Universidade de São Paulo
BRASIL

Kefir administration reduced progression of renal injury in STZ-diabetic rats

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Cristina Stewart Bogsan
Pharmaceutical-Biochemical Technology Department

Outline

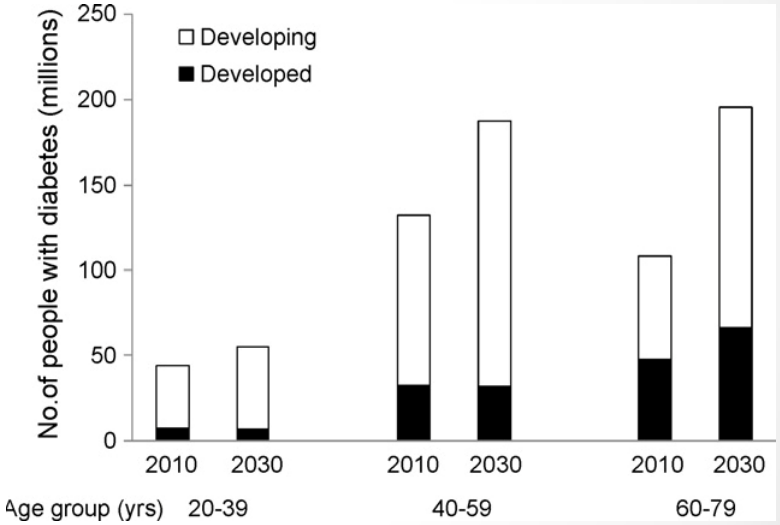
- Diabetic Mellitus;
- Kefir;
- Oxidative stress;
- Aim;
- Protocol;
- Results;
- Conclusion.

Diabetes Mellitus

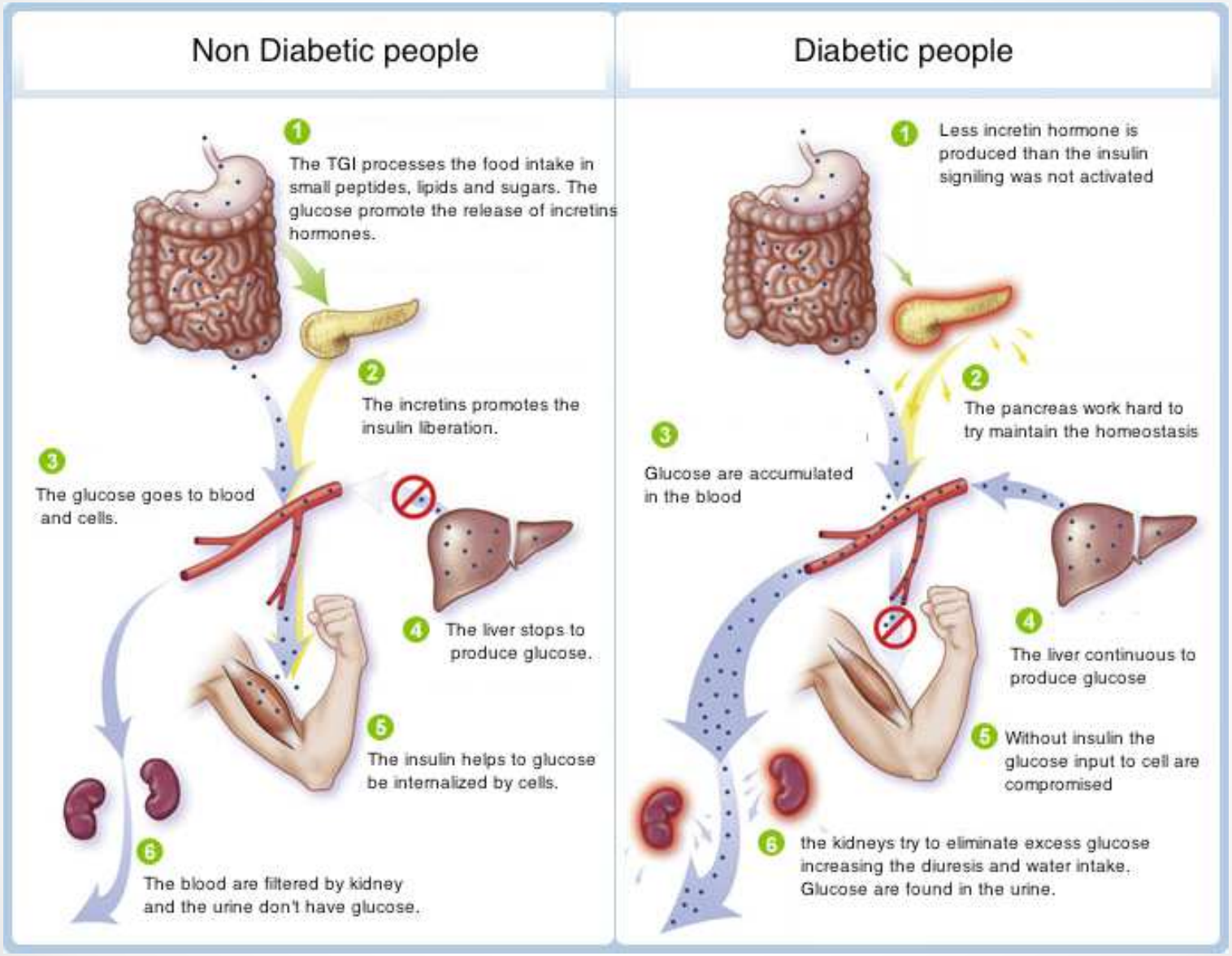
Diabetes Mellitus (DM) is a group of metabolic disorders that have in common the hyperglycemia.

