WAT and muscle tissues [31]. Moreover, Veledo et al. [32] also observed that chronic treatment with TNF-α induces serine phosphorylation of IRS-1 and a decrease in insulin-stimulated IRS-1 tyrosine phosphorylation in human visceral adipocytes. This induction is JNK1/2-dependent, suggesting that activation of JNK1/2 is involved in serine phosphorylation of IRS-1, and consequently in insulin resistance on glucose uptake. Our results, supported by these previous findings, indicate that TNF-α

Table 1
Glucose disappearance rate (K_{itt}), and plasma resistin and TNF-α in control and fluoride groups [mean (±CV) ± SEM, $n = 10$].

Parameter	Control group	Fluoride group
K_{itt}	3.107 (±0.17) + 0.1652	1.994 (±0.23) + 0.1447*
Resistin (ng/ml)	0.5437 (±0.30) + 0.0523	0.9315 (±0.35) + 0.1040*
TNF-α (pg/ml)	7.183 (±0.09) + 0.1961	7.842 (±0.08) + 0.1902*

CV = coefficient of variation.
* $p < 0.05$ compared to control group.

is a significant contributor to the attenuation of insulin action and development of insulin resistance.

Qatanani et al. [21] observed in studies carried out on humanized resistin mice that, when placed on a high-fat diet, these animals showed an increase in WAT resistin expression. These humanized resistin mice also showed a general decrease in insulin sensitivity in insulin tolerance tests, elevated serum insulin levels, impairment of glucose metabolism revealed by the glucose-tolerance test, an increase in serine phosphorylation of IRS-1 in WAT, and a decrease in tyrosine phosphorylation of IRS-1 in muscle and WAT, indicating a relationship between resistin levels and insulin resistance. Banerjee et al. [19] also observed that animals deficient in resistin had improved glucose tolerance and a reduced increase in post-fast blood glucose normally associated with obesity, suggesting a role for resistin in mediating hyperglycemia associated with increased weight.

In a study performed on a community-based cohort, Hivert et al. [20] evaluated the relationship between adipokine levels and insulin resistance, and showed a significant association between resistin and HOMA-IR. This association persisted even after adjustment for body mass index, metabolic syndrome, and adiponectin and TNF-α levels, suggesting a possible link between resistin and insulin resistance. Our results are consistent with these observations, as we observed that rats chronically treated with NaF showed an increase in plasma resistin and a decrease in insulin sensitivity. However, other studies are not in agreement with these results, showing significantly reduced resistin expression in different models of obesity [33].

Menoyo et al. [34] demonstrated that acute treatment of rats with F from a single dose of NaF (16.8 mg/kg bw) induces insulin resistance. Recently, Chiba et al. [6] used the same experimental model of the present study and evaluated the index of HOMA-IR (homeostasis model assessment of insulin resistance), observing that chronic intake of F promotes a decrease in insulin sensitivity in rats. Our results are in agreement with this study, which verified insulin resistance in the F-treated group compared to the control, using the short intravenous insulin tolerance test (Table 1). In contrast, Chehoud et al. [5] found that treatment of rats with a single dose of NaF (1.0 mg F/kg bw) by gavage did not change insulin sensitivity. This divergence in results may be due to the difference in the dose of NaF used.

In the present study, the F dose used was 20 times higher than the proposed maximum allowable value a child can ingest through diet (0.045 mg F/kg bw/day) and during tooth brushing (0.155 mg F/kg bw/day) with a fluoridated dentifrice [35]. This dose was adopted from the Rigalli et al. [3] studies in humans and in rats, in which the authors increased the fluoride dose in rats by this proportion to attain a similar plasmatic peak in the two models. The requirement for a higher dose in rats is probably due to the faster bone turnover rate in rats compared to humans. In our study, the calculation of F concentration in the drinking water was based on the body weight and daily volume of water consumed per rat.

Studies carried out in children [35] have shown that the dentifrice is the main source of fluoride intake, exceeding the daily intake limit able to prevent unwanted degrees of dental fluorosis, which is estimated at around 0.05–0.07 mg F/kg bw/day [2]. Therefore, knowing that chronic NaF intake can alter the carbohydrate metabolism, the use of dentifrices with lower fluoride content is recommended, mainly for diabetic children, for whom excessive fluoride ingestion can lead to health impairment.