DM has become a serious public health problem that affects millions of individuals worldwide (Shaw, 2010).

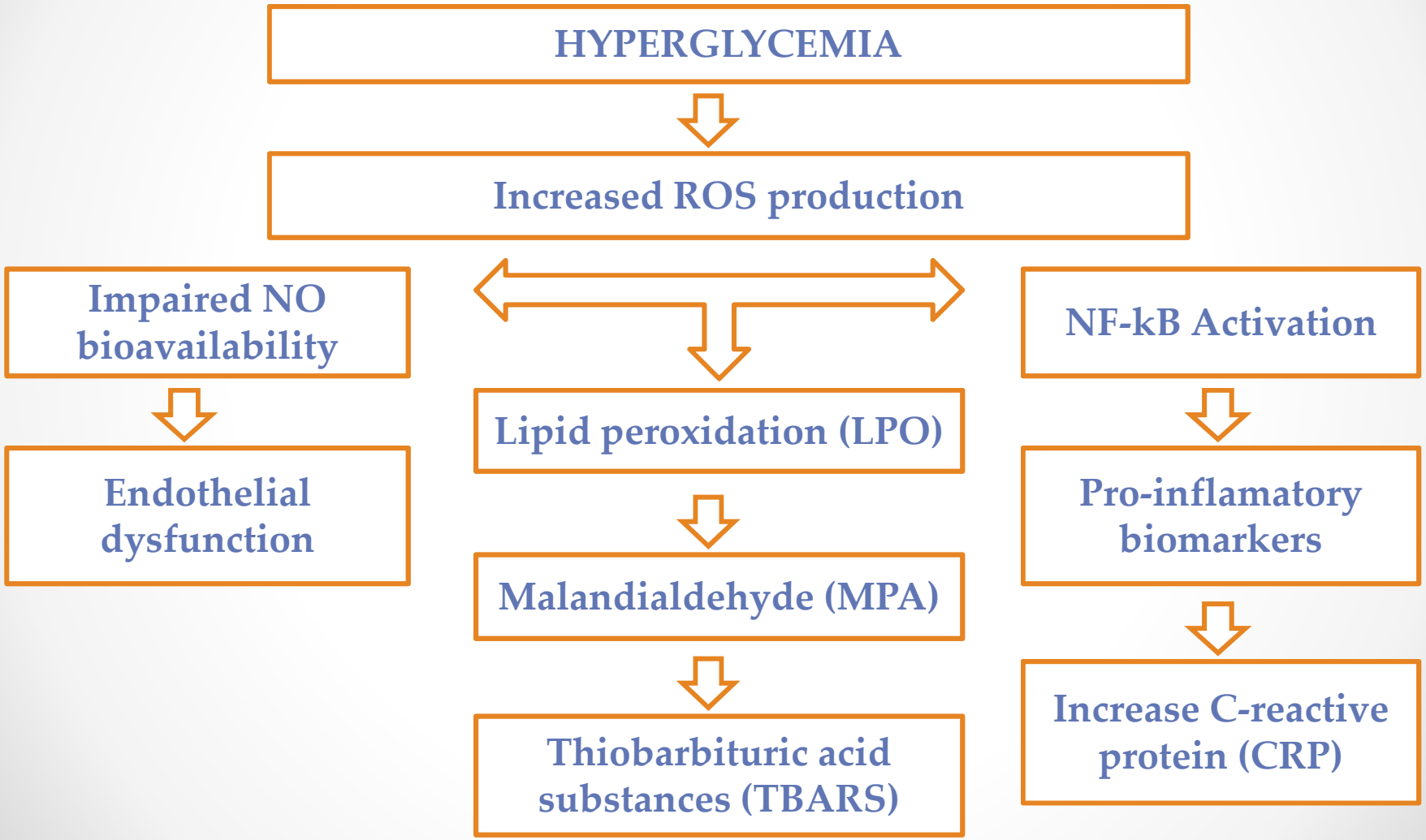
30% of patients develop nephropathy, the main cause of morbidity and mortality in diabetic patients (Molitch et al., 2004).



Source: Shaw, 2010


Non Diabetic people
1
The TGI processes the food intake in small peptides, lipids and sugars. The glucose promote the release of incretins hormones.
2
The incretins promotes the insulin liberation.
3
The glucose goes to blood and cells.
4
The liver stops to produce glucose.
5
The insulin helps to glucose be internalized by cells.
6
The blood are filtered by kidney and the urine don't have glucose.
Diabetic people
1
Less incretin hormone is produced than the insulin signiling was not activated
2
The pancreas work hard to try maintain the homeostasis
3
Glucose are accumulated in the blood
4
The liver continuous to produce glucose
5
Without insulin the glucose input to cell are compromised
6
the kidneys try to eliminate excess glucose increasing the diuresis and water intake. Glucose are found in the urine.

Oxidative Stress in DM

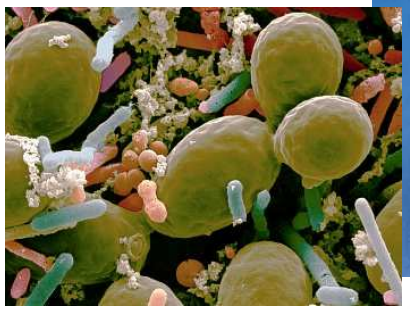


Kefir

- Kefir is a fermented milk that contains a complex symbiotic mixture of Lactic Acid Bacteria (LAB) and Molds.

- The main microorganisms are:
  - *Lactobacillus,*
  - *Lactococcus,*
  - *Leuconostoc,*
  - *Streptococcus,*
  - *Kluyveromyces,*
  - *Saccharomyces,*
  - *Torula.*





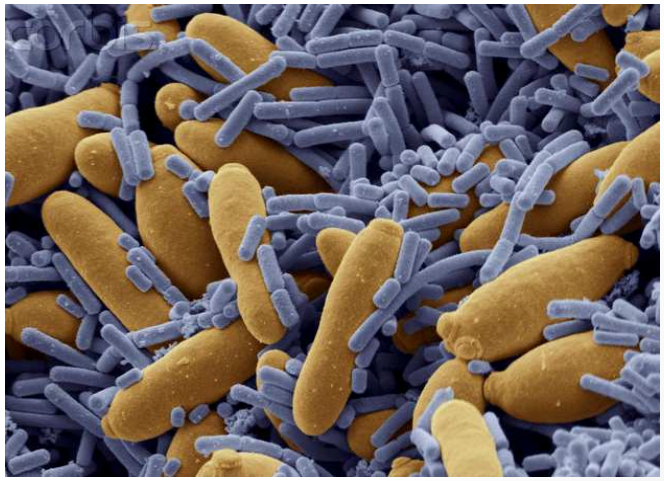
Kefir properties

Kefir is known for **providing benefits** to human health through its **anti-inflammatory, immune-stimulatory and antioxidant properties**.

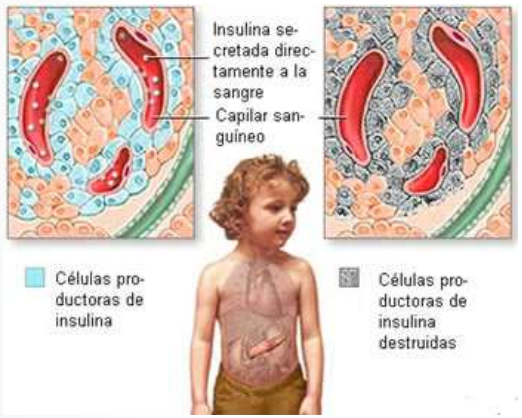
AIM

This study aimed at assessing the effects of **Kefir** on **oxidative stress** in diabetic animals.





STZ - diabetic induction 45 mg/kg iv



Kefir preparation

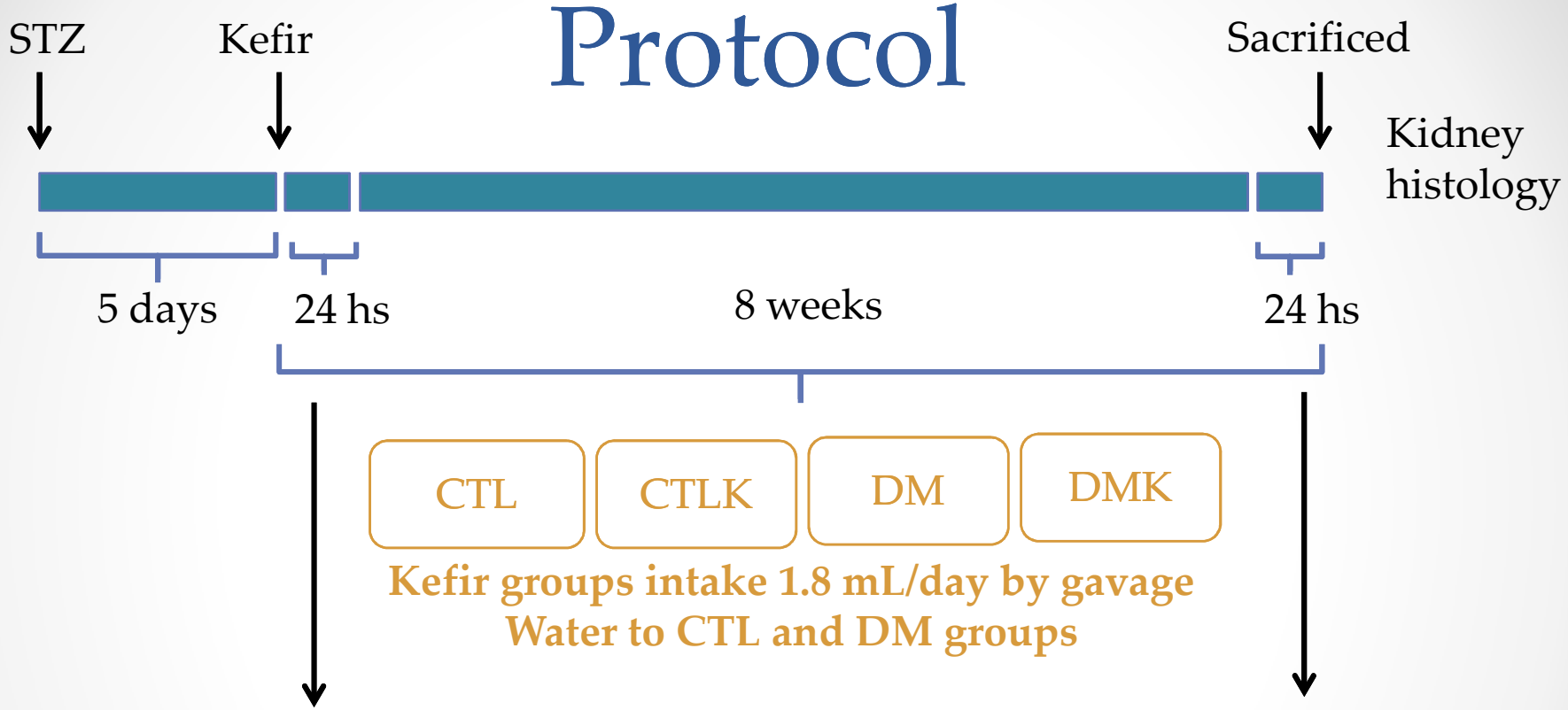
- Skimmed Milk
- 20mg/ 100mL Kefir DA Inoculation
- Fermentation until pH 4.6
- Cooling in ice bath
- Stiring(1min)
- Distribution in 50 mL cups
- Storing (4 °C)
- Kefir fermented milk