In vitro studies using a more acidified toothpaste with F concentrations of 550 μg/g (pH 4.5) [36] and 550 μg/g (pH 5.5) [37] observed that these acidified toothpastes had the same anticariogenic effectiveness as neutral toothpastes (pH 7.0) with 1100 μg/g of F. Vilhena et al. [38] showed in a study performed on 4-year-old schoolchildren living in a fluoridated area, that low-fluoride acidified liquid dentifrice with 550 μg/g (pH 4.5) seems to lead to similar caries progression rates compared to conventional toothpaste with 1100 μg/g (pH 7.0) of F. These acidified toothpastes with low F concentration would be a better option for young children who often ingest large amounts of toothpaste, with a major impact on the health of diabetic children.

5. Conclusions

Based on the present results, we can conclude that chronic treatment with NaF promoted: (1) a decrease in the pp185 (IRS-1/IRS-2) tyrosine phosphorylation status in the WAT; (2) an increase in the IRS-1 serine phosphorylation status in the WAT; (3) an increase in plasma concentrations of TNF-α and resistin; (4) a decrease in insulin sensitivity. Therefore, knowing that chronic NaF intake is capable of decreasing insulin signal and causing insulin resistance, the use of dentifrices with lower F content is recommended, especially for diabetic children, for whom excessive F consumption may lead to health implications.

Acknowledgements

This work was supported by Grants from the São Paulo Research Foundation (FAPESP 2009/00086-7 and 2010/10664-5), Pro-Rector for Research of UNESP (PROPe-UNESP), Foundation for the Development of UNESP (FUNDUNESP) and the National Council for Scientific and Technological Development (CNPq). We would like to thank Mrs. Karina Vieira Casatti (English teacher) for her English technical assistance in the preparation of this manuscript.

References

[1] Y.B.O. Lima, J.A. Cury, Rev. Saúde Públ. 35 (2001) 576–581.
[2] B.A. Burt, J. Dent. Res. 71 (1992) 1228–1237.
[3] A. Rigalli, J.C. Ballina, E. Roveri, R.C. Puche, Calcif. Tissue Int. 46 (1990) 333–338.
[4] A. Rigalli, R. Alloatti, I. Menoyo, R.C. Puche, Arzneimittelforschung 45 (1995) 189–192.
[5] K.A. Chehoud, C.A.S. Garbin, K.T. Sassaki, F.Y. Chiba, D.H. Sumida, Fluoride 41 (2008) 270–275.
[6] F.Y. Chiba, N.H. Colombo, D.J. Shirakashi, W.D.S. Gomes, S.A.S. Moimaz, C.A.S. Garbin, C.A. Silva, D.H. Sumida, Fluoride 43 (2010) 25–30.
[7] F.N. Dost, R.M. Knaus, D.E. Johnson, C.H. Wang, Toxicol. Appl. Pharmacol. 41 (1977) 451–458.
[8] A. Shashi, J.P. Singh, S.P. Thapar, Fluoride 21 (1988) 82–86.
[9] N. Trivedi, A. Mithal, S.K. Gupta, M.M. Godbole, Diabetologia 36 (1993) 826–828.
[10] M. De La Sota, R. Puche, A. Rigalli, L.M. Fernández, S. Benassati, R. Boland, Medicina (B Aires) 57 (1997) 417–420.
[11] Y.P. Xie, X.J. Ge, Y.T. Jiang, M.Y. Feng, Y.H. Fan, F.L. Wang, Chin. J. Endemiol. 19 (2000) 84–86.
[12] G.S. Hotamisligil, N.S. Shargill, B.M. Spiegelman, Science 259 (1993) 87–91.
[13] T. Teruel, R. Hernandez, M. Lorenzo, Diabetes 50 (2001) 2563–2571.
[14] C. Alvaro, T. Teruel, R. Hernandez, M. Lorenzo, J. Biol. Chem. 279 (2004) 17070–17078.
[15] K. Bouzakri, J.R. Zierath, J. Biol. Chem. 282 (2007) 7783–7789.
[16] P. Plomgaard, K. Bouzakri, R. Krogh-Madsen, B. Mittendorfer, J.R. Zierath, B.K. Pedersen, Diabetes 54 (2005) 2939–2945.
[17] K.T. Uysal, S.M. Wiesbrock, G.S. Hotamisligil, Endocrinology 139 (1998) 4832–4838.
[18] C.N. Lumeng, S.M. Deyoung, A.R. Saltiel, Am. J. Physiol. Endocrinol. Metab. 292 (2007) E166–E174.
[19] R.R. Banerjee, S.M. Rangwala, J.S. Shapiro, A.S. Rich, B. Rhoades, Y. Qi, J. Wang, M.W. Rajala, A. Pocai, P.E. Scherer, C.M. Steppan, R.S. Ahima, S. Obici, L. Rossetti, M.A. Lazar, Science 303 (2004) 1195–1998.
[20] M.F. Hivert, L.M. Sullivan, C.S. Fox, D.M. Nathan, R.B. D'Agostino, P.W.F. Wilson, J. Clin. Endocrinol. Metab. 93 (2008) 3165–3172.
[21] M. Qatanani, N.R. Szwergold, D.R. Greaves, R.S. Ahima, M.A. Lazar, J. Clin. Invest. 119 (2009) 531–539.
[22] C.M. Steppan, S.T. Bailey, S. Bhat, E.J. Brown, R.R. Banerjee, C.M. Wright, H.R. Patel, R.S. Ahima, M.A. Lazar, Nature 409 (2001) 307–312.
[23] E. Bonora, P. Moghetti, C. Zancanaro, M. Cigolini, M. Querena, V. Cacciatori, A. Corgnati, M. Muggeo, J. Clin. Endocrinol. Metab. 68 (1989) 374–378.
[24] C.R. Carvalho, S.L. Brenelli, A.C. Silva, A.L. Nunes, L.A. Velloso, M.J. Saad, Endocrinology 137 (1996) 151–159.
[25] F. Viñals, X. Testar, M. Palacin, A. Zorzano, Biochem. J. 291 (1993) 615–622.