Protocol


STZ
Kefir
Sacrificed
Kidney histology
5 days
24 hs
8 weeks
24 hs
CTL
CTLK
DM
DMK
Kefir groups intake 1.8 mL/day by gavage
Water to CTL and DM groups
Water and Chow intake
Diuresis, Weight
Fasting Blood tolerance
Creatinine,Proteinuria, T bars, NO
Water and Chow intake
Diuresis, Weight
Fasting Blood tolerance
Creatinine,Proteinuria, T bars, NO

Metabolic profile, renal function, and oxidative stress

Parameters in experimental animals before treatment with Kefir

Parameters	CTL n=18	DM n=24
Water intake (mL/24 h)	30.0 ±0.9	80.2 ±4.9[c]
Chow intake (g/24 h)	19.1 ±0.4	23.9 ±0.9[c]
Diuresis (mL/24 h)	13.0 ±0.7	61.1 ±4.6[c]
Weight (g/24 h)	269.8 ±5.2	253.7 ±4.0[a]
Fasting blood glucose (mg/dL)	91.8 ±3.5	293.5 ±12.3[c]
NO plasmatic (µM)	89.7 ±12.2	58.9 ±9.0[a]
Excretion NO (µmol/24 h)	15.9 ±2.7	1.4 ±0.2[c]
TBARS plasmatic (nmol/mL)	3.03 ±0.06	3.17 ±0.06
Excretion TBARS (nmol/24 h)	86.9 ±5.3	192.4 ±10.4[c]
Urea plasmatic (mg/dL)	29.2 ±1.9	55.6 ±4.6[c]
Urea urinary (mg/dL)	7,556 ±444	2,403 ±129[c]
Creatinine plasmatic[y] (mg/dL)	0.28 ±0.02	0.33 ±0.01
Creatinine urinary[y] (mg/dL)	138.8 ±20.9	54.0 ±9.9[b]
Proteinuria (nmol/24 h)	11.2 ±0.6	21.4 ±1.0[c]

Values are expressed as means ± SEM. Student's unpaired *t* test.
[y]Corrected values per 100g weight; [a]$p<0.05$; [b]$p<0.01$; [c]$p<0.001$

Metabolic profile, renal function, and oxidative stress

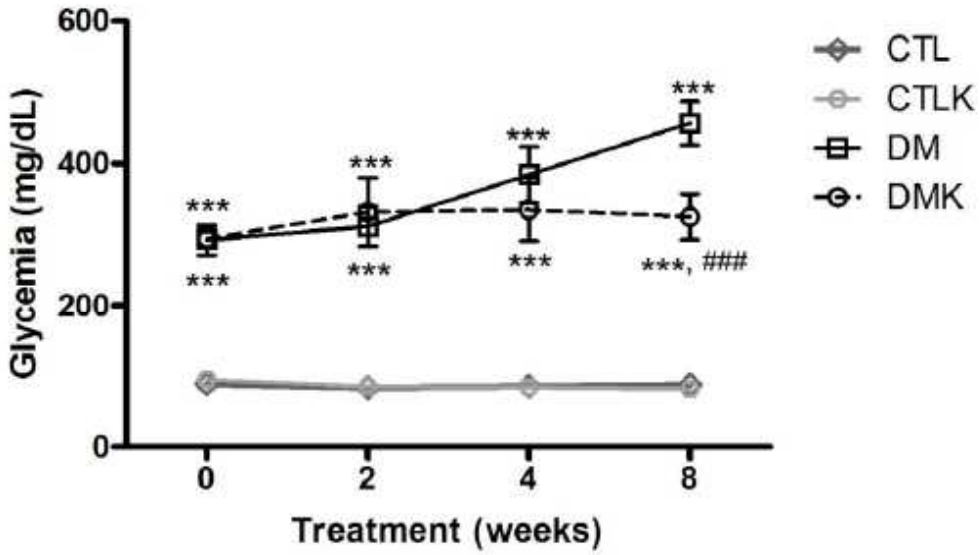
Metabolic profile, renal function, and oxidative stress of the groups after Kefir treatment.

Variables	CTL	CTLK	DM	DMK
Water intake (mL/24 h)	24.8 ± 1.8	25.8 ± 3.7	124.4 ± 12.4[a]	94.1 ± 14.4[b,c]
Chow intake (g/24 h)	17.3 ± 0.6	19.1 ± 1.0	36.9 ± 2.0[a]	30.6 ± 2.3[b,c]
Diuresis (mL/24 h)	13.1 ± 1.0	13.7 ± 0.7	90.9 ± 9.0[a]	70.9 ± 9.4[b,c]
Weight (Δ)	67.5 ± 4.4	69.3 ± 1.6	25.3 ± 4.5[a]	35.3 ± 5.9[b]
Plasmatic urea (mg/dL)	31.9 ± 1.4	36.2 ± 2.5	58.5 ± 3.6[a]	47.6 ± 2.4[b,c]
Urinary urea (mg/dL)	8,691 ± 343	8,423 ± 229	2,006 ± 142[a]	2,996 ± 322[b,c]
Plasmatic creatinine (mg/dL)	0.71 ± 0.05	0.72 ± 0.04	0.75 ± 0.03	0.78 ± 0.05
Urinary creatinine (mg/dL)	131.7 ± 9.2	127.9 ± 4.5	33.2 ± 5.6[a]	34.8 ± 7.8[b]
Proteinuria (nmol/24 h)	10.4 ± 0.8	11.2 ± 0.7	25.5 ± 3.7[a]	21.0 ± 2.8[b]
Plasmatic NO (μM)	66.6 ± 4.3	77.8 ± 6.6	79.2 ± 5.0	76.5 ± 5.4
NO excretion (μmol/24 h)	14.9 ± 3.6	17.5 ± 3.8	2.1 ± 0.7[a]	16.4 ± 4.9
Plasmatic TBARS (nmol/mL)	3.32 ± 0.06	3.16 ± 0.08	3.79 ± 0.10[a]	3.58 ± 0.17
TBARS excretion (nmol/24 h)	81.6 ± 2.1	84.4 ± 4.3	300.4 ± 18.9[a]	248.9 ± 19.2[b,c]

Values are expressed as mean ± SEM. One-way ANOVA followed by Newman–Keuls Multiple Comparison post test. Control (CTL); control Kefir (CTLK); diabetic (DM); diabetic Kefir (DMK); $n = 9–12$/group.

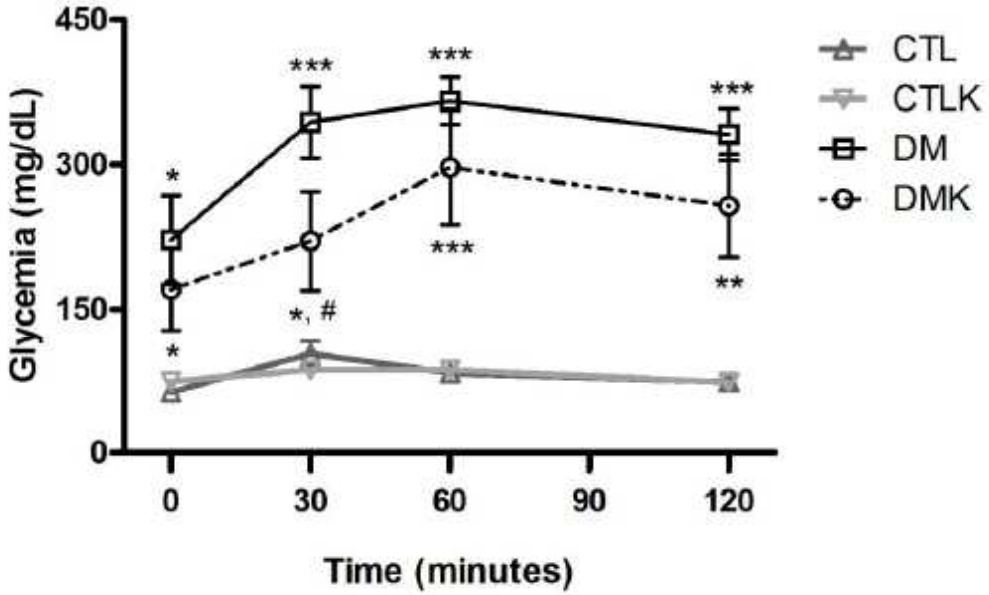
[a] $p < 0.001$ vs CTL.
[b] $p < 0.01$ vs CTLK.
[c] $p < 0.05$ vs DM.