[26] G.S. Hotamisligil, P. Peraldi, A. Budavari, R. Ellis, M.F. White, B.M. Spiegelman, Science 271 (1996) 665–668.
[27] J.B. Carvalheira, E.B. Ribeiro, R.B. Guimarães, M.M. Telles, L.A. Velloso, J.A. Gontijo, Diabetes 51 (2002) A41.
[28] R. Feinstein, H. Kanety, M.Z. Papa, B. Lunenfeld, A. Karasik, J. Biol. Chem. 268 (1993) 26055–26058.
[29] G.S. Hotamisligil, D.L. Murray, L.N. Choy, B.M. Spiegelman, Proc. Natl. Acad. Sci. U. S. A. 91 (1994) 4854–4858.
[30] G. Kroder, B. Bossenmaier, M. Kellerer, E. Capp, B. Stoyanov, A. Muhlhofer, L. Berti, H. Horikoshi, A. Ullrich, H. Haring, J. Clin. Invest. 97 (1996) 1471–1477.
[31] K.T. Uysal, S.M. Wiesbrock, M.W. Marino, G.S. Hotamisligil, Nature 389 (1997) 610–614.
[32] S.F. Veledo, R.V. Bedmar, I.N. Vazquez, M. Lorenzo, J. Clin. Endocrinol. Metab. 94 (2009) 3583–3593.
[33] I. Nagaev, U. Smith, Biochem. Biophys. Res. Commun. 285 (2001) 561–564.
[34] I. Menoyo, R.C. Puche, A. Rigalli, Fluoride 41 (2008) 260–269.
[35] J.P. Pessan, S.M.B. Silva, M.A.R. Buzalaf, J. Appl. Oral Sci. 11 (2003) 150–156.
[36] F.L. Brighenti, A.C.B. Delbem, M.A.R. Buzalaf, F.A.L. Oliveira, D.B. Ribeiro, K.T. Sassaki, Caries Res. 40 (2006) 239–244.
[37] K.M.R.P. Alves, J.P. Pessan, F.L. Brighenti, K.S. Franco, F.A.L. Oliveira, M.A.R. Buzalaf, K.T. Sassaki, A.C.B. Delbem, Caries Res. 41 (2007) 263–267.
[38] F.V. Vilhena, K.P. Olympio, J.R. Lauris, A.C. Delbem, M.A. Buzalaf, Caries Res. 44 (2010) 478–484.