Glycemia levels



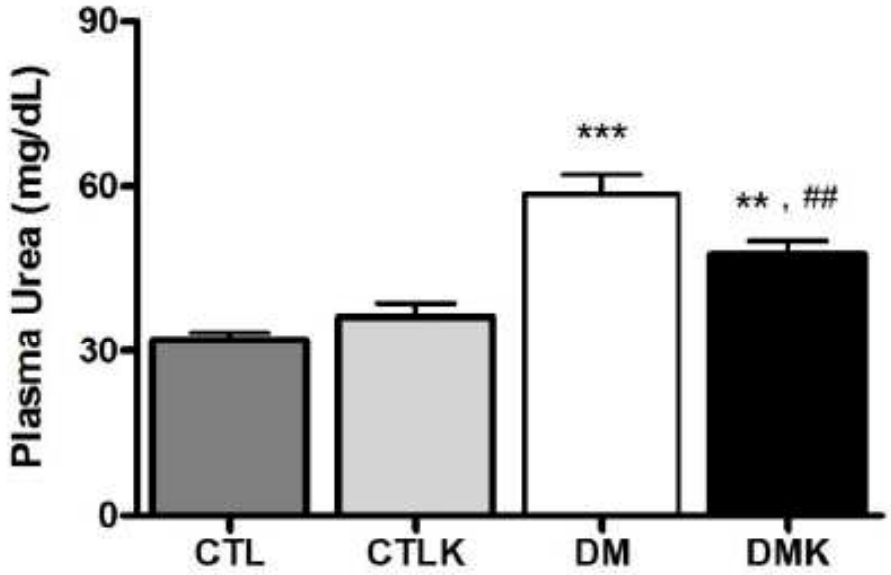
Glycemia levels in the 5th day after diabetes induction (0) and 2-4-8 weeks after Kefir treatment. Control (CTL) n=9; control Kefir (CTLK) n=9; diabetic (DM) n=12; diabetic Kefir (DMK) n=12. Values are expressed as means ± SEM. One-away ANOVA followed by post test Student Newman Keuls. $^{***}p<0.001$ vs controls; $^{###}p<0.001$ vs DM

Oral Glucose Tolerance Test



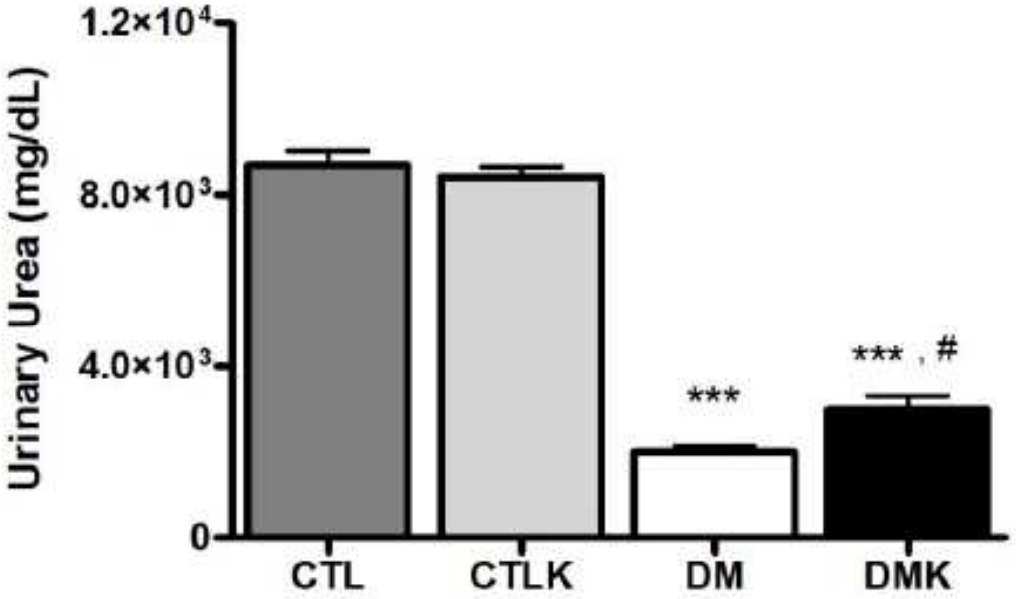
Glycemia levels during oral glucose tolerance test (OGTT) after 8 weeks of Kefir treatment. Control (CTL) n=4; control Kefir (CTLK) n=5; diabetic (DM) n=6; diabetic Kefir (DMK) n=4. Values are expressed as means ±SEM. One-away ANOVA followed by post test Student Newman Keuls. $^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$ vs controls; $^{\#}p<0.05$ vs DM.

Renal function



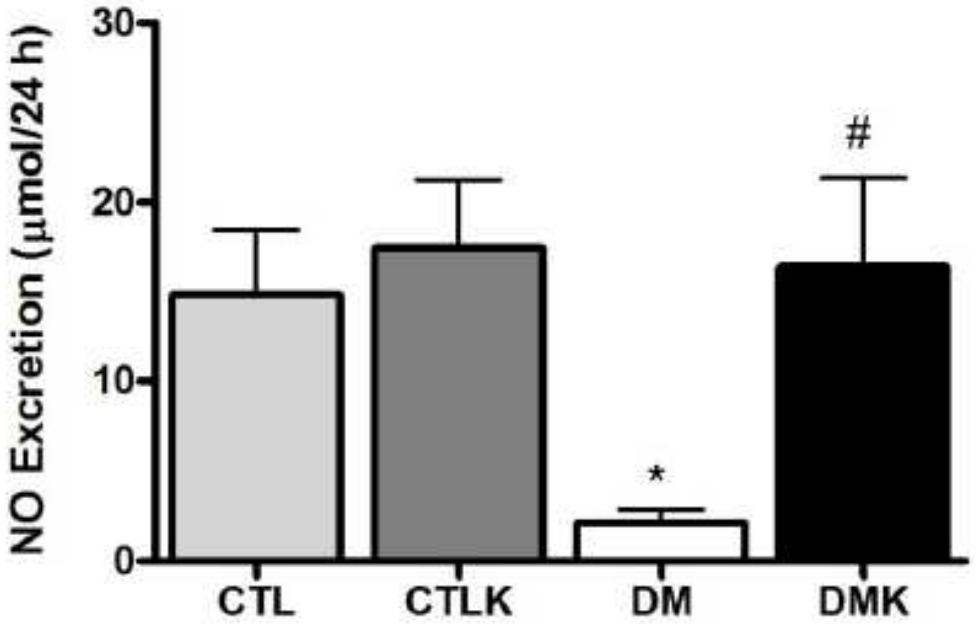
Plasma urea concentration after 8 weeks of Kefir treatment. Control (CTL) n=9; control Kefir (CTLK) n=9; diabetic (DM) n=12; diabetic Kefir (DMK) n=12. Values are expressed as means ±SEM. One-away ANOVA followed by post test Student Newman Keuls, $^{**}p<0.01$, $^{***}p<0.001$ vs controls; $^{\#\#}p<0.01$ vs DM.

Renal function



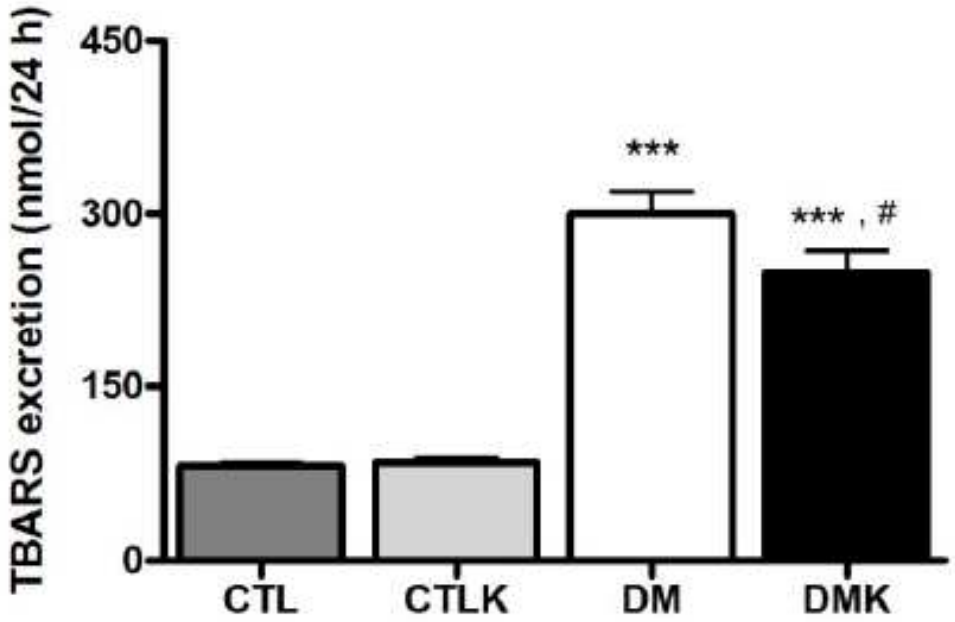
Urinary urea concentration after 8 weeks Kefir treatment. Control (CTL) n=9; control Kefir (CTLK) n=9; diabetic (DM) n=10; diabetic Kefir (DMK) n=11. Values are expressed as means ±SEM. One-away ANOVA followed by post test Student Newman Keuls, $^{***}p<0.001$ vs controls; $^{\#}p<0.05$ vs DM.

Oxidative Stress



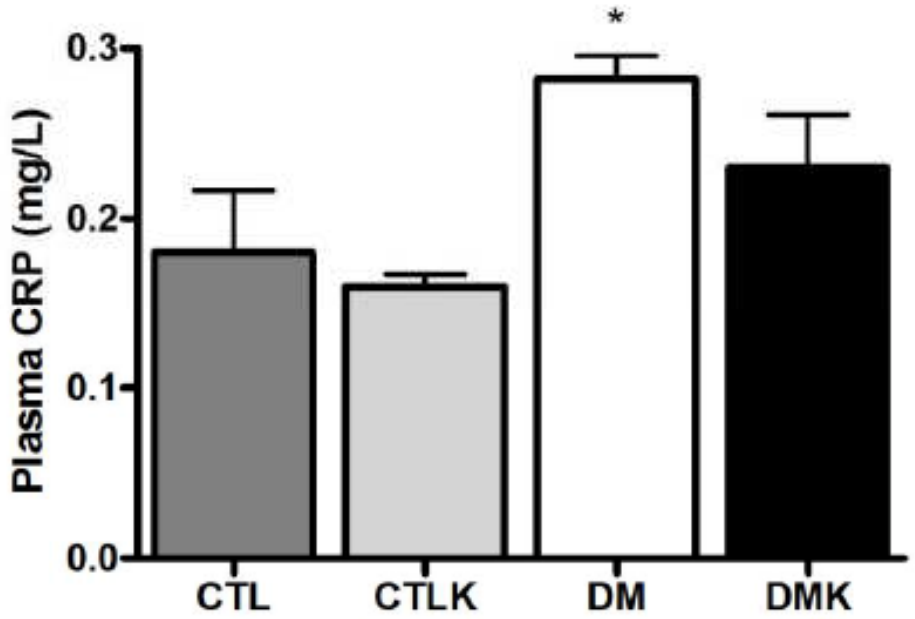
Excretion of nitric oxide (NO) in all groups after 8 weeks of Kefir treatment. Control (CTL) n=9; control Kefir (CTLK) n=9; diabetic (DM) n=11; diabetic Kefir (DMK) n=12. Values are expressed as means ±SEM. One-away ANOVA followed by post test Student Newman Keuls. $^{*}p<0.05$ vs CTL; $^{\#}p<0.05$ vs DM.

Oxidative Stress



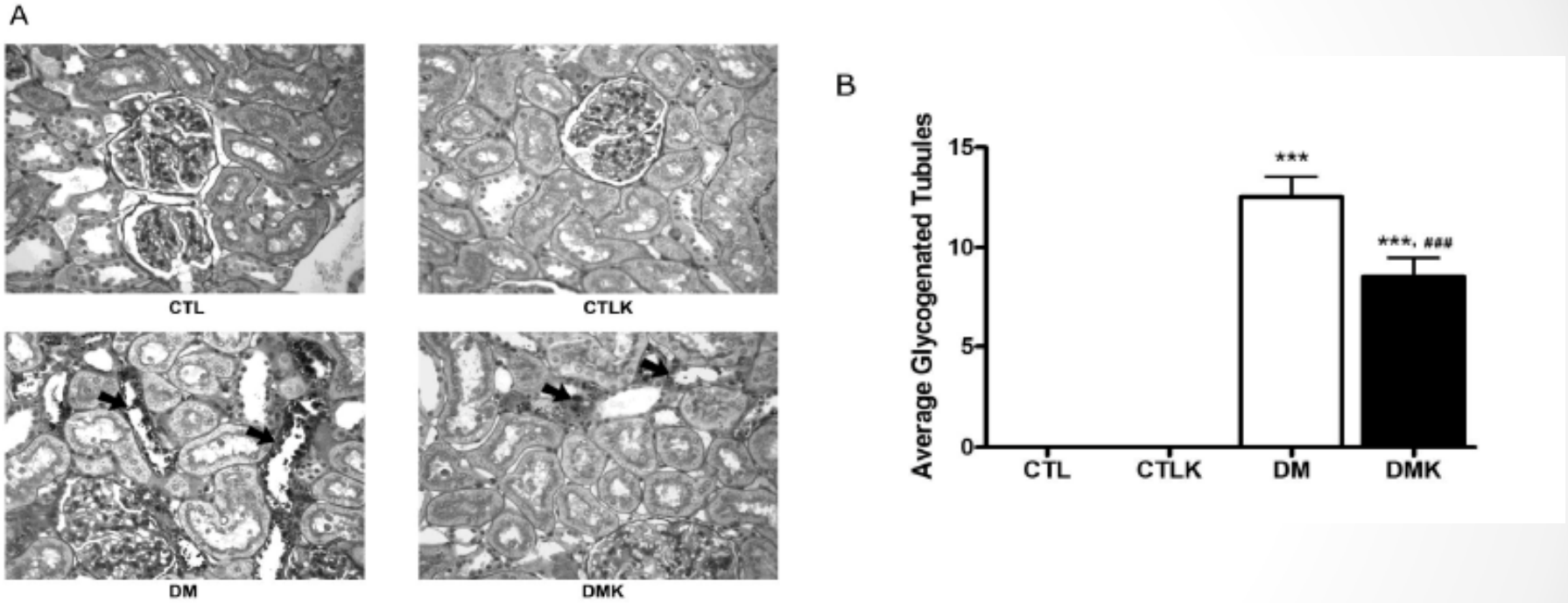
Excretion of thiobarbituric acid reactive substances (TBARS) after 8 weeks of Kefir treatment. Control (CTL) n=9; control Kefir (CTLK) n=9; diabetic (DM) n=10; diabetic Kefir (DMK) n=12. Values are expressed as means ±SEM. One-away ANOVA followed by post test Student Newman Keuls. $^{***}p<0.001$ vs controls; $^{\#}p<0.05$ vs DM.

Inflammatory biomarker



Plasma C-reactive protein (CRP) levels after 8 weeks of Kefir treatment. Control (CTL) n=5; control Kefir (CTLK) n=5; diabetic (DM) n=6; diabetic Kefir (DMK) n=6. Values are expressed as means ±SEM. One-away ANOVA followed by post test Student Newman Keuls. * $p<0.05$ vs CTL.

Kidney histology



Renal histology after the 8th week of Kefir treatment. (A) Kidneys showed glycogen storage in diabetic rats tubules (shown by arrows). (B) Average glycogenated tubules represented graphically in each group (B). Control (CTL); control Kefir (CTLK); diabetic (DM); diabetic Kefir (DMK). Values are expressed as means ±SEM. n=3 per group. One-away ANOVA followed by post test Student Newman Keuls, $^{***}p<0.001$ vs controls; $^{###}p<0.001$ vs DM.

Conclusion

The results obtained in this study show that **Kefir treatment significantly reduced** the progression of STZ-induced **hyperglycemia and oxidative stress** in rats.

Kefir may play a role in **slowing the metabolic changes** that contribute to DM as a non-pharmacological adjuvant .

Acknowledgments

Unifesp

Elisa M.S. Higa[a,]
Giovana R. Punaro[a],
Fabiane R. Maciel[a],
Adelson M. Rodrigues[a],
Silvia S.M. Ihara[b],
Sergio R.R. Araujo[b]

USP

Marcelo M. Rogero[c],
Marice N. Oliveira[d],
Talita R.C. Sanches[e],
Lucia C. Andrade[e,]

This work was supported by:

Coordenação de Aperfeicoamento de Pessoal de Nivel Superior (CAPES),

Fundação de Apoio a Universidade Federal de São Paulo (FAP-Unifesp),

Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP).

Thank you

cris.bogsan@usp.br